# Columella

## Journal of Agricultural and Environmental Sciences

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# PROCEEDINGS

## OF THE



# 16th Alps-Adria Workshop

## Synergism in science

Opatija, Croatia

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Zoltán KENDE

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**Reviews & project communications**

# Prologue

The Alps-Adria scientific cooperation has a long history by now. No one knows whoever has launched the idea to collaborate in the field of various sciences within the region. There is one exact date – namely 2002 – when the first Alps Adria Scientific Workshop was organized in Opatija, initiated by specific committees of the Hungarian Academy of Sciences. Since that time the conference series have been wandering around the region like a "travelling circus". We went as far as Dubrovnik and as high as the Gross Glockner regarding the venues of the scientific meeting. However during the past period several times we returned to the birthplace – Opatija.
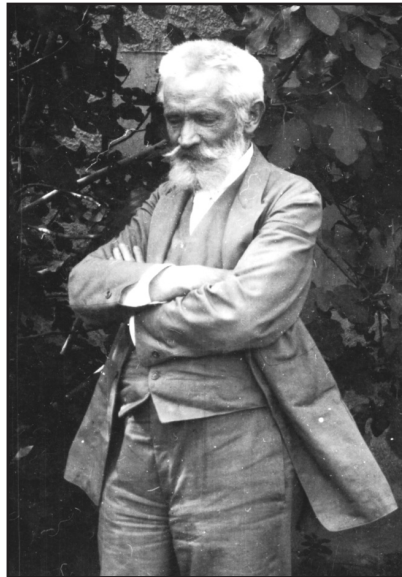


Professor Jenő Cholnoky (1870-1950)

Organizers of the 16th workshop have chosen a unique subject to discuss; economic geography. What is that? What are the aim and the scope of this branch of science? Economic geography is the study of the location, distribution and spatial organization of economic activities across the world. It represents a traditional subfield of the discipline of geography. Economic geography has taken a variety of approaches to many different subject matters, including the location of industries, economies, transportation, trade, development, tourism, land use, the relationship between the environment and the economy, culture-environment interaction, and globalization.

Geography of agriculture is traditionally considered to be the branch of economic geography that investigates those parts of the Earth's surface that are transformed by humans through primary sector activities. It thus focuses on structures of agricultural landscapes and asks for the processes that lead to these spatial patterns. While most research in this area concentrates rather on production than on consumption, a distinction can be made between nomothetic (e.g. distribution of spatial agricultural patterns and processes) and idiographic research (e.g. human-environment interaction and the shaping of agricultural landscapes). The latter approach of agricultural geography is often applied within regional geography.

The conference is dedicated to the memory of Professor Jenő Cholnoky (1870-1950). He was one of the last scientists who's work yielded axiomatic results in various fields like earth sciences, geology, hydrology, geography, ethnography, as well as published and disseminated postulates of his research in relation with society, demography and economy.

The 16th Alps Adria Scientific Workshop is intended to provide floor for scientific lectures, presentation of research results and discussion of the subjects that belong to the broader field of economic geography. The conference is clustered into three branches: natural sciences, including environment, earth sciences, soil science, hydrology and geography. Life sciences, covering the fields of agronomy, biology, land use, crop production, animal husbandry. Social sciences, including economy, health, society, demography, tourism and migration.

The conference is held in the Grand Hotel Adriatic, a really comfortable, friendly and cooperative hotel on the Adriatic and one of the most popular convention venues in Croatia. Situated in the beautiful surroundings of the Opatija Riviera this unique sea side resort will link science and leisure, touristic and intellectual interest. The historical roots of Dalmatia, the royal dignity of Abbazia, and the mutual interest of the participating scientists will result in a successful conference.

Opatija, April 2017

*Márton Jolánkai*

# The effects of wastewater irrigation on the yield of energy willow and soil sodicity

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Abstract: However, lot of surface water is available for irrigation in Hungary now; in the near future the access for fresh water can be limited due to the more and more competition for resources. In this context, farmers expected to be forced to use moderate saline or saline water for irrigation also. In our research, waste water was used for irrigation of energy willow in lysimeter experiment in 2015. There were applied 7 irrigation treatments and one rain-fed control in four replications during the vegetation period. In each 1 m³ lysimeter were planted two willows which were harvested in 2015 December. Dry and wet biomass, dry matter content of the willow and the exchangeable sodium content and ammonium-lactate soluble sodium (AL-Na) content of the soil were investigated in order to evaluate the impact of waste water on the willow yield and the soil salinization. According to our results, the improved waste water treatment (diluted and containing added gypsum repair material) produce the same yield like raw waste water and control irrigation treatment. At the same time, the soil from the significant sodium accumulation was prevented by this treatment. The improvement of waste water quality could be represented by a practical solution of moderate saline water use for irrigation.

Keywords: exchangeable sodium percentage, ammonium-lactate soluble sodium, lysimeter, gypsum

## Introduction

Sustainable soil and water management and use of alternative water resources for agricultural production are one of the key elements of the fight against the continuous increase in global population (Singh, 2015) and climate change. The new water resources play determining role because of the water scarcity (Francés et al., 2017) in addition to water and energy saving irrigation methods. For the sustainable soil management the irrigation water quality has to be prosperous to avoid soil degradation (e.g. salinization) (Singh, 2015; Elgallal et al., 2016). More and more water types are used for irrigation despite of having high specific electrical conductivity above 2000 or even 6000 µs/cm (Tzanakakis et al., 2011; Myers et al., 1998). At the same time, in nowadays this water situation require rethinking and renewal of the irrigation water qualification system also in Hungary to create more efficient and sophisticated regulations for irrigation water qualification. With a new or modified system more water resources can became applicable for irrigation purposes nonetheless without soil salinization. In this paper a new type of diluted and contained repair added gypsum waste water through the soil sodium accumulation parameters and energy willow yield was evaluated.

## Materials and methods

The experiments were conducted at the National Agricultural Research and Innovation Centre (NAIK), Research Department of Irrigation and Water Management (OVKI) in

Szarvas, Hungary. The experiment was set up in the NAIK ÖVKI Lysimeter Station in 2014 in 64, 1 m³ vessels with energy willow. The two willow clones (no. 77, 82) were selected by the Forest Research Institute of Püspökladány Experimental Station of NAIK. In this experiment the soil of clone no. 82 was examined in 24 vessels in three replications in three depths (0-20, 20-40, 40-60 cm). The mean temperature at the irrigation period (June-September) in 2015 was 22.3°C and the precipitation was 137.2 mm. Between 19 June 2015 and 18 September 2015 irrigation occurred 12 times with 3 doses 15, 30 and 60 mm with two water types. First one originated from the Oxbow Lake of Körös River (K15, K30, K60) with excellent water quality according to Filep's classification (Stefanovits, 2010) while the other one was a wastewater (W15, W30, W60) from an intensive African catfish farm in Szarvas with high sodium, total dissolved salt and hydrogen carbonate content (Table 1). Beside the rainfed treatment (Control), one wastewater based irrigation water type (HG60) was used for irrigation (only 60 mm doses) which was diluted with River Körös water and added gypsum to improve sodium-calcium rate of the waste water (Table 1).

Table 1: Chemical parameters of the different irrigation water types

|  | River Körös | Wastewater | HG60* | Analytical method |
|---|---|---|---|---|
| pH (KCl) | 7.49 | 7.46 | 6.71 | MSZ EN ISO 10523:2012 |
| Specific electrical conductivity (20 °C) (µS/cm) | 436 | 1310 | 924 | MSZ EN 27888:1998 |
| Bicarbonate (mg/l) | 227 | 949 | 398 | MSZ ISO 9963-1:1998 |
| Calcium (mg/l) | 48.3 | 20.0 | 98.9 | MSZ 1484-3:2006 |
| Potassium (mg/l) | 3.94 | 6.19 | 4.55 |  |
| Magnesium (mg/l) | 12.6 | 9.42 | 11.9 |  |
| Sodium (mg/l) | 44.6 | 291 | 107 |  |
| Na% * | 35.4 | 86.8 | 43.6 | calculated |
| SAR * | 1.48 | 13.4 | 2.71 | calculated |

* SAR=Na/((Ca+Mg)/2)1/2 (Richards, 1954 In: Ayers and Westcot,1994)
* Na%=Na/(Ca+Mg+Na+K)*100 (Darab K. – Ferecz K., 1969)
* HG60=diluted and improved irrigation water

The soil samples were collected before and after the irrigation period and analysed in the Laboratory for Environmental Analytics (NAIK ÖVKI). The statistical calculation was performed in SPSS 22.0 Statistics Software (T-Test, ANOVA).

## Results and discussion

The mean value of the exchangeable sodium percentage of the all soil sample (72) from the experiment was 1.72% in springtime and 2.70% after the irrigation period, in autumn. The minimum measured value was 0.96% in spring and 1.19% in autumn. The maximum value was 2.65% and 7.18% in these sampling times.

Table 2: Alteration of the exchangeable sodium percentage (%) during irrigation time in the different treatments (According to MSZ-08-0214-2:1978)

| Depth of soil (cm) | W15 | W30 | W60 | HG60 | K15 | K30 | K60 | Control |
|---|---|---|---|---|---|---|---|---|
| 0-20 cm | 2.70* | 4.34** | 3.72 | 0.13 | 0.34* | 0.49 | -0.10 | 0.57** |
| 20-40 cm | 0.43* | 2.92** | 2.77** | -0.24 | 0.24 | 0.14 | -0.24 | 0.58** |
| 40-60 cm | 0.14 | 1.47* | 1.54* | 0.16 | -0.03 | 0.11 | 0.72 | 0.54* |

Values represents the subtraction of exchangeable sodium percentage (%) in spring and in autumn (*:$p<0.05$ **: $p<0.01$ ***: $p<0.001$)

The highest increase occurred in the treatment W30 (>+4%). In the Control treatment the increase was also significant in all layers but the growth was below 1%. In the treatment W60 the increase was significant just in the subsurface layer between 20 and 40 cm despite of the high values. In the treatments irrigated with Körös River water the increase was not significant (or negligible in K15) and in one case decrease occurred (K60). In treatment HG60 there were no significant increase.

*Table 3: Alteration of the ammonium-lactate soluble sodium content (mg/kg) in the soil during irrigation time in the different treatments*

| Depth of soil (cm) | W15 | W30 | W60 | HG60 | K15 | K30 | K60 | Control |
|---|---|---|---|---|---|---|---|---|
| 0-20 cm | 182** | 289* | 323* | 48** | 45** | 58** | 25** | -5 |
| 20-40 cm | 57* | 220** | 156** | 25* | 42*** | 50** | 13 | -2 |
| 40-60 cm | 45* | 112* | 68* | 30** | 31* | 43** | 10 | 10* |

*Values represents the subtraction of AL-Na (mg/kg) in spring and in autumn (\*:p<0.05 \*\*: p<0.01 \*\*\*: p<0.001).*

The mean value of the AL-Na content was 75.1 mg/kg in springtime and 153.1 mg/kg after irrigation period. The minimum value of the AL-Na content was 43.9 mg/kg and 79.3 mg/kg before and after irrigation. The maximum measured value was 119 and 492 mg/kg, in spring and autumn, respectively. The significant increase occurred in all treatments, but in the control was not remarkable (Table 3). In the treatments with wastewater the mean alteration in all layers was 161 mg/kg while this value was only 35 mg/kg in HG60 and the same in the three treatments with River Körös. The increase was the highest in the surface layers in all treatments similarly as the exchangeable sodium percentage. The treated wastewater irrigation (HG60) had the similar impact on the AL-Na content of the soil like the Körös River water (K30 and K60).

*Table 4: Biomass production (g/m²) and dry matter content (%) of the willow in the different treatments*

| Treatment | Wet yield (g/m²) | Dry yield (g/m²) | Dry matter (%) |
|---|---|---|---|
| Control | 1445[a] | 716[a] | 49.6 |
| K15 | 2418[b] | 1199[ab] | 49.6 |
| W15 | 2745[bc] | 1361[bc] | 49.5 |
| K30 | 2975[bc] | 1492[bc] | 50.2 |
| W30 | 3573[cd] | 1796[cd] | 50.3 |
| K60 | 4433[de] | 2289[de] | 51.6 |
| HG60 | 4443[de] | 2278[de] | 51.1 |
| W60 | 4568[e] | 2332[e] | 51.0 |

[abcde]: *Homogenous subsets of the Tukey's Test*

According to the measurements of the wet and dry biomass production of the willow, the highest productivity was achieved in the treatments with 60 mm irrigation doses. There were no significant differences between these three treatments despite of the distinct irrigation water quality (K60, HG60, W60), (Table 4). The highest dry matter contents were also in these treatments, but there was no significant difference between the other irrigations.

**Conclusions**

In the treatments with Körös River water irrigation, the exchangeable sodium content did not increase significantly and the AL-Na increased less than in treatments with wastewater. In the treatments with diluted and improved water quality (HG60) the examined soil

parameters values were the same like in the treatment with the Körös River irrigation, which has appropriate, distinguished water quality according to the Hungarian classifying. According to Jalali et al. (2008) the exchangeable sodium percentage (ESP) of the soil was increased with 12% when the initial ESP was 9% and the total salt content of the irrigation water was 6040 µS/cm and the SAR value was 25.3 in a leaching experiment with soil columns. According to our results irrigation water with 43.6 Na% did not cause higher sodium accumulation than irrigation water with 35.4 Na%. Despite of the distinct irrigation water qualities, there were no significant differences in the wet and dry yield of willow between the treatments with same irrigation water quantity. According to Wang et al. (2016) three years of continuous irrigation with saline water (with 3000 mg/l total salt content) also did not remarkably reduce the yield of spring maize (average yield 7811 kg/ha) compared to the fresh water irrigation (70 mg/l). The main results of the experiment is that with applying of the diluted and improved irrigation water mix the wastewater can be reused and can retain for the soil water balance, the soil salinization avoidable, nonetheless the willow yield is the same like with the classic, prosperous water quantity.

## Acknowledgement

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# Model study to investigate the toxic interaction between glyphosate herbicide and lead acetate on chicken embryos

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Abstract: The aim of this study was to determine the individual and combined toxic effects of Glialka Star herbicide (360 g/l) and lead acetate on the development of chicken embryos. The eggs were injected by 0.1 ml of lead acetate solution (0.01%; 0.1%) and/or by 0.1 ml of Glialka Star (glyphosate, 360 g/l; 2%). The treatments were performed on day 0 of incubation, and the embryos were examined on day 19 by the followings: rate of mortality of embryo, body mass, type of developmental anomalies by macroscopic examination. The body weight was evaluated statistically by the one-way ANOVA with Tukey and Dunnett post-test, the mortality of embryo and the developmental anomalies was analysed by Fisher test. Single treatment of both items and their combination increased the mortality of embryo but the single treatment of herbicide caused significant difference. The combination of Glialka Star and lead acetate significantly reduced the body weight of embryos but no significant difference was observed due to the single administration of the herbicide. Developmental abnormalities were observed sporadically either single or concomitant treatment. Based on the results there is a possibly additive toxic interaction between the lead acetate and Glialka Star that can highly reduce the viability of the embryos or can lead to extinction of wild birds.

Keywords: chicken embryo, lead acetate, toxic interaction, developmental abnormality, glyphosate

## Introduction

The chemical plant protecting process is one of the most important polluting activities in the agricultural production. The ecosystem of a given habitat can be contaminated simultaneously by sprayed pesticides and other xenobiotics, e.g. heavy metals due to the agricultural activities during the plant protecting processes. Therefore, the chemical load can be occurred as a complex problem, so the combined toxic effect, i.e. toxic interaction of at least two substances can expected and the components can modify the effect of each other. Recently, the examination of the combination of heavy metals and other chemicals gained significant ground in both avian (Fejes et al., 2001; Kertész, 2001) and mammalian (Institóris et al., 2001; Pecze et al., 2001) toxicology research studies. Furthermore, the interaction effects are examined not only in the field of ecotoxicology, but also in all other areas that deal with health care and chemical safety issues (Oskarsson, 1983; Danielsson et al., 1984; Speijers and Speijers, 2004).

The different agricultural areas offer sources of food, shelter and breeding places to wild birds, therefore the sprayed pesticide and other chemical substances can contaminate not only the adults, but the embryos developing in egg, as well. The eggs of the wild birds may be exposed to different chemicals on the cultivated lands at the same time and their toxic effects may appear in embryo mortality and developmental anomalies.

Teratological tests carried out on avian embryos provide useful data for environmental protection and facilitate the development of environmental-friendly chemical plant protection techniques (Várnagy et al., 1996). The aim of our study was to examine the toxic effect and interaction of lead acetate and a glyphosate containing herbicide (Glialka Star) on chicken embryos after administration of single compounds and simultaneously by injection technique.

**Materials and methods**

Farm chicken eggs with good fertile potential (Goldavis Ltd., Hungary) were used in the experiment. The eggs based on their size and weight were divided into six homogenous groups (40 eggs in each), and were incubated in Ragus type table incubator (Vienna, Austria) ensuring the required temperature (37–38ºC), the relative humidity (65–70%) and the daily rotation.

The eggs were treated with a final volume of 0.1 ml solution or emulsion of the test items, directly into the air-chamber with a pipette on the first day of incubation. The egg-shell was bored through before the injection, and it was sealed with paraffin after treatment (Clegg, 1964). During the single and simultaneous administration lead acetate (Reanal-Ker Ltd., Budapest) with a concentration of 0.01% or 0.1% and 2% of Glialka Star glyphosate containing herbicide (Monsanto Hungary Ltd., 360 g/l) corresponding to that used in plant protection practice were applied. The control group was treated with avian physiological saline solution (NaCl 0.75%). The details of the experimental design are presented in *Table 1*.

All eggs and embryos were examined and processed on day 19 of incubation. During the processing rate of embryo mortality, body mass of embryos and type of developmental anomalies were registered.

The distribution of body weight of the live embryos was controlled by Comparison-Quantile Plot and was analysed statistically by One-Way ANOVA. Data of groups were compared by Tukey and Dunnett tests. The statistical analysis of the results of embryo mortality and developmental abnormalities were performed by Fisher's exact test (Baráth et al., 1996).

*Table 1. Experimental design*

| Group | No of egg | Treatment (concentration) | |
|---|---|---|---|
| | | Lead acetate | Glialka Star |
| I (control) | 40 | - | - |
| II | 40 | 0.01% | - |
| III | 40 | 0.1% | - |
| IV | 40 | - | 2% |
| V | 40 | 0.01% | 2% |
| VI | 40 | 0.1% | 2% |

**Results and discussion**

The average body weight of the embryos was 19.26±1.37 g and 18.31±1.71 g in Group II and Group III that was significantly lower as compared to the control group (22.58±1.98 g, p=0.001). Due to glyphosate treatment (2%) the body weight was 21.98±1.92 g that was significantly lower than the control. The simultaneous administration of lead acetate (0.01%) and glyphosate resulted significant decrease (p=0.05) of average body weight (Group V: 20.48±1.28 g) as compared to the control (p=0.05) (*Figure 1*). The combination of 0.1% concentration of lead acetate and 2% glyphosate caused 97.50% mortality of the embryos.The results of the embryo mortality is presented in *Table 2*.

*Figure 1. Body weight (g) of the chicken embryos on day 19 of incubation from teratogenicity test on Glialka Star and lead acetate after single and simultaneous administration*
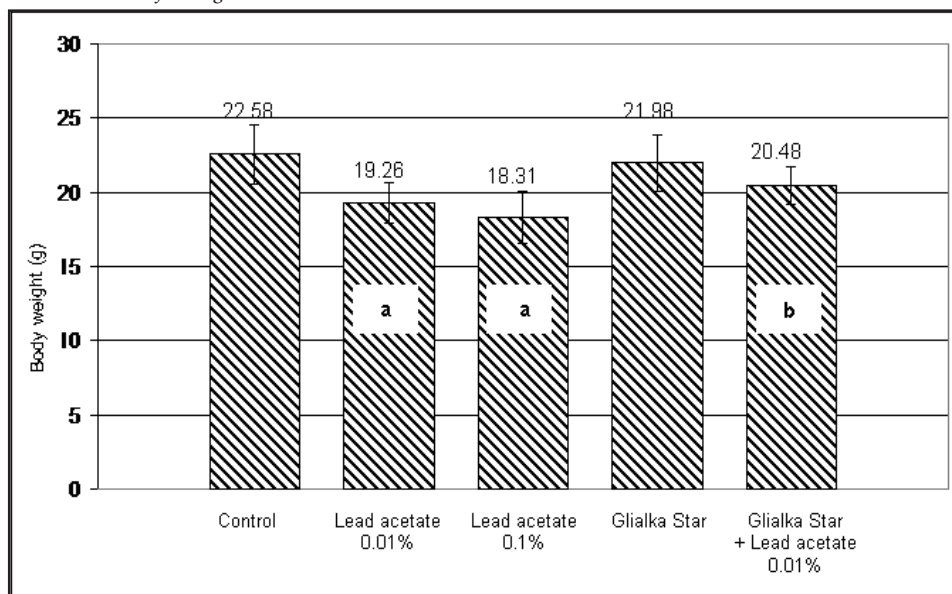


a: Significance decrease as compared to the control(p=0.001)
b: Significance decrease as compared to the control(p=0.05)

There were two died embryos in the control group (5%). The single administration of lead acetate increased the mortality up to 20% and 27.50% in Group II and Group III, respectively. The changes were not statistically different as compared to the Group I (control). The application of 2% Glialka Star caused 57.50% of mortality in the treated embryos in Group IV that was significant as compared to the control (p=0.001).

*Table 2. Embryonic death and developmental anomalies from teratogenicity test of lead acetate and Glialka Star in chicken embryos after single and combined administration*

| Group | Treatment | No of embryos showing abnormality/No of live embryos | Death No/ Total eggs | Rate of developmental anomalies (%) | Mortality (%) |
|---|---|---|---|---|---|
| I | Control | 0/38 | 2/40 | 0 | 5.00 |
| II | Lead acetate 0.01% | 1/32 | 8/40 | 3.12 | 20.00 |
| III | Lead acetate 0.1% | 2/29 | 11/40 | 6.89 | 27.50 |
| IV | GLIALKA STAR | 0/17 | 23/40[a] | 0 | 57.50 |
| V | GLIALKA STAR + Lead acetate 0.01% | 0/32 | 8/40 | 0 | 20.00 |
| VI | GLIALKA STAR + Lead acetate 0.1% | 1/1 | 39/40 | 100.00 | 97.50 |

a: Significant difference as compared to the control (p=0.001)

Due to the simultaneous administration of 0.01% lead acetate and 2% glyphosate (Group V) induced not significant increase of embryo mortality (20%), and simultaneous administration of 0.1% lead acetate and 2% Glialka Star (Group VI) the rate of mortality increased up to 97.50%.

Developmental abnormalities were not recorded in the control group (*Table 2*). The 0.1 and 0.01% lead acetate induced leg deformation and open abdomen (Group II: 1, Group III: 2 embryos) without statistical difference as compared to Group I.  Teratogenic malformations were not registered due to the single administration of Gialka Star (Group IV) and its application with 0.01% lead acetate (Group V). However, the higher concentration of lead acetate (0.1%) and 2% glyphosate containing product induced leg deformation, growth retardation and beak malformation in the survivor embryos (Group VI).

The results of the individual teratogenicity studies on lead acetate in chicken are in accordance with results of toxicity studies in other species. Depending on the dose, lead has embryotoxic potential and may cause developmental anomalies (Ferm and Carpenter, 1967; Várnagy and Budai, 1995). Similar results were found in chicken embryos treated with 0.01% lead acetate (lower body weight, higher rate of embryo mortality) but the developmental anomalies were not significant versus the control group (Juhász, 2009).

Glyphosate containing RoundUp herbicide was examined by other researchers in Wistar rats. Dams were treated orally with 500, 750 and 1000 mg/kg glyphosate via drinking water. The results showed 50% mortality rate of dams treated with 1000 mg/kg glyphosate. Skeletal alterations were observed in foetuses of the dams in Groups treated with 500, 750 or 1000 mg/kg. Based on these data can be concluded that the glyphosate containing RoundUp is toxic to dams and induces developmental retardation of the foetal skeleton (Dallegrave et al., 2003).

Generally, the simultaneous application of heavy metals and pesticides may cause significant increase of their toxic effect in comparison with the individual toxicity of the applied components. It was particularly distinct due to the combined administration of Glialka Star herbicide.

According to the published literature the toxicity of many pesticide combinations is at least additive. In some cases pesticide mixtures, if they particularly contain insecticide component, have been shown to be synergistic effects, with reported increase in toxicity up to 100-fold. However, these effects are species, time and dose dependent, therefore difficult to predict it routinely (Thompson, 1996).

**Conclusions**

The single treatment of lead acetate with 0.1 and 0.01% concentration induced embryotoxic effect in chicken embryo which manifested in significant decrease of body weight and elevated rate of embryo mortality.

The glyphosate containing Glialka Star plant protection product with herbicidal action was also embryotoxic on chicken embryos and resulted not significant reduce of body weight and statistically significant increase of mortality. Due to the simultaneous application of lead acetate and Glialka Star the embryo mortality was statistically higher than the individual effect.

Developmental abnormalities were sporadically observed due to the single and concomitant administration (leg and beak deformation, growth retardation, open abdomen).

Based on the results, there are presumably addition-type toxic interaction between lead acetate and Glialka Star herbicide, that highly reduce the viability of the embryos.

### Acknowledgement

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# 16th Alps-Adria Workshop

## Synergism in science

# The effect of sowing date and plant density on yield elements of different winter oilseed rape (*Brassica napus var. napus f. biennis L.*) genotypes

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Abstract: Rapeseed is worldwide the 3rd, while regarding Hungary – beside sunflower – the 2nd most important oil crop produced. In our country winter oilseed rape is grown. Its cultivation area has been increasing since 1990; currently it varies between 200 and 250 thousand ha. The variety/hybrid palette has changed significantly during the past decade. As the result of this process hybrids are produced regarding the national average on 90%, while varieties on 10% of the total 230 thousand hectares production area. The experiment was set up at the Látókép Plant Production Research Site of the University of Debrecen. Three different sowing times were investigated and three different plant densities were set in 2015 200, 350 and 500 thousand plant/ha$^{-1}$. Uniform nutrient supply and a row spacing of 45 cm were applied. Winter wheat was used as pre-crop. Plant physiological measurements (relative chlorophyll content analysis (SPAD) and leaf area index (LAI)) were made in the populations of the Arkaso and Hybridrock rapeseed hybrids and Ontario variety. Relative chlorophyll content (SPAD) and leaf area index (LAI) were monitored in 7 different measurement times. Plant densities were counted before the winter and after the winter in the all experiment. The primary and secondary braniching, number of siliquae and seed yield have been determined.

Keywords: winter oil seed rape, sowing date, plant density, overwinter, yield quantity

## Introduction

Demand on plant oil has shown increasing tendency in the past decades both world- and countrywide. Regarding the most important produced oil crops rapeseed is ranked 3rd worldwide and 2nd in Hungary at present (Pepó-Vincze, 2015). According to the data of the Central Bureau for Statistics (2015) rapeseed was sown in Hungary on a territory of 225 thousand hectares. Its sowing area was 9 thousand hectares higher than that of the previous year, but regarding the 2010-2014 average, that showed significant deviations in sowing area, this area was only 8 thousand hectares higher. In the 2015 57% of the total area sown with rapeseed was located in Central Hungary and in the Transdanubian region, while 43% in the regions Northern-Hungary and Northern Great Plain. According to Eőri (2012) the least suitable production regions in Hungary are the northeaster counties and the regions by the river Tisza due to the cold, continental winter weather conditions. Strong night radiation increases the risk of late frosts as well. Concerning the statistical data it can be stated that most of the freeze in rapeseed populations occur in the region Great Plain, especially in case – due to any reason – a given rapeseed population was not well-prepared for the winter. Therefore an important aspect of this research is to choose the optimal sowing time and plant density in order to have favourably developed rapeseed population in the autumn period that is less sensitive towards winter frost. Sváb and Simits (1980) analysed different experimental data and concluded that there was a positive relationship between yield and the total applied nitrogen fertilizer-amount, as well as the depth of soil tillage, but there was a negative correlation between yield and spring weed coverage, just as sowing time. Yield exceed 2.5 t ha$^{-1}$ overall the country, but this can be improved to a significant extent. Potential yield capacity of rapeseed is about 6 t grain yield ha$^{-1}$. However, criteria of that are perfect winter hardiness, good regeneration ability, optimal plant density, just as tolerance to late sowing time etc. (Röbbelen, 1975).

According to the results of Blum (2009) there is a difference in the production technology of varieties and hybrids regarding seed rate and spring nutrient supply. Necessity of autumn N-fertilization, just as the use of growth regulators in the autumn and spring periods are determined by sowing time, the development of the given population and the properties of the applied varieties or hybrids. In their experiments Risnoreanu and Buzdugan (2011) found the sowing time interval 5-10 September to be optimal. 17-25 August is considered as optimal sowing time in Germany. In accordance with German researchers each day delay in sowing results in yield loss (Eőri, 2012). However, Schuster (1965) stated that yield loss is not realized linear. Rapeseed population sown in September produced half the yield of that sown in August. In the experiments of Sharafizadeh et al. (2012) sowing time affected rapeseed yield to a significant extent.

## Materials and methods

Our experiments were set up on calcareous chernozem soil in the Hajdúság, 15 km from Debrecen at the Látókép Plant Production Research Site of the University of Debrecen. The soil is characterized by favorable physical, chemical and biological traits. The humus content of the calcareous chernozem soil of the experiment is 2.76%, its AL extractable $P_2O_5$ content is 133 mg kg-1, its AL extractable $K_2O$ value is 240 mg $kg^{-1}$. The soil has favorable water management conditions. The soil saturated up to the field water capacity can store 578 mm water in the 0-2 m layer, 50% of which is disposable water. The experiment design was set as split-plot, plot areas were 36 $m^2$ in four replications and we used two hybrids (Arkaso and Hybridrock) and one varieties (Ontario). Three different sowing times were investigated in the experimental year (2015/2016). Three different plant densities were set: 200, 350 and 500 thousand plants $ha^{-1}$. Uniform nutrient supply and a row spacing of 45 cm were applied. Winter wheat was used as pre-crop. In the crop year were an early sowing date: August 28 (SD1), average: September 12 (SD2), late: September 23 (SD3). The harvest was done with a SAMPO plot combine harvester.

In the crop year 2015/2016 altogether 694.6 mm precipitation fell during the vegetation of rapeseed (1 August 2015 – 30 June2016). This amount was about one and a half times higher than the several years' average (Table 1.). The significant amount of precipitation in August (84 mm) was higher than the several years' average value (60.7 mm), which enabled the execution of soil preparation works in a rather good quality. September and October were very wet. This was favourable from the aspect of uniform emergence and adequate early development of rapeseed populations. Vegetative development of rapeseed populations was favoured by the weather conditions of autumn months. An amount of precipitation that fell in October (86.6 mm) was higher than the several years' average (30.8 mm). The monthly average temperature (10.0 ˚C) was similar to the several years' average (10.3 ˚C). Measured average temperature at the experimental field was higher in November (5.3 ˚C) than the average value (4.5 ˚C). Due to the combined effect of the mentioned factors, just as the optimal applied agrotechnical management, rapeseed populations started winter in favourable development stage. Monthly temperature average values were higher than the several years' average values – except for October and May.

For the statistical evaluation of the experiment, SPSS 13.0 for Windows and Microsoft Excel 2010 programs are used. The statistical evaluation, the bifactorial variance analysis and correlation analysis were done according to Sváb (1981), with regression equations.

In the correlation analysis, the following types of correlations according to the r values were determined: r<0.4: loose, 0.4-0.7: medium, 0.7-0.9: tight, >0.9: strong.

*Table 1: Amount of precipitation (mm) and temperature (˚C) during rapeseed vegetation period (Debrecen)*

| | | | **Months** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | VIII. | IX. | X. | XI. | XII. | I. | II. | III. | IV. | V. | VI. | **Total/ Average** |
| **Precipitation (mm)** | **2015/2016** | | 84 | 49 | 87 | 43 | 13 | 59 | 79 | 51 | 15 | 69 | 146 | **694,6** |
| | **30 year's average** | | 60,7 | 38 | 31 | 45 | 44 | 37 | 30 | 34 | 42 | 59 | 79,5 | **499,6** |
| **Temperature (°C)** | **2015/2016** | | 23,3 | 18 | 10 | 5,3 | 2,2 | -2,3 | 5,5 | 6,4 | 13 | 16 | 20,1 | **10,6** |
| | **30 year's average** | | 19,6 | 16 | 10 | 4,5 | -0,2 | -3 | 0,2 | 5 | 11 | 16 | 18,7 | **8,9** |

## Results and discussion

Freezing rate was studied in the crop year 2015/2016 in case of two hybrids (Arkaso and Hybridrock) and a variety (Ontario) for three different applied plant densities and three different sowing times. The number of primary and secondary branches just as the number of siliquae per plant were registered (Table 2.) in case of the application of different plant densities and sowing times. It can be stated that parallel to increasing plant density the rate of winter loss increased as well. Results of the measurements confirm that the lower plant density was applied at sowing produced higher number of primary and secondary branches, just as the higher number of seed shells could be registered.

*Table 2: Number of registered primary and secondary branches and number of shell of the studied rapeseed genotypes in 2015/2016 (Debrecen)*

| | | **Primary branches** | | | **Secondary branches** | | | **Number of siliquae** | | | **Winter loss (%)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Plant density (thousand ha⁻¹)** | | | | | | | | | | | |
| **Sowing date** | **Genotype** | 200 t ha⁻¹ | 350 t ha⁻¹ | 500 t ha⁻¹ | 200 t ha⁻¹ | 350 t ha⁻¹ | 500 t ha⁻¹ | 200 t ha⁻¹ | 350 t ha⁻¹ | 500 t ha⁻¹ | 200 t ha⁻¹ | 350 t ha⁻¹ | 500 t ha⁻¹ |
| SD1 | 1 | 14.8 | 10.5 | 9 | 24.5 | 5.3 | 5.3 | 890 | 450 | 440 | 12% | 18% | 28% |
| | 2 | 15 | 10.5 | 7.8 | 38.8 | 16.8 | 4.3 | 1409 | 803 | 358 | 18% | 27% | 44% |
| | 3 | 12.8 | 11.5 | 13.3 | 32 | 9 | 16.5 | 1207 | 529 | 691 | 11% | 21% | 23% |
| SD2 | 1 | 13.3 | 9.8 | 7.8 | 28.8 | 8.8 | 9 | 1144 | 536 | 425 | 13% | 16% | 17% |
| | 2 | 10 | 9.3 | 9 | 12.3 | 10.3 | 4.3 | 620 | 549 | 421 | 14% | 16% | 18% |
| | 3 | 12 | 11 | 9.5 | 17 | 13.8 | 6 | 714 | 733 | 382 | 14% | 14% | 15% |
| SD3 | 1 | 9.3 | 7 | 7.3 | 15.8 | 2 | 0 | 707 | 301 | 256 | 15% | 26% | 29% |
| | 2 | 8.8 | 6.8 | 6.8 | 6 | 1 | 6.3 | 383 | 298 | 333 | 15% | 30% | 31% |
| | 3 | 9.3 | 8 | 6.8 | 6.8 | 5.3 | 3 | 581 | 550 | 257 | 20% | 21% | 28% |

Correlation analysis results of data in the crop year 2015/2016 are shown in Table 3.

Medium negative correlation was found between sowing time and the number of primary (r=-0.673) and secondary (r=-0.501) branches, just as the number of siliquae (r=-0.488). As the later sowing was done, the less number of primary and secondary branches, consequently the less number of siliquae was developed. Medium negative correlation between sowing time and seed yield (r=-0.686) explains that in the earlier sowing dates the higher seed yield could be produced. Sowing time affects plant physiological processes. Rapeseed seed yield is determined by the number of siliquae that develop on secondary branches. Sowing time affects the number of branches negatively and thus the number of siliquae, consequently seed yield as well. Strong positive correlation was found between primary and secondary branches (r=0.870), and between primary branches and the number of siliquae (r=0.863). The number of primary branches determines the number of secondary branches, that determines the number of siliquae developed per plant – this is confirmed by the strong positive correlation (r=0.979). As less loss of the studied population occurs from winter to spring, the higher number of primary (r=-0.556) and secondary (r=-0.612) branches will be developed, consequently the higher number of siliquae will be developed later. This is confirmed by the medium negative correlation that was found between all three studied parameters.

*Table 3: Correlation analysis of the studied parameters (Debrecen, 2015/2016)*

| Parameters | Sowing date | Genotype | Plant density | Primary branches | Secondary branches | Number of iliquea | Yield | Winter loss |
|---|---|---|---|---|---|---|---|---|
| Sowing date | 1 | 0 | 0 | -0.673** | -0.501** | -0.488** | -0.686** | 0.104 |
| Genotype | 0 | 1 | 1.000** | 0.104 | 0.047 | 0.078 | 0.162 | 0.019 |
| Plant density | 0 | 1.000** | 1 | 0.104 | 0.047 | 0.078 | 0.162 | 0.019 |
| Primary branches | -0.673** | 0.104 | 0.104 | 1 | 0.870** | 0.863** | 0.341 | -0.556** |
| Secondary branches | -0.501** | 0.047 | 0.047 | 0.870** | 1 | 0.979** | 0.298 | -0.612** |
| Number of siliquae | -0.488** | 0.078 | 0.078 | 0.863** | 0.979** | 1 | 0.317 | -0.594** |
| Yield | -0.686** | 0.162 | 0.162 | 0.341 | 0.298 | 0.317 | 1 | 0.087 |
| Winter loss | 0.104 | 0.019 | 0.019 | -0.556** | -0.612** | -0.594** | 0.087 | 1 |

** Correlation is significant at the 0.01 level (2-tailed).

Table 4. shows rapeseed seed yield obtained in the study. The highest seed yield was obtained in the early sowing time (5475 kg ha$^{-1}$). Regarding the applied sowing dates following yield results were registered: early sowing time: 5475 kg ha$^{-1}$, optimal sowing time: 4760 kg ha$^{-1}$ and late sowing time: 4590 kg ha$^{-1}$.

*Table 4: Rapeseed yield of the studied rapeseed genotypes in 2015/2016 (Debrecen)*

| Genotype | Sowing date | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | SD1 | | | SD2 | | | SD3 | | |
| | 200 t ha$^{-1}$ | 350 t ha$^{-1}$ | 500 t ha$^{-1}$ | 200 t ha$^{-1}$ | 350 t ha$^{-1}$ | 500 t ha$^{-1}$ | 200 t ha$^{-1}$ | 350 t ha$^{-1}$ | 500 t ha$^{-1}$ |
| 1 | 4312 | 4668 | 5006 | 4196 | 4682 | **4760** | 4571 | 4584 | 4293 |
| 2 | 5160 | 5415 | **5475** | 4389 | 4485 | 4387 | 4043 | 3408 | 4104 |
| 3 | 4890 | 4912 | 5104 | 4752 | 4757 | 4696 | 4521 | **4590** | 4373 |

*Genotype 1: Ontario,  Genotype 2: Hybridrock, Genotype 3: Arkaso* LSD 5%

## Conclusions

 In the later sowing dates, the lower number of primary and secondary branches, thus the lower number of siliquae per plant were developed. Higher seed yield was obtained in the earlier sowing time: early sowing time: 5475 kg ha$^{-1}$, optimal sowing time 4760 kg ha$^{-1}$ and late sowing time 4590 kg ha$^{-1}$. Sowing time had negative impact on the number of branches, number of siliquae and seed yield. With the increasing plant density the rate of losses during the winter increased as well.

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16th Alps-Adria Workshop

Synergism in science

# Responses of maize (*Zea mays* L.) roots to soil condition in an extreme growing season

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Abstract: Maize (*Zea mays* L.) covers the greatest area in Hungarian crop production relation. While tillage effects on soil condition and through the physical changes influence the depth and formation of the crop rooting. Maize root development was investigated in a long-term experiment on a Chernozem soil at the Experimental and Training Farm of the Szent István University (47°68'N, 19°60' E, 130 m a.s.l.) near the town Hatvan, Hungary. The objective was to compare mass and formation of the maize (*Zea mays* L.) roots in soils prepared by three different tillage systems. Methods of the primary tillage were suitable for conventional, soil remedying and the conservation principles that are ploughing (P, 30-32 cm), subsoiling (S, 40-45 cm) and tine tillage (T, 18—22 cm). The soil state was favourably loosened in the first three months, including at planting, then the soil became settled owing to the repeatedly, and often torrential rains. The maize roots has optimally grown in the first three months, and reached the maximal length, and later, probably due to the deterioration of the loosened state, it has not lengthened. The T treatment had the longest root (45.5 cm) and the highest root mass (8.84 t/ ha) which differed significantly ($P<0.05$) from values obtained at the S and the P treatments. The results had particular importance in the contexts of the extreme growing season. Due to the soil condition assessments, more useful data were available to specify the effects of repeated rains on maize production.

Keywords: soil tillage, climate extreme, loosened soil layer, root biomass

## Introduction

In Hungary the arable land covers 4,332 000 ha, and sowing area of maize covers about 1,150 000 ha (KSH, 2015). Three soil types are dominated in maize production, namely Luvisols, Chernozems and Vertisols (these together cover 70 % of the total area, Michéli et al., 2014). In Hungarian relation some 40 % of the total sown land is tilled by plough, 25 % by subsoilers and 22% by cultivators (Dekemati et al., 2016). A number of authors emphasised that damage caused by climate conditions could be diminished, however site adopted solutions are to be applied (Chen and Weil, 2011). Preserving damages on wet soils has become an acute issue during the autumnal primary tillage. Birkás (2008) calls attention to the maize requirements, including deeper loosened layer without compaction damage. Soil compaction is known to be a limited factor in the root development and moreover that restricts the water and/or nutrients uptake (Yaduvanshi and Ashwini, 2015). Soil tillage may a key factor in crop root development through improving physical factors that are water, aeration, temperature and penetration resistance (Dwyer et al., 1996, Kadžiené et al., 2011). Chen and Weil (2011) found that greater diameter of roots is more capable to penetrate compacted soil layer than roots with smaller diameter. Barber (1971) and Kovar et al. (1992) stated that different water regimes may influence on the root formation, therefore under various soil tillage methods root development can significantly be different. Birkás (2011) highlights the importance to use climate focused soil management with the primary goal of reducing climate-induced stresses through improving soil quality. The objective of this study was to compare mass and formation of the maize (*Zea mays* L.) roots in soils prepared by three different tillage systems. The hypothesis was that the deeper loosened layer may give the optimal conditions for maize root development.

**Materials and methods**

Maize root development was investigated in a long-term experiment on a Chernozem soil (WRB 2006) at the Experimental and Training Farm of the Szent István University (47°68'N, 19°60' E, 130 m a.s.l.) near the town Hatvan, Hungary. In this region, long-term yearly precipitation averages 580 mm (313 mm fall in the growing season). The measurements were conducted in year 2016, when amount of precipitation run to 731 mm, and from this 413 mm fell in the growing season. The experiment was arranged in a randomised block design with four replicates, and area of each plot is 2340 m² (13 m x 180 m). Six treatments are applied in the experiment (Birkás et al., 2015) however only three were selected for investigation. These treatments were suitable for conventional, soil remedying and the conservation principles that are ploughing (P, 30-32 cm), subsoiling (S, 40-45 cm) and tine tillage (T, 18-22 cm). Primary tillage was completed on 28th October, 2015, sowing on 8th April, 2016, and harvest on 24th October, 2016. Soil moisture content has exceeded the optimum (26m/m%) at the time of primary tillage causing unfavourable deformation in soil mainly at P treatment. There were no similar limiting factors at maize sowing. The maize cultivars (Limagrain 33.30 hybrid) corresponded to the site relation. The rate of fertilizer for maize met the requirements, that are N 120, P 90, and K 70 kg/ha. Samples to check soil condition – penetration resistance, moisture content, crumb' ratio – were taken in 30-day intervals in five replications. Impacts of the rain stress on soil condition were also surveyed. Shortly before the harvest three soil cores (30x30x50 cm) were taken per plot at three separate locations in four repetitions. Roots immediately cleaned from the soil and measured the length and then the biomass after air drying. The statistical evaluation was made by Microsoft Office Excel software package.

**Results and discussion**

The precipitation was 57% higher in the growing season compared to the multi-year average. In May, there was a day when 46 mm of rain fell, coupled a hail aggravating the situation. Moreover, intense rainfalls had repeatedly occurred during summer. Monthly amount of precipitation (Table 1.) was less unfavourable than intensity and distribution of rains which proved to be more extreme.

*Table 1. Monthly amount of precipitation in the growing season (Hatvan, 2016 )*

|  | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | **Dec** |
| **mm** | - | - | - | 21 | 106 | 44 | 107 | 55 | 47.5 | 33 | - | - |

Due to the abundant rains soils has continuously settled. Values of the soil penetration resistance were quite low at the sowing time (≤1.8MPa) at both treatments. These values had doubled (~3.7MPa) till the harvest time at same soil moisture content (31-30 m/m% in average of the 0-60 cm soil layer). Authors previously stated that tillage affects the development of roots (e.g. Barber, 1971). As Unger and Kaspar (1994) noted, soil compaction is a limiting factor of the root growth, and the climate extremities, through soil state deterioration are also modifying factors. Soil state measurements gave real information to the evaluation of maize rooting. The former pan compacted layer had really extended at the P treatment, and for this reason, depth of the rooting was the shortest (34 cm). The loosened state had fairly remained at the T treatment consequently depth of the

rooting (47 cm) surpassed the depth of the tillage. Values of the deviation were low at the treatments where soil state remained (T) or worsened (P) to the same extent (Table 2.). The length of the maize root was found to be unequal at the S treatment, similarly to the uneven loosened state.

*Table 2. Values of root length*

| Treatments | 1. repetition | 2. Repetition | 3. Repetition | 4. Repetition | Average (cm) | Deviation |
|---|---|---|---|---|---|---|
| Tine tillage | 47 | 46 | 46 | 44 | 45.75 | 1.26 |
| Ploughing | 34 | 36 | 37 | 35 | 35 | 1.29 |
| Subsoiling | 39 | 41 | 40 | 44 | 41 | 2.16 |

The difference between dry root biomass at different soil tillage and soil condition could be proven statistically ($P < 0.001$, Figure 1.). Highest (8.8 t/ha) dry root biomass was measured in tine tilled (T) soil, and the lowest (7.2 t/ha) in the subsoiled (S) treatment. These favourable effects were confirmed by yield data (no discussed in this paper), too.
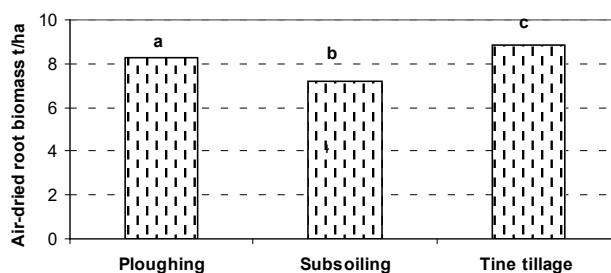


Figure 1. Dry root biomass of maize at different tillage treatments in year 2016

Note: $LSD_{0.05}$: 0.848 t/ha

According to the publications concerned, depth and quality of tillage are important primary factors of the root formation (Bennie and Botha, 1986; Unger and Kaspar, 1994). However, as Dekemati et al. (2016) highlighted, that the consequences of a rainy growing season can really be unpredictable.

Further experience was gained in this rainy season. The higher soil moisture content at the time of primary tillage increased the production risk through soil state damage which were mostly visible, however less detectable by instrumental measurements. Kneading and puddling of the soil by plough are visible and the pan compaction is opened by profile excavation. Damaging the soil structure in wet soil by subsoiler is related to the tool design. In our case a moderated soil kneading occurred at subsoiling, however, the quality of the loosening was poorer compared to the loosening created by tine tillage. The favourable soil conditions at planting time gave minimal guarantees to prevent soil state deterioration in he given rainy season. As Birkás et al. (2015) and Dekemati et al. (2016) noted that there is a demand for the elaboration of methods suitable for conservation tillage of wet soils. The growing season in year 2016 was considered to be favourable in terms of the amount of precipitation. It may outline that the impact of the repeated rains, and the extreme distribution proved to be more harmful to the soil quality condition. Among the negative phenomena of the rainy season were the crumb deterioration, the siltation of the small soil particles, the

leaching the small particles to the deeper soil layer (increasing the compaction risk) and the hard crust formation in the soil surface. In the viewpoint of the soil, damages caused in the rainy seasons require step-by-step remediation in the next seasons.

## Conclusions

The unpredictable weather is considered to be much danger today, particularly for production of crops – mainly for maize – that are more sensitive to the climate induced soil condition deterioration during growing season. In our case, the experience gained in the long-term experiment give a chance to recognize the climate induced hazards in the future. The data were stated that the maize root may grow most favourably in soil prepared by less physical damages in the tillage season. This requirement fulfilled satisfactory by tine tillage. The dry root biomass may the highest at soil condition which has suffered the least deterioration in the rainy season. Less soil damage was realized by tine, when pan compaction occurrence was negligible. Soils will really be exposed to the climate stresses. Vulnerability of soils has already become an acute problem for agricultural production, and it will be even more complex problem in future decades.

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# Measuring and mapping within-field soil moisture content for precision (site-specific) plant production

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Abstract: Precision (site-specific) agriculture requires within-field information. Within-field information is based on site-specifically collected data. In order to be able to make decisions site-specifically, the spatial resolution of the data has to be sufficient for mapping. Traditional (hand collected) data collection methods for soil moisture content distribution mapping are time and workforce consuming; moreover, this method does not provide enough information for reliable soil moisture content distribution maps. In this paper authors report the results of the measurements based on traditional data collection as well as a result of a new measurement instrument (Veris-3100). Both methods aimed to map the spatial distribution of soil moisture content within a 23ha agricultural land. Veris technologies instrument results soil apparent electrical conductivity ($EC_a$) data. In our particular research field, we have investigated the connection between this ($EC_a$) parameter and soil moisture content, and have found strong correlation between the two datasets. We have found that on-the-go soil properties measurement instrument provides spatially well distributed data for mapping various soil parameters, and based on the ($EC_a$) data, soil moisture content distribution can be mapped with much better spatial resolution than mapping based on the traditional sampling method.

Keywords: Precision agriculture, within-field soil moisture content distribution mapping

## Introduction

Precision agriculture relies on site-specifically collected information. As soil moisture content directly influences yield, mapping within-field soil moisture content differences provides information for agricultural management practices (Milics, 2013). The standard method for soil moisture content measurement is based on hand collected sampling, and oven drying, however this method is time and workforce consuming. This method does not provide a spatially well distributed and sufficient amount of data for mapping the distribution of soil moisture content for large areas. For this reason, authors have investigated an alternative solution. Soil moisture measurement in precision agriculture is in the research focus in order to find an efficient way for measuring soil moisture content (Balla and Jolánkai, 2012). Soil apparent electrical conductivity is one possibility to measure indirectly soil moisture content (Milics et al., 2012, Nagy et al., 2013; Balla et al., 2013). Soil apparent electrical conductivity measurement in this particular field proved that mapping soil moisture content within-field results additional information for precision agriculture practice (Milics et al., 2017). Mapping the pattern of the agricultural fields later can be the input information for precision irrigation.

## Materials and methods

Measurements were carried out in the 23ha experimental research field belonging to Széchenyi István University in the vicinity of Mosonmagyaróvár, Hungary [N47°54'20.00"; E17°15'10.00"]. The study field is an agricultural land – alluvial plain of the Leitha River – on which precision agriculture has been applied since 2001. The field cannot be characterized by one typical soil profile, as a buried riverbed (former Leitha)

crosses it. The humus content in the upper 0.2 m layer is between 1.4–2.8%.

Soil sampling locations were determined based on earlier experiments (Fig. 1a). In the case of hand-collected, undisturbed core sampling, moisture content was determined by means of the gravimetric method. Six undisturbed soil samples (50 mm in height) were taken consecutively from each sampling site. The sampling depth was thus 0.3 m. Soil samples were dried to a constant weight (>24 hr) in the oven at a temperature of 105 °C. After drying, the gravimetric and volumetric moisture content was determined. Volumetric moisture contents (θ) were calculated from the known volume (100 cm$^3$) of the core soil sampling rings. From the same locations soil samples were collected and sent
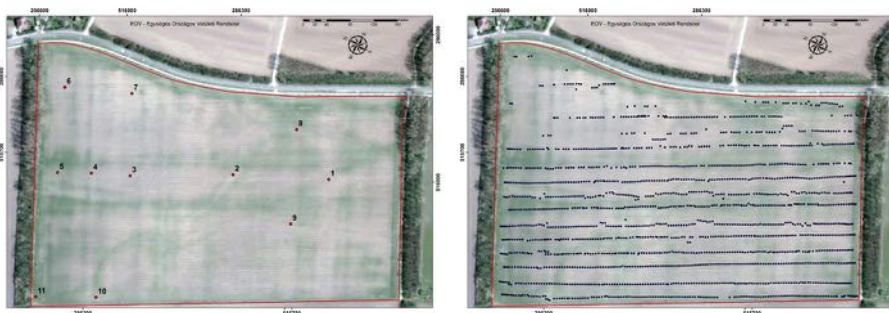


*Figure 1: Location of the experimental field and the soil sampling points by hand collection (a) and on-the-go (b) methods*

for laboratory analysis.

The soil electrical conductivity $EC_a$ was measured by a Veris Soil EC-3100 (Salina, KS, USA) instrument. The most important parts of the Veris-3100 meter are the Coulter-Electrode blades (6 pcs) with 430 mm diameters, which are electrically insulated from the frame. The Coulter-Electrode Blades are arranged symmetrically. The device measures the bulk apparent electrical conductivity of the soil at depths of 0–0.3 m and 0–0.9 m at the same time. In this study only data from the depth of 0–0.3 m was used, as the reference soil samples were also collected from this depth. Soil data collection was in much denser locations (Fig. 1b). Measurements were carried out in 28th of October 2016.

All measured data was integrated to ArcMap 10.0 software. For mapping, kriging interpolation method was used.

**Results and discussion**

Soil moisture content map based on the data of measurement of hand collected and oven dried samples showed some differences in the field; however, the map did not show the assumed pattern that was expected based on earlier experiments (Fig. 3a).

Measured soil moisture content data, laboratory reference data and on-the-go collected $EC_a$ values were analysed by linear regression. In 2016 we have found strong correlation between $EC_a$ and gravimetric soil moisture content (w), and $EC_a$ and volumetric soil moisture content: $R^2$=0.8972 and 0.8251, respectively (Tab. 1). Other results based on laboratory analyses also showed strong correlation with $EC_a$.

Based on the equation derived from the linear correlation regression (Fig. 2), all $EC_a$ values were converted to soil moisture content values.

Table 1: Correlation matrix of selected soil properties

|  | ECa | w | θ | $K_A$ | OM | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|
| ECa | 1 | 0.8972 | 0.8251 | 0.9672 | 0.8505 | 0.8834 | -0.5577 | 0.8811 |
| w |  | 1 | 0.9099 | 0.9348 | 0.7684 | 0.9917 | -0.5407 | 0.965 |
| θ |  |  | 1 | 0.8369 | 0.6991 | 0.8801 | -0.4247 | 0.8675 |
| $K_A$ |  |  |  | 1 | 0.8061 | 0.92 | -0.6403 | 0.8726 |
| OM |  |  |  |  | 1 | 0.7949 | -0.3771 | 0.834 |
| Mg |  |  |  |  |  | 1 | -0.5004 | 0.9673 |
| Zn |  |  |  |  |  |  | 1 | 0.4387 |
| Cu |  |  |  |  |  |  |  | 1 |
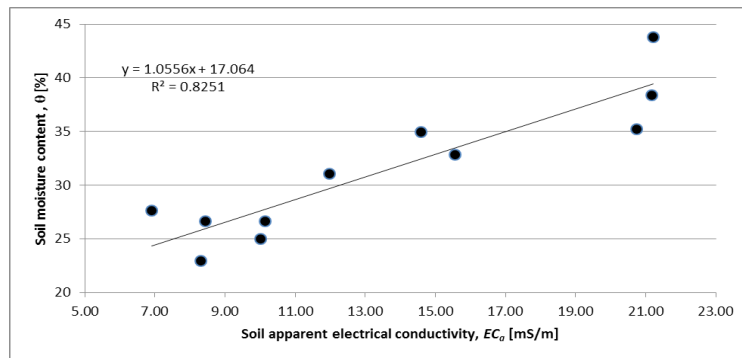


Figure 2: Results of the linear regression analysis between ECa and soil moisture content (θ)

Soil moisture distribution map was created by means of applying agricultural GIS mapping tools (Fig. 3b). As it is clearly visible on the map, the pattern of soil moisture content
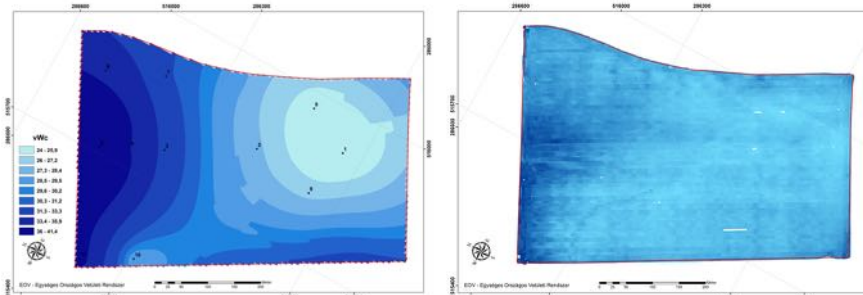


Figure 3: Location of the experimental field and the soil sampling points

differs from the map created from hand collected and spatially less well distributed data.

## Conclusions

In precision agriculture having accurate information about the habitat is the basic prerequisite of all agricultural activities, as plants growing conditions are influenced by genetic, ecological and production technology factors together, which can change

and vary considerably even within one agricultural field. Manual soil moisture content measuring does not provide spatially well distributes and dense data for mapping within field differences. It can be replaced by Veris Soil EC-3100 mapping of the soil apparent electrical conductivity (ECa). Further research is needed to define how the connection between soil moisture content and soil apparent electrical conductivity changes in the case of the different soil types, physical characters and salinity of the soils.

We state that soil moisture content map can be created from the measured soil apparent electrical conductivity values.

### Acknowledgement

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# The natural viral infections of the weedy *Panicum miliaceum* (L.)

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Abstract: Common millet is a dangerous weed in Hungary, not only on a maize field, but also in other crops like potato, sunflower, wheat and other cereals. It can widely spread because of the lack of seed dormancy. This weed is a close relative to wheat, so we wanted to investigate, which wheat viruses can infect of the common millet (*Panicum miliaceum*).

Forty-five millet leaf samples were collected from the fields in 2014 and 2015 near Keszthely and Lesencefalu, from Hungarian Transdanubia. After the collection, we immediately froze the samples, and stored at -20 °Celsius. We used the DAS ELISA serological method to determine wheat viruses from the leaves.

Nineteen of 45 collected leaf samplesgave positive results. Simple virus infection were realised in 15 samples, but complex infections were also detected in 4 samples.The samples were infected with Wheat streak mosaic virus in 22%, with Wheat dwarf virus and Barley stripe mosaic virus in 13%, with Barley yellow dwarf virus in 4% and with Barley stripe mosaic virus in 2%. Brome mosaic virus (BMV) was not detected from the collected samples. Complex infections of WDV and WSMV as well as WDV, WSMV and BYDV were also detected in three and one samples, respectively.

After the first investigation we collected another samples too, to continue our examination. Our results indicate, that millet as weed can play a major role in the distribution of different cereal virus species.

Keywords: millet, maize, virus, cereal

## Introduction

Field crops play a key role in both food and animal feed worldwide. It is therefore worrying that the damages caused by the viral diseases show growing trend in the recent years. Prevention is the only effective protection against viruses. The millet is not just a weed, it has a really strong competitive harm, and also plays a role in spreading cereal viruses. Several viruses have been identified amongst the wild and cultivated millet species in several cases. Firstly Wheat streak mosaic virus has been identified from the millet species *P. capillare* in Australia and the United States (Christian and Willis 1993, Coutts 2008). In the work of Lapierre and Signoret (2004) it is mentioned, that the Barley yellow mosaic virus is a pathogen of the millet species. This fact carries several problems: while a lot of alien millet species have recently been identified, there is a high risk of carrying wheat viruses, as well. Therefore, the goal of our study was to examine the dominant millet species, *Panicum miliaceum* (L.) and its viral infection with the most dangerous wheat viruses in Hungary. The millet causes a major problem in maize, but in the recent years, we found it in other cultures too, like wheat stubble and potato (Pásztor and Nádasy 2016). That's the first signal of the mild climate change in Hungary, the millet, being a hot-consuming species, is spreading into other cultures. Because of that, the wheat is highly compromised, because of the wheat viruses. So, our goal was to examine this threat, especially its viral infection.

**Materials and methods**

45 millet (*Panicum miliaceum* L.) leaf samples were collected during the investigation in September of 2014 and 2015. We collected 35 samples from the grain-field stubble near Lesencefalu, and 10 samples from Keszthely. The collected samples showed signs of a viral infection. The laboratory test requires, that all the samples need to be packed individually in polyethylene bags and stored at -20 °Celsius.

The most commonly used serological test was used, the double antibody sandwich DAS ELISA test. We used reagents from the LOEWE Biochemica (Brome mosaic virus, Brome dwarf mosaic virus, Brome treak mosaic virus, Barley stripe mosaic virus, Barley yellow dwarf virus, Wheat dwarf mosaic virus and Wheat dwarf virus).

The colour of the samples was evaluated with Multiscan RC Elisa Reader at 405 nm. The samples, which extinction value was three times higher than the negative control, were considered positive.

**Results and discussion**

From the 45 leaf samples in 19 cases have been proven viral infection. The most serious infection has been diagnosed in the case of Wheat streak mosaic virus; we diagnosed 10 samples with this virus. In six-six samples the Wheat dwarf virus and barley stripe mosaic virus were also identified, among these two Barley yellow dwarf virus and one Brome streak mosaic virus infection were find. But, we can't identify the Brome mosaic virus (Fig.1, Fig. 2). We found complex infection also. Three samples with two viruses (WDV, WSMV), and one sample with three viruses (WDV, WSMV, BYDV) were infected.
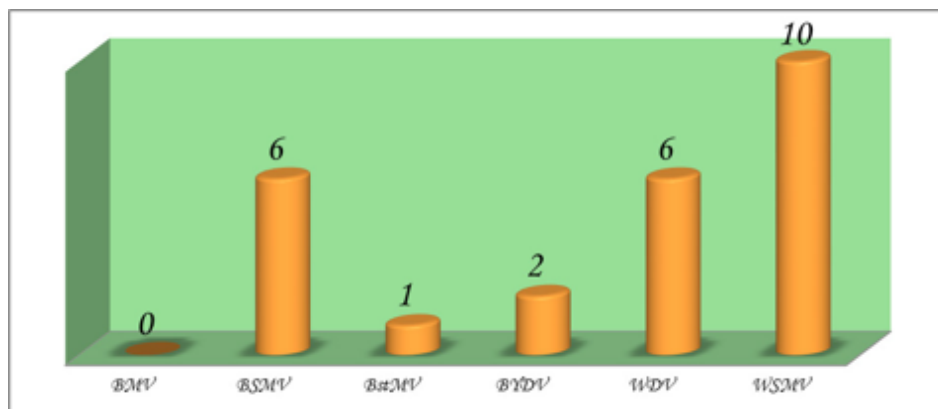


*Figure 1. The number of different virus infected samples.*
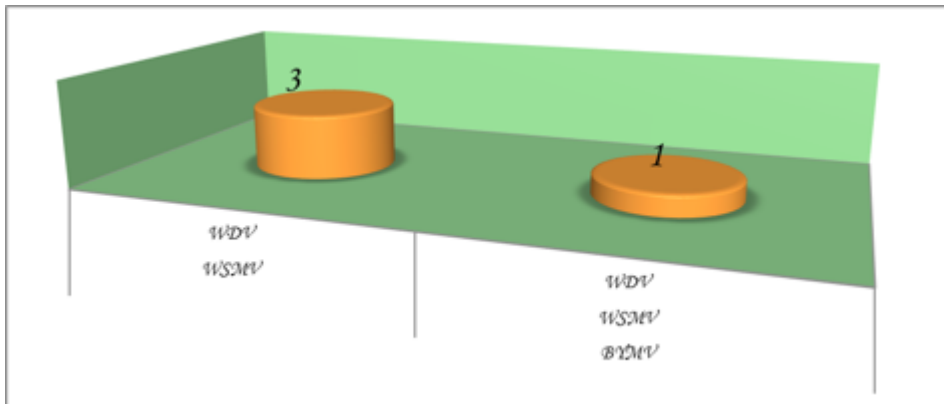
*Figure 2. The complex virus infections*

**Conclusions**

The results show, that the weedy subspecies of the broomcorn millet can play a significant role in spreading cereal viruses into the cereals. This study is unique, because so many viral diseases in such complexity was never been identified by the *Panicum miliaceum* species. Our main priority is the successful weed control, and prevention the spreading of new alien millet species.

The reduced agricultural technology promotes the spread of the pests, like new weed species and also the virus vectors and viruses. But if we stubble the fields and carry out the cultivation works in time, the spreading of the pests can be reduced. If it possible, it is advisable to choose resistant varieties in crop production.

To the good production of virus resistant varieties important the optimal plant nutrition, because of this help the genetically determined natural defence mechanism of plant. The forecast plays a key role in protection against vectors. The massive proliferation of pests can be significantly reduced by prevention, therefore the transmission of the virus. It is strongly recommended the further investigation of the millet species, because we can find them in other cultures too, and there are a lot more viruses, that can infect the millet and therefore the cultivated plant too.

For example, in Hungary the millet species causes a major problem in maize fields, but it can easy happen, that the two maize viruses (the Maize mosaic virus and the Sugarcane mosaic virus) can also infect those species. We need to extend our examination on the alien millet species too.

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# Abiotic stress impacts caused by weather and nutrient replenishment on the yield of maize (*Zea mays* L)

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Abstract:   In the long-term experiment established by the University of Debrecen, we were examining how the amount and distribution of yearly precipitation, as well as nutrient replenishment affect maize productivity during the growing season in 2014, 2015 and 2016.

Environmental factors have a significant impact on yield. In all examined cases, there was a significant ($P<0.001$) difference between crop years. The most significant difference was observed between the yield of 2015 and 2016 (3475 kg ha$^{-1}$, $P<0.001$). The growing season of 2016 – which ended with significant precipitation surplus (+110 mm) – provided the availability of nutrients, thereby resulting in the highest yield both in treatments A and B.

Averaged over the examined years, treatment B resulted in a non-significant yield increasing effect (603 kg ha$^{-1}$) in comparison with treatment A. When evaluating the different crop years one by one, it can be concluded that there was a significant yield difference in 2015 (1036 kg ha$^{-1}$; $P<0.05$) (averaged over the different fertiliser treatments), despite the fact that it was a dry year. This phenomenon is explained by the fact that the silking of the examined maize hybrid ended before the dry period and the unfavourable effect of weather could be reduced with a high amount of nutrient replenishment.

Keywords: maize, nutrient replenishment, environmental factors

## Introduction

The intensive growth of world population and the change of dietary habits prompt agriculture to increase productivity at an accelerating rate. Food production has to be increased with 70% by 2050 (FAO 2009), as the world's population is expected to be higher by 2.2 billion people and the quantity of cereals has to be increased by one billion in order to provide food for the population. However, in order to do this, increased amount of irrigation water must be applied and mineral fertilisation accessible for growing crops also needs to be performed (Yang et al. 2006).

Various research findings show that maize yield is mainly related to fertilisation, which has a more significant impact than genotype and all other examined production technological factors (crop rotation, tillage, irrigation, sowing density and crop year) (Berzsenyi and Győrffy 1995; Nagy 2012).

Nitrogen fertiliser is an essential crop nutrient which increases vegetative mass and plays a fundamental role in increasing yield (Modhej et al. 2008) and it also has an impact on yield quality (Ványiné and Nagy 2012), as well as access to other elements (Bruns and Ebelhar 2006). 61% of nitrogen taken up by maize is transferred into the grain yield (Berzsenyi 2013). A sufficient amount of nitrogen must be available in the whole vegetation period. In the case of N deficiency, yield loss is inevitable (Alvarez and Grigera 2005). The amount of N fertiliser used in excess of the crop's needs reduces economicalness and harms the environment (Nagy 2012). Phosphorus is also an indispensable nutrient and the most important element of the generative development and energy supply of maize. Consequently, phosphorus deficiency causes metabolic disturbance, the water balance of

crops deteriorate, while silking and ripening delay. However, phosphorus oversupply leads to significant nutrient imbalance. The excess phosphorus resulting from P-Zn antagonism results in relative Zn deficiency, which has a negative impact on yield quantity and quality (Szakál et al. 2004). 70% of the phosphorus taken up during the vegetation period is transferred into the grain yield, which shows the significant role of phosphorus in yield formation (Berzsenyi 2013).

Potassium affects photosynthesis (Árendás et al. 1998), increases the active water uptake of crops (Mengel et al. 2001) and it has a role in the process of opening and closing stomata. Balanced potassium supply improves stress tolerance (frost and drought tolerance) (Sárdi 1999). Insufficient potassium supply leads to significant yield decrease.NPK fertilisation can be used to increase yield, while the unwanted impact of certain agrotechnical factors can also be mitigated (Széll et al. 2004). However, nutrient conversion greatly depends on crop year (Csathó et al. 1991; Wiswakumar 2008). The unfavourable impact of crop year can be avoided or mitigated with rational nutrient and water management.  This research carried out in different crop years (2014, 2015 and 2016) focused on revealing how the amount of yearly precipitation and its distribution over the growing season, as well as nutrient replenishment affect maize productivity. It was also the aim of this research to identify the NPK ratio, which results in the highest yield at the lowest cost.

**Material and methods**

The examinations were performed in Eastern Hungary (horizontal degree: 47°56', longitudinal degree: 21°44') *in a long-term small plot field experiment on calcareous chernozem soil with deep humus layer formed on loess. The experiment had a strip plot design and four replications and it was carried out under natural precipitation supply conditions between 2014-2016.* The examined hybrid was FAO 490 in all three years. The proportions of the constant NPK fertiliser active ingredient doses of treatment "A" were 1 N : 0.75 $P_2O_5$ : 0.88 $K_2O$. The nitrogen base dose was 30 kg ha$^{-1}$. In addition to non-fertilised control, we applied 1, 2, 3, 4 and 5 times this dose. Increasing N dose and the same P and K doses (184 kg ha$^{-1}$ $P_2O_5$ and 216 kg ha$^{-1}$ $K_2O$) were applied in treatment "B". The harvested grain yield was provided for 14% moisture content. Weather was evaluated based on the data measured and logged by the automatic weather station installed on the experiment site. *In 2014*, the amount of precipitation in the growing season was 385 mm, which was 13% higher than the 50-year-average (Figure 1). There were two significantly drier months during the growing season: 2% of precipitation was observed in June and 12% in August. The 40 mm rainfall observed in April is considered to be the average amount. In May, the amount of precipitation was 10 mm more than the multiple year average. In July (128 mm) and September (96 mm), the amount of rainfall was more than twice higher than the average. The number of wet days (61) when rainfall reached 10 mm was 47, while there were 11 days when rainfall was above 10 mm. Rainfall was above 20 mm for three days altogether. The mean temperature of the growing season was 0.2 °C higher than the 50-year average. April was significantly warmer (+1.7 °C). The mean temperature of May (-1.1 °C) and August (-0.4 °C) was lower than the multiple year average. The mean temperature of June and July was in accordance with the average, while it was 0.8 °C higher in September. *In 2015*, the average precipitation sum of the growing season was 285 mm, which is 84% of the 50-year-average (Figure 1). May was somewhat drier than usual, the amount of rainfall was 90% of the average. June and July were rather dry. In

June, the amount of rainfall was 9 mm lower than the normal value, while the precipitation in July was 25 mm lower. The amount of rainfall in August is significant, as 24 mm excess was measured. In September, rainfall was lower than the average, as its amount did not reach 65% of the average. There were 43 rainy days in the growing season of 2015, and the amount of rainfall exceeded 1 mm during 32 of these days. For 9 days, precipitation was above 10 mm, while there were only two days with rainfall above 20 mm. The mean temperature of the growing season was higher (+1.0 °C) than the multiple year average. The mean temperature values of April and May were below the multiple year average. The rest of the observed months were significantly warmer than the average. The biggest positive anomaly was observed in August, when the monthly mean temperature was 3.0 °C higher than the 50-year average. This value is followed by those of September (+1.9 °C), July (+1.7 °C) and June (+0.8 °C).

*Figure 1. Precipitation and temperature trends in the growing season (Debrecen, 2014–2016)*
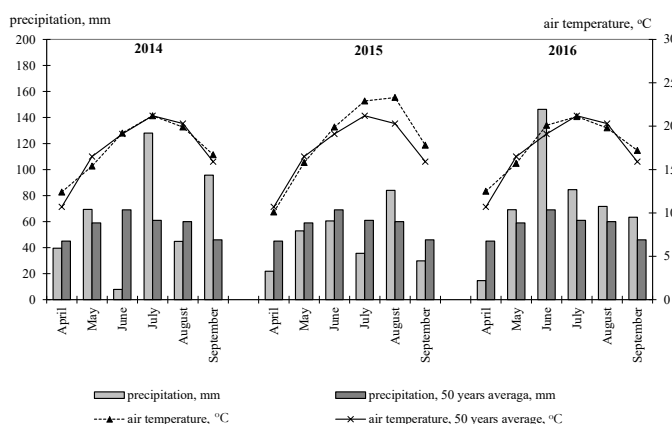


The growing season of *2016* was rich in rainfall (Figure 1). The precipitation sum of 2016 (450 mm) was 110 mm higher than the 50-year average (340 mm). April was dry, as there was less than 15 mm rain, which is well below the multiple year average of 45 mm. There was 69 mm rain in May, which was 17% higher than the 50-year average. The amount of rainfall in June (146 mm) was more than twice as much as the average precipitation sum (69). Unfortunately, more than one third of this rain (45 mm) arrived in only one day. There was significant excess rain also in July, August and September. We observed 39% excess rain in July, 20% in August and 37% in September in comparison with the 50-year average. Both in August and September, more than half of all precipitation arrived in one day – on 21st August and 21st September. There were 48 rainy days in the growing season, of which there were 40 days when the amount of rainfall was above 10 mm. Precipitation increased 20 mm for five days and there were only three days when the amount of rainfall was lower than 10 mm. Altogether, the mean temperature of the growing season was 16.5 °C, which differed from the 50-year average by only a few tenths. The month of sowing was much warmer than the average (+1.8 °C), while May was 0.9 °C colder. June was more than 1 °C warmer than the 50-year average, while the temperature in July was in accordance with the average. In August, the amount of average temperature decrease was

0.5 °C, while September was warmer again, exceeding the average by 1.3 °C. *Statistical analysis.* The correlation between the dependent variable (yield) and the production factor (fertilisation, irrigation) was evaluated using a general linear model (GLM). Duncan's test was used to compare yield to its mean values. The correlation between dependent variables was evaluated using a linear function. Functions were fitted with regression analysis and by minimising the sum of squared deviations. The correctness of fitting the functions was determined using the R value and the mean squared error. Evaluation was performed using SPSS for Windows 14.0.

## Results

The yield quantified for each crop year – averaged over the different treatments – shows significant differences. The yield difference was significant in all three years at a 0.1% level. The most significant difference was observed between the dry year of 2015 and the wet year of 2016 (3475 kg ha$^{-1}$). Based on the paired T test of the fertilisation treatment, it can be concluded that treatment "B" (10.876 t ha$^{-1}$) provided better conditions for maize, but the 603 kg ha$^{-1}$ excess yield was not shown to be significant difference in comparison with treatment "A" (10.273 t ha$^{-1}$). By examining the two fertilisation treatments, it can be concluded that treatment "B" was more successful in 2014 – averaged over the different treatments – by 1006 kg ha$^{-1}$, but the difference is not significant. In the dry year of 2015, higher yield was observed on fields where the fertilisation treatment "B" was applied and the difference (1306 kg ha$^{-1}$) was significant ($P<0.05$). There was no notable difference between the two treatments in 2016 (Table 1).

*Table 1. The impact of crop year and fertilisation treatments on maize yield (Debrecen, 2014–2016)*

| | Average yield (t ha$^{-1}$) | | |
|---|---|---|---|
| Fertilisation treatment | 2014 | 2015 | 2016 |
| "A" | 9.460[ns] | 8.490[*] | 12.870[ns] |
| "B" | 10.466 | 9.796 | 12.366 |

Legend: *P=0.05, ns= not significant

As a result of examining the significance of fertilisation treatments carried out each year, it was shown that larger fertiliser doses had a favourable effect in *2014*. However, the effect of the fertiliser doses of 120:92:108, 150:115:135, 120:184:216, 180:184:216, 240:184:216 and 300:184:216 kg NPK ha$^{-1}$ cannot be separated from each other. All six treatments were equally shown to provide significantly better impacts in terms of yield compared to the non-treated control and the 30:23:27, 60:184:216 and 60:46:54 kg NPK ha$^{-1}$ treatments. Compared to the non-treated control, the response to the higher doses of 30:23:27, 60:46:54 and 60:184:216 kg NPK ha$^{-1}$ was properly realised in yield, but the Duncan's test showed that the yield resulting from these four fertilisation treatments were classified into the same homogeneous group. The yield of the 90:69:81 kg NPK ha$^{-1}$ treatment (9.506 t ha$^{-1}$) was significantly different from the yield resulting from lower and higher fertiliser doses. In 2014, yield increased as a result of nutrient supply and the highest value was observed in the case of 120:92:108 kg NPK ha$^{-1}$ (11.546 t ha$^{-1}$) (Figure 2). There was a notable difference (3658 t ha$^{-1}$) between the yield of the non-treated control and the fertilised plots in the driest year of *2015*. Even the lowest dose of 30:23:27 kg NPK ha$^{-1}$ resulted in an excess yield of 2074 kg ha$^{-1}$, but the impact of this treatment did not show any significant yield increase in comparison with the other treatments of treatment "A". There was a slight, non-significant difference between the impact of the NPK fertiliser doses evaluated in treatment "B". In this year, the fertiliser level of 90:69:81 kg NPK ha$^{-1}$ was shown to have a favourable effect (Figure 2).

*Figure 2. The impact of fertilisation on maize yield (Debrecen, 2014–2016)*
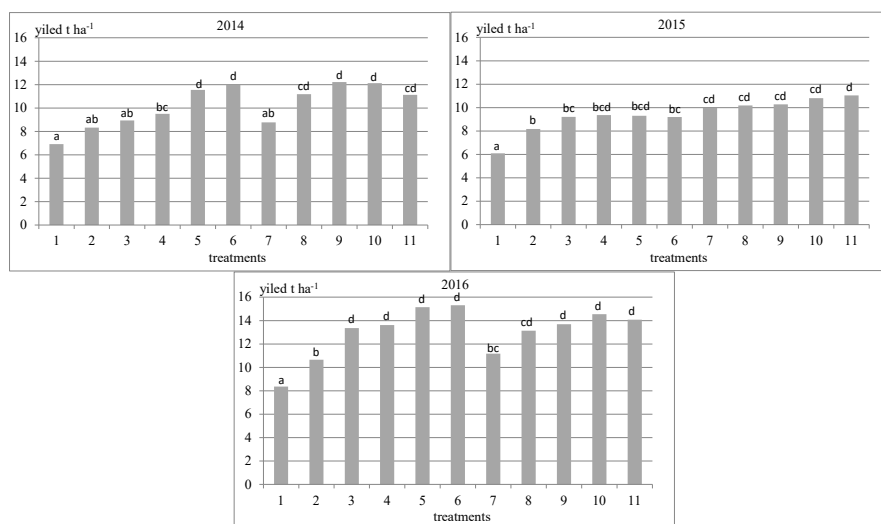


Legend: Columns indicated with different letters significantly differ from each other at the level of P≤0.05, based on Duncan's test

The more favourable weather impact of *2016* made larger yields possible (13403 kg ha^{-1}). The yield of the non-treated control was 8.353 t ha^{-1}, which was properly separated from the other fertiliser levels. There was no significant yield increase between the doses of 30:23:27 and 60:184:216 81 kg NPK ha^{-1} (519 kg/ha). Similarly, no significant difference was observed between the doses of 60:184:216 81 and 120:184:216 kg NPK ha^{-1} (1958 kg ha^{-1}). The other fertiliser treatments carried out in the experiment resulted in nearly identical yields, which formed a homogeneous group based on the Duncan's test. In 2016, the highest yield resulted from the fertiliser dose of 60:46:54 kg NPK ha^{-1} (Figure 2).

**Conclusions**

Based on the MQ value of the multivariate ANOVA, the environmental factor had the most significant yield impact (at a level of 0.1%), averaged over the three examined years. Treatment "B" was not shown to be significantly better than treatment "A". The different fertiliser doses used in these treatments had a significant impact (P<0.001). Similarly to the conclusions of Izsáki (2008), the highest and economically attainable yield was not observed in the same year during either of the examined years. In 2014, the highest yield resulted from the 180:184:216 kg NPK ha^{-1} treatment, which was only 6% higher than the yield resulting from 120:92:108 kg NPK ha^{-1}, which provided the highest significant yield. In 2015, the difference between the results of the statistically significant 90:69:81 kg NPK ha^{-1} and the extreme 300:184:216 NPK ha^{-1} treatments was 18%. In 2016, there was a 16% difference between the treatment of 60:46:54 NPK ha^{-1} which was statistically significant and the treatment of 150:115:135 NPK ha^{-1} which provided the highest yield. Averaged over the three examined years, 90:69:81 kg NPK ha^{-1} was the most effective treatment. Similarly to the results of Pepó (2012), it was shown with statistical methods that crop year greatly affects the impact of applied fertilisers. The most effective NPK ha^{-1} level resulted in 23% yield difference between the rainfall-deficient (-55 mm) year of 2015 and

2014, when the amount of rainfall was 45 mm higher than the 50-year average, while it was 43% in 2016, when the precipitation surplus was 110 mm. The difference between the yields of the two years with excess rainfall (2014 and 2016) was the lowest (16%).

## Acknowledgement

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# Inland excess water hazard on the flat lands in Hungary

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Abstract: Inland excess water is surplus surface water forming due to the lack of runoff, insufficient absorption capability of soil or the upwelling of groundwater. This interrelated natural and human induced phenomenon causes several problems in the flat-land regions of Hungary, which cover nearly half of the country. Reasonable and preventive management of agricultural areas requires satisfactory information on the spatial and temporal distribution of excess water. Excess water is a complex process whose characteristics can only be determined through taking numerous factors into consideration. GIS together with large-scale spatial information on those factors, which significantly affect formulation of excess water, can provide suitable background for the compilation of hazard map. One well-defined and quantified parameter representing the affect of relief, groundwater, agro-geology, hydrometeorology, land use and soil properties on the formulation of excess water was defined and derived. Multiple linear regression analysis was used for modeling the joint effect of the selected environmental factors on inundation. The result of the applied regression kriging process is a map which spatially predicts inundation frequency. The final hazard map can be used in spatial planning, as it is valuable in numerous land-related activities (land use and agricultural planning, water management procedures, water-oriented cultivation systems, wetland restoration, etc.).

Keywords: excess water, hazard, land use, GIS

## Introduction

Inland excess water is surplus surface water forming due to the lack of runoff (Rakonczai et al., 2011). This water related phenomenon causes several problems in the agricultural lands in Hungary. Most of the excess water definitions have a common part that inland excess water is a temporary water inundation that occurs in flat-lands due to both precipitation and groundwater emerging (water uprush) on the surface as substantial sources (Pálfai, 2001). Damage caused by excess waters can be occurred about 1.8 million hectares, from which 60% is located in the arable-land. The area affected by inundation in every 5 years is 150000 hectares on average. The possibility of the amount and distribution of extreme precipitation is increased in Hungary, as it is written in VAHAVA (Change-Effect-Response) project (Várallyay, 2007). Extreme precipitations of recent years have made seasonal and permanent waterlogging the most serious agro-environmental problem in the Great Hungarian Plain (Koncsos, 2011). As a consequence, preventive management of land requires sufficient information on the spatial and temporal distribution of inland excess water (Bozán and Tamás, 2008). The non-uniform distribution of atmospheric precipitation combined with heterogeneous relief and soils with unfavourable physical/hydrophysical properties are the reasons of extreme moisture regime: the simultaneous hazard of waterlogging or over-moistening and drought-sensitivity in extensive areas, sometimes on the same places within a short period (Várallyay, 2003; 2004). Mapping of excess water hazard is a great challenge since excess water is a complex process. Its characteristics can only be determined by taking numerous factors into consideration. Geographical Information System (GIS) together with large-scale spatial information on

those factors, which significantly affect formulation of excess water, can provide suitable background for the compilation of maps with the expected accuracy. Due to the more than 10 years research and development work, the NAIK ÖVKI compiled the Hungarian Excess Water Hazard Map for the lowlands which can suitable for numerical characterization of the hazarded areas by excess water. Our further research and development have placed great emphasis on the consideration of other influential factors (additional variables) in order to refine the sensitivity of mapping (e.g. confined groundwater, irrigated and meliorated areas, NATURA2000, discharge of canal networks, excess water regulation, excess water retention reservoir, etc.).

**Materials and methods**

Limited numbers of affecting environmental factors were considered, and information on these factors was collected and arranged. The affects of soil, agro-geology, relief, groundwater, land use and hydrometeorology were represented by one parameter. Essentially each influential factor was typified by only one value at a given place. In this way the formation of excess water was defined and quantified. The effect of soil on the formation of inland excess water was modeled and spatially represented by the soil's water management characteristics, i.e. soil water conductivity. The effect of (agro)geology was modeled and spatially represented by a complex index, taking into consideration the depth and thickness of the uppermost aquitard. The effect of groundwater was modeled and spatially represented by the standard depth of groundwater, i.e. the average of its ten highest values within the last 50 years. The effect of land use was modeled and spatially represented by a numeric coefficient based on the National CORINE Land Cover database (Büttner et al., 2004) and individually attributed to its categories using expert judgement. The effect of hydrometeorology on the formation of inland excess water was modeled and spatially represented by a humidity index (10% possibility of occurrence of root square of sum of monthly weighted precipitation and sum of monthly weighted potential evapotranspiration ratio). A map displaying the relative frequency of inland excess water events was also compiled. Its source was the seasonal mapping of areas damaged by inland excess water. Data were provided by the responsible Water Directorates from the period between 1962 and 2010. Multiple linear regression analysis (MLRA) was used for modeling the joint effect of the selected environmental factors on inundation. First, principal component analysis (PCA) was performed on the affecting environmental auxiliary information and the resultant principal components (PCs) were used in the further procedures. Since PCs are orthogonal and independent, they satisfy the requirements of the MLRA and decrease multicollinearity. The PCs were used as explanatory variables and the inundation data as response variable in MLRA, applying stepwise selection at the 0.05 significance level. The coefficient of determination varied between 0.15% and 0.25% in the course of the 100 runs. The best performing case was selected for further processing. MLRA only partly explains the spatial variability (pattern) of the distribution of inland excess water. On the other hand, taking the role of environmental factors by the linear model into account, it eliminates the trend. Kriging of the MLRA residuals provides the stochastic component. Kriging requires knowledge of the spatial auto-correlation, estimated by the semivariogram of the variable to be spatially predicted, which in our case is the MLRA residual. An exponential semivariogram model was fitted to the experimental semivariogram (range ¼ 10,623 metres, sill ¼ 24.5 and nugget ¼ 17.2), which was then applied to calculate the kriging weights in the spatial interpolation. Superposing the

regression and interpolation results provided the overall RK prediction of the inundation hazard (Pásztor et al., 2016).

**Results and discussion**

Regression kriging is a spatial prediction technique that combines the regression of the dependent variable on auxiliary (additional) variables with kriging of the regression residuals. The resulted final map is the Complex Excess Water Hazard Map (Figure 1.) is suitable for comparing different kind of areas and determining the relationship between different influential factors. According to the final map the Hungarian lowland areas are hazarded by excess water inundations in different rates. Highly and extremely hazarded areas can find along the rivers and the Great Hungarian Plain in Upper-Tisza region (Bereg, Tisza-Szamos köz, Szamos-Kraszna köz, Rétköz, Bodrogköz, Taktaköz), Hortobágy river valley, Jászság and Nagykunság, Körös River valley, Lower-Tisza valley, and west part of Danube-Tisza Interfluve. The Little Hungarian Plain (Fertő-Hanság) belongs to this category, meanwhile from the Transdanubian region there are some small places where the excess waters can cause damages (Sárvíz). Moderate hazarded areas can find on the plateaus (Danube-Tisza Interfluve, Nyírség), but excess water inundations can appear on the Békés-Csanád Loess Plateau due to the water uprush.
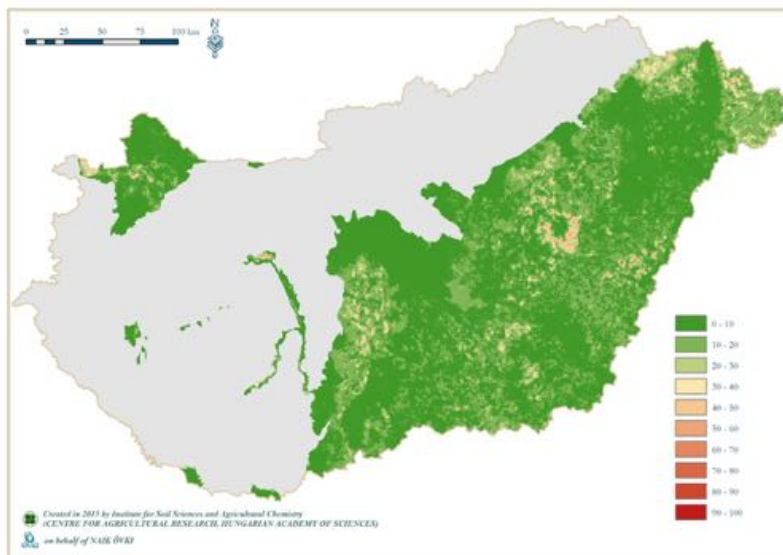


*Figure 1. Complex Excess Water Hazard Map*

**Conclusions**

Reasonable and preventive management of agricultural areas requires satisfactory information on the spatial and temporal distribution of excess water. Excess water is a complex process whose characteristics can only be determined through taking numerous factors into consideration. Due to the more than 10 years research and development work the NAIK ÖVKI compiled the Hungarian Excess Water Hazard Map for the lowlands

which can suitable for numerical characterization of the hazarded areas by excess water. The result of the applied regression kriging process is a map which spatially predicts inundation frequency. The final hazard map can be used in spatial planning, as it is valuable in numerous land-related. The presented method could be further refined. The application of regression kriging together with the applied multiple, virtual sampling allows the consideration of multiple data layers for each affecting factor. The environmental correlation expressed by multiple linear regression could be substituted by further, knowledge based, data mining methods for improving the modeling of the complex relationship between inundation and its affecting factors. Our further research and development have placed great emphasis on the consideration of other influential factors in order to refine the sensitivity of mapping. The groundwater factor can refine with the database of confined groundwater and flow system. It is important to develop the land use factor, because there are so many additional variables which have direct and indirect effects on the development of excess water inundation (i.e. irrigated and meliorated areas, NATURA2000, discharge of canal networks, excess water regulation, excess water retention reservoir, actual agricultural techniques, etc.). Remote sensing data services are becoming more and more affordable, improved reference data series on the spatial distribution of inland excess water events could be incorporated.

## Acknowledgement

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# Impacts of arbuscular mycorrhizal fungi on plant growth and yield of three pepper genotypes

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Abstract: Field study was implemented at the experiment station of Szent Istvan University, Godollo, Hungary to investigate the effects of arbuscular mycorrhizal (AM) fungi on plant growth, yield of three pepper cultivars during the plant growth. The experiment was arranged in randomized complete block design with two factors of pepper cultivars and AM applications. Three pepper varieties were Karpia, Karpex, Kaptur while AM applications consisted of AM mixture with 6 different AM species and non-inoculated treatment. Greater shoot fresh weight in AM plants were observed while values of root fresh weight, shoot and root dry weight remained unchanged in inoculated plants, except the increases recorded in Karpex cultivar. AM application also enhanced significantly fruit yield in Karpia and Kaptur but not in Karpex cv. In addition, AM treated plants showed improved root colonization of AM fungi.

Keywords: Arbuscular mycorrhizal fungi, pepper, growth, fruit yield.

## Introduction

Pepper (*Capsicum annuum* L.) is one of the main horticultural vegetables and cultivated worldwide due to important nutritional and economic values. In addition to pepper cultivars, pepper production and quality are diversed owing to various stress conditions that which often loses 70 % of yield forming a barrier in pepper cultivation (Gajanayake et al., 2011). The exploitation of symbiotic feature of AM fungi is one of the efficient approaches to improve crop tolerance to unfavored environment (Birhane et al., 2012). In fact, AM fungi are probably the most ubiquitous soil microbe that can colonize 80% of terrestrial plant species consisted of many important crops (Smith and Read, 2008). Many beneficial effects from mycorrhizal colonization including increased seedling survival, enhanced growth, fruit yield and quality, uniformity of horticultural crops, and earlier and increased flowering as well as induced resistance to abiotic and biotic stresses have been reported (Estrada-Luna et al., 2000; Estrada-Luna & Davies, 2003; Garmendia et al., 2004; Alejo-Iturvide et al., 2008; Mena-Violante et al., 2006; Kaya et al., 2009; Ruscitti et al., 2011; Ortas et al., 2011; Franco et al., 2013).

Although use of the AMF is widely investigated in many plants, little attention has been paid to use different cultivars as target plants for inoculation. Therefore, the aim of this study was to examine the potential of AM mixture for growth, fruit yield in different pepper genotypes under field conditions.

## Materials and methods

Three sweet pepper (*Capsicum annuum* L.) hybrids, Karpia, Karpex and Kaptur were used for this study at experimental station of Szent István University, Gödöllő, Hungary. Under field, treatments including inoculation of AM mixture or no inoculation (control) and three cultivars were arranged in randomized complete block design with 30 replications each treatment. Pepper seedlings were grown in greenhouse in 7 weeks before planting in field. Mycorrhizal Inoculation with commercial product Symbivit® (mixture of *Glomus intraradices, G. mosseae, G. etunicatum, G. claroideum, G. microaggregatum,*

*G. geosporum*) (Symbiom Ltd., Lanskroun, Czech Republic; www.symbiom.cz), was utilized at 25 g of inoculum per pepper seedling at planting. **Assessment of mycorrhizal colonization** by gridline intersect method (Giovannetti & Mosse, 1980) after staining roots (Vierheilig et al.; 1998) with five plants per treatment. **Plant biomass and fruit yield**: The pepper harvesting was performed randomly by hand at the biological maturity stage and evaluated for each treatment. All data were evaluated by two-way factorial analysis of variance (ANOVA) and Tukey's Post hoc test at $P < 0.05$ by SAS 9.1 software.

## Results and discussion

Pepper cultivar Karpex inoculated with AM mixture exhibited higher root fresh and dry weight than the counterpart while conversely, in Karpia and Kaptur root fresh and dry weight in AM plants were reduced and remained unchanged, respectively in relation to non-inoculated plants (Figure 1). Noticeably, all varieties pretreated with AM showed higher shoot fresh weight than their counterparts, however, the increase in dry shoot weight was only observed in Karpex cultivar applied by AM, not Karpia and Kaptur.
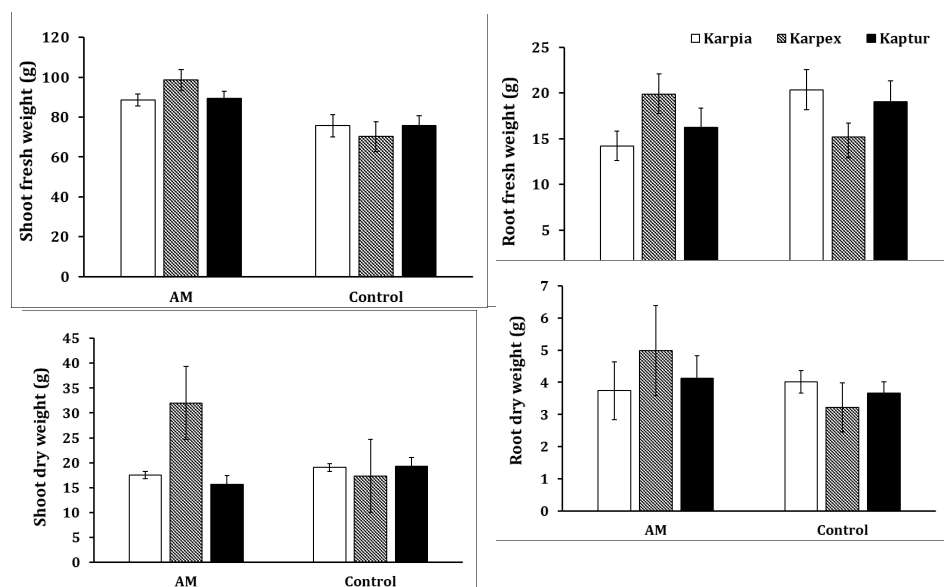


*Figure 1. Effect of AM inoculation on root fresh weight (A - upper left), root dry weight (B - upper right), shoot fresh weight (C - lower left) and shoot dry weight (D - lower right) of three pepper cultivars, Karpia, Karpex, Kaptur. Each bar presents mean ± standard deviation*

AM inoculated plants in all pepper cultivars showed the greater AM colonization rate in roots than non-inoculated controls (Figure 2). Interestingly, fruit yield in cultivars inoculated with AM improved significantly than non-inoculated plants, nevertheless, Karpex cv. has the similar fruit yield to their control. The results of inclined biomass production, fruit yield are accordance to numerous reports showing that AM inoculation enhanced root and shoot dry weight, fruit yield in pepper plants (Abdel Latef & Chaoxing, 2014; Abdel Latef, 2013; Boonlue et al., 2012; Tanwar et al., 2013). The most common explanation is that AM symbiosis enhance absorption of soil mineral nutrients, water through the AM hyphae network (Smith & Read, 2008), induced resistance or tolerance

to various stresses (Garmendia et al., 2004; Alejo-Iturvide el al., 2008; Mena-Violante et al., 2006; Kaya et al., 2009; Ruscitti el al., 2011). However, no beneficial effect from AM inoculation on pepper plants were also found in other reports (Russo, 2006; Russo & Perkins-Veazie, 2010).
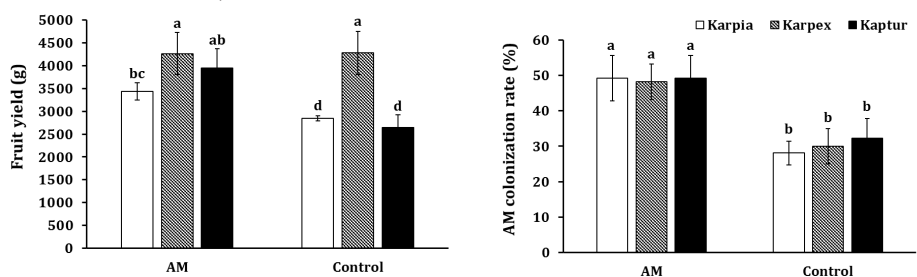


*Figure 2. Arbuscular mycorrhizal colonization rate (A) and fruit yield (B) of AM inoculated and control plants of three pepper cultivars, Karpia, Karpex, Kaptur. AM, Arbuscular mycorrhizal fungi; Each bar presents mean ± standard deviation. Different letters denote significant differences among treatments according to Tukey's post hoc test (P < 0.05) among treatments.*

## Conclusions

This study showed that application of AM mixture generally enhanced the growth, fruit yield of pepper plants but not in all cultivars tested under field conditions.

## Acknowledgement

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# Wheat genotypes under reduced nitrogen supply: changes in chlorophyll fluorescence parameters

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Abstract: Lowering chemical fertilizer input and breeding plants with better nitrogen use efficiency is one of the main goal of research on plant nutrition. It is therefore of major importance to identify the critical steps controlling plant nitrogen use efficiency (NUE). Based on identifying nitrogen sensitive genotypes with every trait, which is participates in this features, we can step forward to have breed crop lines regarding NUE. NUE is largely influenced by the plant's photosynthetic efficiency. Chlorophyll fluorescence (Chl-fl) is connected to the primary reactions of photosynthesis. Parameters of Chl-fl induction curve provide parameters which are used to characterise plant conditions *in vivo*. The Chl-fl parameters were measured at tillering of 31 wheat genotypes under reduced nitrogen content. Several parameters of Chl fluorescence including potential (Fv/Fm) and actual photochemical activity (Yield), non-photochemical quenching (NPQ), vitality index (RFD), etc. were detected with a PAM 2001 modulated light fluorometer. Dry weight of root and shoots and number of tillers also were investigated. Based on our results, some genotypes under reduced nitrogen supply are characterized by similar photochemical activity than under optimal nitrogen nutrition. These genotypes may have the basis of new breeding lines after detailed investigations. Chl-fl parameters's sensitivity for detecting nitrogen deficiency is different, but some of them are really applicable for describing nitrogen lack.

Keywords: wheat, nitrogen, chlorophyll fluorescence

## Introduction

Nitrogen is important in supporting growth and providing high yields. Insufficient nitrogen application causes reduction in plant production, but nitrogen in excess means environmental, health and economic problems. The increased nitrogen-input and global nitrogen-flows pose environmental (Galloway et al., 2008) and nutrient imbalance problems in agricultural development (Vitousek et al., 2009). Beside the question of sustainable agriculture, the demand for fertilizers remains strong. The world nitrogen-fertilizer consumption had decupled in a last 50 years (IFA Statistics, 2010). Two crops, wheat and rice, which have key roles in feeding people, consume 70% of the chemical fertilizers. Unfortunately, the utilization efficiency of nitrogenous fertilizers of these plants under field conditions is around 50% and 25-30%, respectively. A crucial challenge for intensive but green agriculture is to improve nitrogen use efficiency (NUE) so that yields can be improved or maintained with reduced N inputs. Nitrogen use efficiency can be defined as the amount of biomass or yield produced per unit of nitrogen fertiliser applied (Vitousek, 1982). Several commonly used practices have been documented to improve nitrogen use efficiency, like selecting a variety or hybrid with a high harvest index (ratio of grain yield to total plant mass; Bufogle et al., 1997); selecting the proper nutrient rate through soil testing or realistic yield expectations and expected crop removal; matching application timing with crop uptake patterns (Scharf and Alley, 1993); banding fertilizer sources (Eghball and Sander, 2001). Nitrogen use efficiency is largely influenced by the plant's photosynthetic activity. The main objective of our research is to establish the relationships between nitrogen-use efficiency and photosynthetic parameters, like chlorophyll fluorescence, in different wheat genotypes under controlled conditions. We

investigate parameters, which are able to use detecting different N supply conditions of plant.

**Materials and methods**

The experimental plant was wheat (*Triticum aestivum L.*), 31 different genotypes (Babona, Bánkúti M, Békés, Berény, Fóti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marcaltői, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyrozvágyi, Porteleki, Rétsági, Ricsei, Saturnus, Sirtaki, Szajlai, Toldi, Urai) were investigated using two different nitrogen supply (optimal and ¼ part of optimal). The environmental conditions of climate-room (University of Debrecen, Department of Agricultural Botany, Crop Physiology and Biotechnology) were controlled. All of the measurements were in tillering growth stage with three replicates. Dry weight of roots and shoots were determined separately with thermogravimetric method under 65°C. The numbers of shoots were also counted. The photochemical activity was established by *in vivo* chlorophyll fluorescence (Chl-fl ) method described by Schreiber et al., 1986 with chlorophyll fluorometer (PAM-2001, WALZ Gmbh, Germany). Several parameters of Chl-fl including potential (Fv/Fm) and actual photochemical activity (Yield), non-photochemical quenching (NPQ), vitality index (RFD), electron transport rate (ETR) were detected. For preparing results Microsoft Office Excell 2007 and SigmaPlot for Windows Version 12.0 programmes were applied. Significant differences between treatments were signed with star (*): $p<0,05*$, $p<0,01**$, $p<0,001***$

**Results and discussion**

Root has a high influence on plant production by its uptaking activity. Besides this activity root size and architecture also has a crucial role in efficient nutrient absorption in the case of nitrogen as well (Xu et al., 2012). Root allows nitrogen enter into the cell by transporters of root cell plasma membrane, on the other hand responses with the balance of growth and development for local signals of nitrogen (Krapp et al., 2014). Following of dry weight changes by the effect of reduced nitrogen amount may have an important data in the differences of genotypes. The figure 1. shows the alternation in root's dry weight by the effect of lower nitrogen supply in different wheat genotypes. In the most investigated genotypes the reduced nitrogen application induced higher root dry weight. Serious nitrogen deprivation leads to short primary roots and decline the ratio of lateral roots. Babona (0.75g ±0,09) and Hyfi (0.74g ±0,091) have relatively high values of root dry weight under low nitrogen condition. Medium nitrogen deficiency can force auxin production which induces the growth of lateral roots (Gruber et al., 2013). Nitrogen, which was taken up by roots will be transported almost immediately to the shoot. Shoot has also an important role in plant development, thus its advancement, size determinant in yield. The figure 2. shows the values of shoot's dry weight by the effect of optimal and lower nitrogen supply in different wheat genotypes. According to our results the reduced nitrogen supply did not caused dry weight loss in Mulan, Mv. Karéj and Mv. Lucilla. No significant differences was experienced in these genotypes, the reduced nitrogen amount did not means deficiency. Optimal N nutrition caused generally higher number of shoots, but in some genotypes the reduced N amount resulted in similar number of shoots, than the optimal supply (Babona, Bánkúti Marquis, HB-04, Sirtaki) (data not showed).
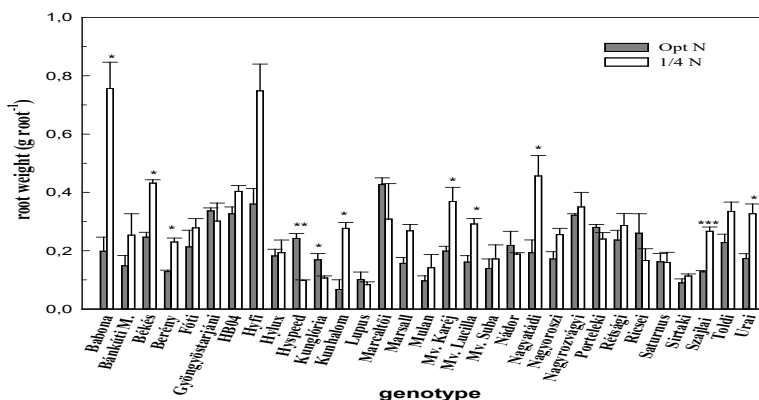
Figure 1 Changes of dry weight of roots (g root$^{-1}$) by the effect of different amount of nitrogen (N) supply (Opt. N: optimal, ¼ part of optimal: (1/4N) in wheat genotypes (Babona, Bánkúti M, Békés, Berény, Fóti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marcaltői, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyrozvágyi, Porteleki, Rétsági, Ricsei, Saturnus, Sirtaki, Szajlai, Toldi, Urai). n=3, ±s.e. (N effects compare to the control value p≤0,05*, p<0,01**, p<0,001***
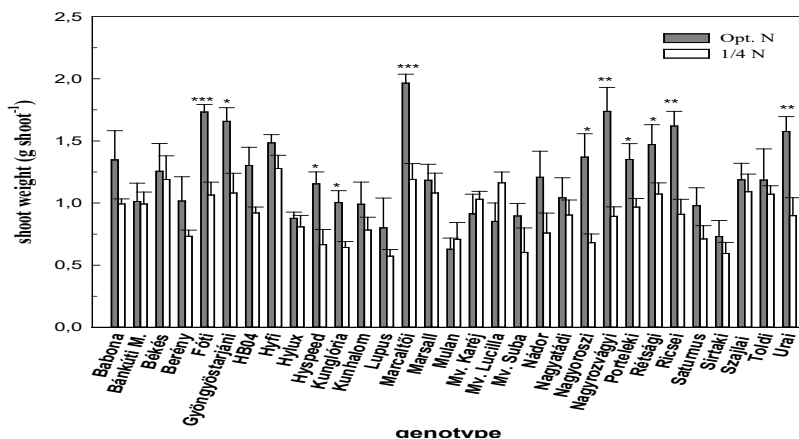


Figure 2 Changes of dry weight of shoots (g shoot$^{-1}$) by the effect of different amount of nitrogen (N) supply (Opt. N: optimal, ¼ part of optimal: (1/4N) in wheat genotypes (Babona, Bánkúti M, Békés, Berény, Fóti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marcaltői, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyrozvágyi, Porteleki, Rétsági, Ricsei, Saturnus, Sirtaki, Szajlai, Toldi, Urai). n=3, ±s.e. (N effects compare to the control value p≤0,05*, p<0,01**, p<0,001***)

Nitrogen nutrition influences the plant photosynthetic capacity through the decrease of synthesis of several key photosynthetic enzymes, especially of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), thus affecting the carbon assimilation, and subsequently also the photochemical processes in thylakoid membranes (Harbinson et al., 1990). Chlorophyll fluorescence measurements give us several useful parameters for characterizing photochemical activity of leaf. The optimal value of Fv/Fm according to Björkmann and Demming-Adams (1987) is 0.832 ±0,004. In the case of Bánkuti M., Békés, Fóti, Kunglória, Rétsági, Szajlai, Toldi and Urai genotypes no significant differences were expressed between the two treatments. It means, that the reduced N supply did not caused incline in the maximal photochemical efficiency of PSII in these genotypes.
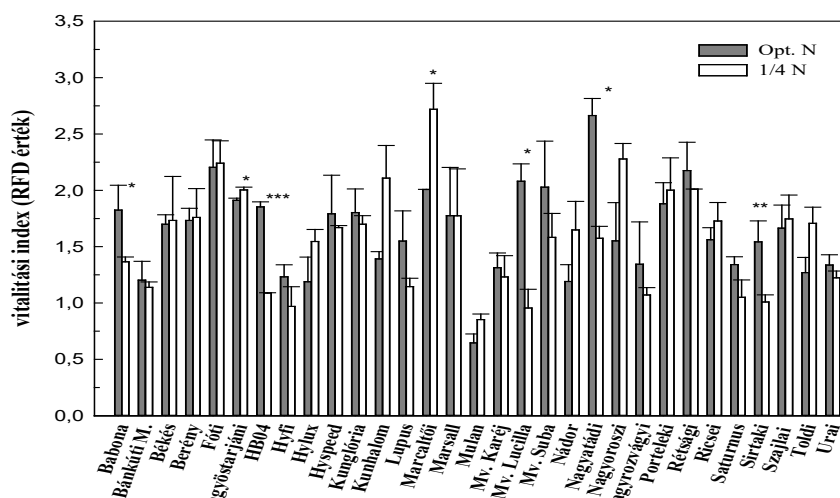
*Figure 3 Changes of potential photochemical activity (Fv/Fm) by the effect of different amount of nitrogen (N) supply (Opt. N: optimal, ¼ part of optimal: (1/4N) in wheat genotypes (Babona, Bánkúti M, Békés, Berény, Fóti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marcaltői, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyrozvágyi, Porteleki, Rétsági, Ricsei, Saturnus, Sirtaki, Szajlai, Toldi, Urai). n=3, ±s.e. (N effects compare to the control value p≤0,05*, p<0,01**)*
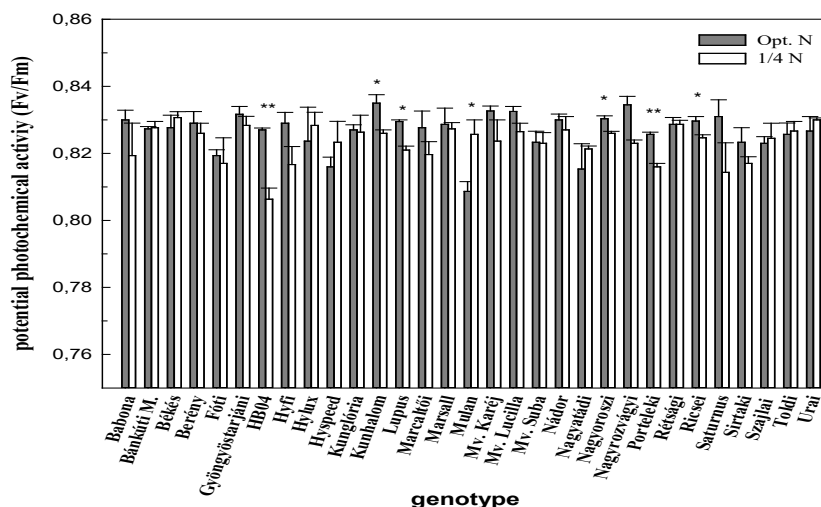


*Figure 4 Changes of vitality index (RFD) by the effect of different amount of nitrogen (N) supply (Opt. N: optimal, ¼ part of optimal: (1/4N) in wheat genotypes (Babona, Bánkúti M, Békés, Berény, Fóti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marcaltői, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyrozvágyi, Porteleki, Rétsági, Ricsei, Saturnus, Sirtaki, Szajlai, Toldi, Urai). n=3, ±s.e. (N effects compare to the control value p≤0,05*, p<0,01**, p<0,001***)*

The relative fluorescence decrease also was measured by Lichtenthaler and Rinderle (1988) who published this parameter as vitality index (RFD). When the value of RFD

decreases under 1, it indicates stress of plants. In our experiments based on this parameters the reduced N application did not cause stress in this early growth stage of 25 genotypes. In the case of six genotypes the nitrogen deprivation generate stress situation: Hyfi, Mulan, HB04, Nagyrozvágyi, Saturnus, Sirtaki 1,009 (Figure 4). From the other examined Chl-fl parameters, the 1-qp/NPQ ratio was the parameter, which showed differences among genotypes in terms of treatments.

## Conclusions

In terms of root dry weight application the reduced nitrogen amount induced higher production, than in optimal nitrogen supply. Optimal nitrogen nutrition caused generally higher number of shoots, but in some genotypes the reduced nitrogen amount resulted in similar number of shoots, than the optimal supply. Interestingly, the reduced nitrogen supply did not caused dry weight loss of shoots in Mulan, Mv. Karéj and Mv. Lucilla. Among the investigated chlorophyll fluorescence parameters some are particularly adaptable for characterization of nitrogen demand of genotypes in the early growth stage. There was a genotypic variation in photochemical activity, and it was influenced by different nitrogen nutrition. The value of 1-qP/NPQ was found to be a suitable parameter for characterizing the intrinsic ability of PSII to balance photochemical and non-photochemical quenching under the given nitrogen supply. According to our results some genotypes's (GK. Békés, MV. Marsall, MV Toldi, Rétsági, Szajlai) photochemical activity are similar to optimal under reduced nitrogen supply.  These wheats are utilizable in breeding wheat lines with sufficient productivity under relatively low nitrogen supply.

## Acknowledgement

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# Activated charcoal improves growth of *F. imperialis* propagated by indirect organogenesis

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Abstract: *Fritillaria imperialis* L production is a challenge for the producers since its regeneration from seeds needs several years. In vitro micropropagation is considered the most proper technique to guarantee a rapid, effective and healthy regeneration. In vitro culture effects of different doses (0.0, 0.5, 1.5, 2.5 g/L) of activated charcoal (AC), and three levels (0.0, 1.5, 1.75 mg/L) of 6-Benzylaminopurine (BAP) on the growth of *F. imperialis* plantlets were determined. The growth medium contained same concentrations of auxins (0.8 mg/L NAA and 0.4 mg/L IAA) for all treatments. The number of shoots, roots, and leaves in regenerated plantlets were increased by adding 2.5 g/L of AC in growth medium containing 1.75 mg/L of BAP. No effects of different cytokinin concentrations were observed on shoot length but added AC significantly improved the shoot length all amounts studied. High concentration of BAP (1.75 mg/L) gave adverse impact on root length in all treatments, but 1.5 g/L AC enhanced the root length and better interacted with cytokinin hormone at 1.5 mg/L level.

Keywords: Crown imperial, activated charcoal, micropropagation, indirect organogenesis

## Introduction

*Fritillaria imperialis* L. (Liliaceae), widely known as crown imperial or Tears of Mary because of great drops of nectar at the petal base, is a perennial plant with ornamental and cut flower use. Besides, it is important for medicine because it contains steroidal alkaloids with various pharmaceutical properties (Gao et al., 1999; Perry and Metzger, 1980; Wang et al., 2005). The species of the Fritillaria genus are distributed in different parts of the world including Turkey, Iraq, Iran, Asia Minor, South Asia, South Eastern Asia and some parts of USA. In the natural habitat, a full-sized Fritillaria needs nearly five years to grow from the seed. On the other hand, the natural propagation rate of most geophytes is relatively low. This often hampers the large scale cultivation of these plants. Since it has small numbers of scales, the amount of meristematic cells in the plant is rather restricted. In vitro propagation methods are essential components of plant genetic resources management and they are becoming increasingly important for conservation of rare and endangered plant species (Almeida et al., 2005; Bhatia et al., 2002). The application of tissue culture techniques may allow rapid and large-scale propagation of uniform plants for field condition. Thus, other techniques such as vegetative propagation and micropropagation were used. On the other side, wild population of *F. imperialis* is highly heterozygote and non-uniform, so for the conservation of different *F. imperialis* populations, the regeneration pathway must be highly genotype-independent and applicable for a wide number of genotypes. Direct organogenesis pathways (regeneration without crossing callus) and indirect organogenesis pathway (regeneration with crossing callus) have been applied for overcoming genotype-dependency and retaining long-time to totipotency in cumin (Ebrahimie et al., 2006b).

In plant tissue culture, activated charcoal (AC) is widely used to stimulate rooting of

micropropagated plantlets, since it enhances the adsorption of metal irons, vitamins, plant growth regulators, and darkening the media (Dumas and Monteuuis, 1995). Moreover, AC adsorbs harmful substances produced either by the media or the explant (Fridborg and Eriksson, 1975; Fridborg et al., 1978).

One of the most important factor in vitro culture especially in proliferation stage is cytokinin hormone. It is well known that cytokinins play multiple roles in the plant development such as promotion of cell, division and cell expansion and protein synthesis stimulation in plant. The present study investigates effects of different doses of activated charcoal AC, different concentrations of 6-Benzylaminopurine (BAP), and their interaction on growth of in vitro micropropagted *F. imperialis*.

## Materials and methods

*F. imperialis* wild grown bulbs obtained from a free trader in Adiyaman were used as plant material. Murashige and Skoog (Murashige and Skoog, 1962) (MS) basal medium was used in this experiment. The medium was gelled with 5.5 g/L agar plant (Duchefa Biocheme) in addition to 30 g/L sucrose as energy and carbohydrate source. The pH of the medium was adjusted to 5.5±0.9 before adding agar and prior autoclaving. After that, the medium was put in autoclave at 121 $^0$C for 15 minute sterilization process. The cultures were saved in a growth room at 25±2 $^0$C with 65% relative humidity, under white fluorescent light for 16h light photoperiod.

The bulbs were washing thoroughly under running tap water to remove the soil, dead parts and insects on the surface, and then, they were rinsed three times with distilled water as well, then after bulbs were cut to smaller pieces. Afterwards, these pieces were pre-treated with 1% (w/v) sodium hypochlorite with Tween-20 for 15 minutes. The prepared cuts were washed extensively with sterile water more than 3- 4 times. This process was completed under controlled and sterilized conditions. These surface sterilized pieces were cut at 1.5 cm width and 2 cm length, then after cultured on MS medium containing 0.125 mg/L Thidiazuron (TDZ).

From the generated callus, medium size pieces '2cm*2cm' were selected and cultured in MS supplemented with different doses (0.0, 0.5, 1.5, 2.5 g/L) of AC, and three levels (0.0, 1.5, 1.75 mg/L) of BAP.

Statistical analysis: Statistical analyses were performed using the software IBM SPSS Statistics for Windows, Version 22.0. Analysis of variances was done by two way ANOVA to separate the activated charcoal effect, hormones effect, and their interaction. Tukey's tests were performed for every possible pairwise comparison.

## Results and discussion

In this study, effects of 0, 0.5, 1.5 and 2.5 g/L of activated charcoal (AC) alone or combined with three concentrations (0.0, 1.5, 1.75 mg/L) of 6-Benzylaminopurine (BAP) on growth parameters were evaluated on *Fritellaria imperialis* plantlets, cultured by indirect organogenesis. Number and length of the roots were observed after 9 and 13 weeks of treatment.

Adding activated charcoal to the growth media without cytokinin hormone increased the number in a dose-dependent manner reaching 10.2 and 36.0 at the 9th and 13th weeks of growth, respectively (Figure 1).

Of all conditions studied, the greatest increase in number of roots was observed when explants were grown in a medium containing the highest dose of AC (2.5 g/L) with the highest concentration of BAP (1.75 mg/L) for both 9 (32.0±2.5) and 13 (54.8±7.6) weeks (Figure 1). This observation might be related to the ability of activated charcoal to
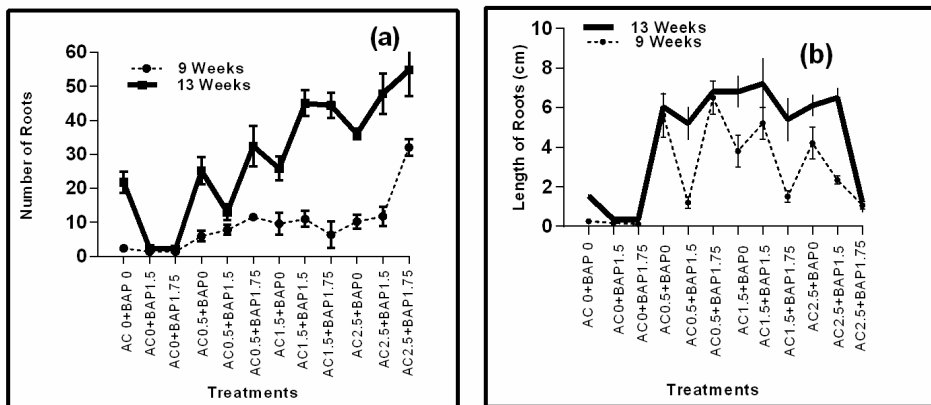


adsorb all compounds in explants and media, also continuing division of the cells in the mersitematic region (Nhut et al., 2001).

*Figure 1. Number of roots, and length of roots of F. imperialis plantlets, after 9, and 13 week. (a) Number of roots. (b) length of roots in centimetre. Vertical bars represent standard deviation (n=5).*

On the other hand, the lowest numbers were counted in the treatments with no AC and higher concentrations of BAP; 1.38±0.5 and 2.20±0.8 for 9 and 13 weeks, respectively. This possibly related to the present of phenolic compounds and toxic brown pigments in the growth medium, as it is also reported by Fridborg et al. (1978), who found phenolic compounds and other metabolites in a medium without activated charcoal resulted in embryogenesis and morphogenesis inhibition.

Despite, differences within the same treatment, adding AC to the medium did positively affect the length of roots reaching the highest value 7.2 cm in ($AC_{1.5}$, $BAP_{1.5}$) treatment at week 13, meanwhile raising the AC dose to 2.5 g/L did not enhance the root length furthermore and gave an adverse effect especially when combined with 1.75 mg/L BAP. Excessive doses of AC in growth media can lessen its adsorption capacity due to many factors such as density, purity of the activated charcoal and the pH of the MS media (Druart and Wulf, 1993).

In contrast to our results, Rahimi et al. (2014) found no difference between treatments with or without activated cahrcoal, when they added 1g/L activated charcoal to 1/2 strength MS media for *F. imperialis*.

As it is shown in (Table 1), the superior dose of AC (2.5 g/L) along with a moderate concentration of BAP (1.5 mg/L) gave the highest number of shoots (32.4±5.0 and

43.2±3.1) after 9 and 13 weeks, respectively. The lowest number of shoots (10.4±2.7) were noticed with no activated charcoal at the same concentration of BAP in 9 weeks. In week 13, however, the lowest number of shoots were observed (11.6± 2.07) only when no BAP or AC were added to the media.

The fact that a similar observation was also made for shoot numbers, which showed its highest numer at the highest concentration of AC, might be another evidence to support that the use of activated charcoal in the growth media stimulates and feeds the tissue more effectively, which in turn helps in modification and differentiation of the explant (Thomas, 2008).According to (Pan and Staden, 1998), AC can inhibit the toxic effect of phenolic components and help plant parts to uptake the nutritional compounds from the media, resulting in better growth.

Shoot length appeared to be affected greatly by both BAP and AC concentrations, and their combination providing us with various results. For samples plantlets treated for 9 weeks generated the longest shoot (1.12±0.10) at moderate concentrations (1.5 g/L of AC and 1.5 mg/L BAP). When the duration of the treatment was extended to 13 weeks, the longest shoot (1.50±0.12) was generated from the sample treated with relatively low concentration of AC (0.5 g/L) and the highest concentration (1.75 mg/L) of BAP (Table 1). In our study, higher concentrations of hormone affected shoot formation possibly related to the physiology and the multiformity of plantlets and the synthesis of endogenous hormones in the plantlets, different endogenous hormones of explants may differ in their ability to react to exogenously applied plant growth regulators (Winkelmann and Serek, 2005).

*Table 1. Number of shoots, and length of shoots of F. imperialis plantlets, after 9, and 13 weeks. Means with different letters are significantly different at (p<0.05), determined by Tukey's HSD test (Mean ± SD, n= 5). Capital letters represent AC effect, small letters represent BAP effect.*

| AC g/L | BAP mg/L | Number of shoots | | Length of shoots | |
|---|---|---|---|---|---|
| | | 9 weeks | 13 weeks | 9 weeks | 13 weeks |
| $AC_0$ | $Cyt_0$ | $10.6^{Aa}\pm2.4$ | $11.6^{Aa}\pm 2.07$ | $0.25^{Aa}\pm0.07$ | $0.90^{Ab}\pm 0.25$ |
| | $Cyt_{1.5}$ | $10.4^{Aa}\pm2.7$ | $11.8^{Aa}\pm 3.11$ | $0.32^{Aa}\pm0.08$ | $0.38^{Aa}\pm 0.17$ |
| | $Cyt_{1.75}$ | $12.8^{Aa}\pm4.4$ | $12.2^{Aa}\pm 3.11$ | $0.40A^{Bb}\pm0.18$ | $0.40^{Aa}\pm 0.18$ |
| $AC_{0.5}$ | $Cyt_0$ | $11.8^{Aa}\pm3.3$ | $33.6^{Bb}\pm 2.3$ | $0.96^{Ca}\pm0.05$ | $1.1A^{Ba}\pm 0.10$ |
| | $Cyt_{1.5}$ | $12.8^{Aa}\pm1.6$ | $23.4^{Ba}\pm 1.1$ | $1.02^{Ba}\pm0.10$ | $1.22^{Ba}\pm 0.16$ |
| | $Cyt_{1.75}$ | $23.4^{Bb}\pm3.9$ | $31.8^{Cb}\pm 1.6$ | $0.90^{Ba}\pm0.07$ | $1.80^{Cb}\pm 0.27$ |
| $AC_{1.5}$ | $Cyt_0$ | $13.8^{Aa}\pm3.3$ | $14.00^{Aa}\pm6.8$ | $0.78^{BCa}b\pm0.3$ | $1.32^{Ba}\pm0.39$ |
| | $Cyt_{1.5}$ | $26.4^{Bb}\pm4.2$ | $31.20^{Cc}\pm2.1$ | $1.12^{Bb}\pm0.10$ | $1.50^{Ca}\pm0.12$ |
| | $Cyt_{1.75}$ | $15.0^{Aa}\pm1.5$ | $23.60^{Bb}\pm2.0$ | $0.50^{Aa}\pm0.10$ | $1.24^{Ba}\pm0.25$ |
| $AC_{2.5}$ | $Cyt_0$ | $15.0^{Aa}\pm2.2$ | $33.8^{Aa}\pm 1.9$ | $0.48^{Ba}\pm0.08$ | $1.62^{Bb}\pm 0.43$ |
| | $Cyt_{1.5}$ | $32.4^{Bc}\pm5.0$ | $43.2^{Db}\pm 3.1$ | $0.98^{Bb}\pm0.04$ | $1.10^{Ba}\pm 0.10$ |
| | $Cyt_{1.75}$ | $26.0^{Bb}\pm2.9$ | $36.2^{Ca}\pm 4.0$ | $0.90^{Bb}\pm0.17$ | $1.30^{Ba}\pm 0.15$ |

Higher concentrations of AC and BAP (2.5 g/L, 1.75 mg/L raised number of leaves (32.40±2.0 and 46.4±3.6) after 9 and 13 weeks respectively (Table 2). The minimum number of leaves was observed when there was no AC but the highest amount of BAP (1.75 mg/L) in the media. Even though increasing AC or BAP alone did not create a regular pattern on the number of leaves in any duration, after AC level was reached to 1.5 g/L, BAP concentration apperaed to be possitivley correlated with leaf number in week 13. As it has been reported previously, in our experiments, MS medium suplemented with activated charcoal also provided a better leaf growth compared to a medium without

activaed charcoal (Rittirat et al., 2012). Consisiting with results by Rittirat et al. (2012), in our experiment, MS medium suplemented with activated charcoal provided a better leaf growth compared to a medium without activaed charcoal. Higher number of leaves obtained by adding activated charcoal related to the attribution of AC in adsorption of inhibitory compounds in the culture medium (Fridborg et al., 1978).

Table 2. *Number of leaves of F. imperialis plantlets.after 9, and 13 week. Means with different letters are significantly different at (p<0.05) as determined by Tukey's HSD test (Mean ± SD, n= 5).Capital letters represent AC effect, small letters represent BAP effect.*

| AC g/L | BAP mg/L | Number of leaves | |
|---|---|---|---|
| | | 9 weeks | 13 weeks |
| | $Cyt_0$ | $1.60^{Aa}\pm0.5$ | $8.0^{Ab}\pm1.2$ |
| | $Cyt_{1.5}$ | $1.40^{Aa}\pm0.5$ | $2.2^{Aa}\pm0.8$ |
| $AC_0$ | $Cyt_{1.75}$ | $1.20^{Aa}\pm0.4$ | $1.6^{Aa}\pm0.5$ |
| | $Cyt_0$ | $6.60^{Ca}\pm1.1$ | $15.2^{Bb}\pm2.3$ |
| | $Cyt_{1.5}$ | $5.60^{Ba}\pm0.5$ | $10.0^{Ba}\pm0.7$ |
| $AC_{0.5}$ | $Cyt_{1.75}$ | $6.80^{Ba}\pm1.3$ | $13.2^{Cb}\pm1.3$ |
| | $Cyt_0$ | $3.00^{Aa}\pm0.7$ | $16.6^{Bb}\pm5.6$ |
| | $Cyt_{1.5}$ | $10.0^{Cb}\pm2.1$ | $19.6^{Db}\pm1.1$ |
| $AC_{1.5}$ | $Cyt_{1.75}$ | $5.20^{Ba}\pm1.3$ | $5.8^{Ba}\pm1.7$ |
| | $Cyt_0$ | $5.00^{Ba}\pm1.0$ | $11.8^{Aa}\pm1.4$ |
| | $Cyt_{1.5}$ | $12.00^{Cb}\pm2.8$ | $15.4^{Ca}\pm1.8$ |
| $AC_{2.5}$ | $Cyt_{1.75}$ | $32.40^{Cc}\pm2.0$ | $46.4^{Db}\pm3.6$ |

## Conclusions

Results of this experiment provide invulnerable evidence that activated charcoal can be added to the culture media for propagation of *F. imperials* by indirect organogenesis to improve the growth of regenerated plantlets, but excessive doses of activated charcoal should be avoided to prevent adverse results. Further studies are needed to standardize the propagation, since *F. imperialis* is slow in growth and culture medium faces unfavorable changes in pH and nutrient depletion.
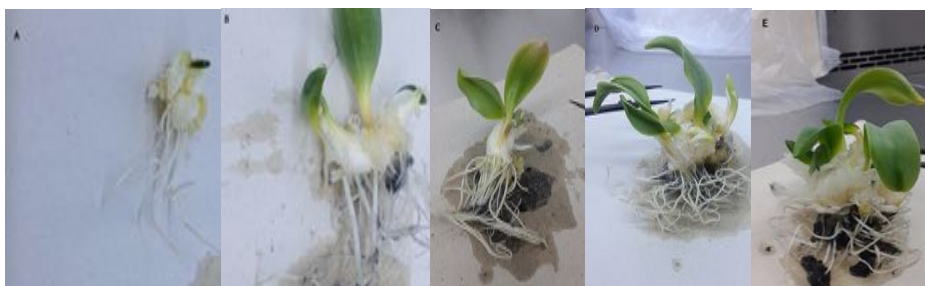


Figure 2. *Plantlets of F. imperialis grown in different media: A- MS medium without AC,  B- MS medium with 0.5 g/ L AC, C- MS medium with 1.5 g/ L AC, D- MS medium with 2.5 g/ L AC,  E- MS medium with 1.5 g/ L AC and 1.5 mg/L BAP.*

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# Effects of soil tillage systems and fertilization on the $CO_2$ emission of chernozem soil

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Abstract: The correlation between carbon dioxide emission and agriculture has been a crucial issue for humanity for decades. As a result of global climate change, this correlation deserves special attention mainly in terms of how greenhouse gas emission can be reduced. For this reason, this subject becomes increasingly important. $CO_2$ is a very important compound and it is one of the greenhouse gases which not only offsets the cooling of the Earth, but too high $CO_2$ emission leads to global warming. By saving the carbon in the soil, it is possible to protect the organic materials in the soil and improve soil fertility. The performed measurements enabled us to study the relationships between the different types of cultivation and carbon dioxide emission.

Keywords: carbon dioxide, emission, greenhouse effect, cultivation, fertilizer

## Introduction

Global climate change has been one of the main subjects and challenges for humanity for a long time. 20% of greenhouse gases - which significantly contribute to climate change - result from agricultural activities. More specifically, 50-75% of anthropogenic methane emissions and 5% of carbon dioxide are related to agriculture. Indirectly, 14% GHG emission is caused by deforestation and the burning of biomass (Gyuricza et al., 2002). Carbon dioxide emission derives from the decrease of organic matter, but emission is influenced by soil moisture, temperature, soil quality and cultivation (Fogarassy et al., 2008).

By performing environmental friendly agriculture, the carbon dioxide emission deriving from the soil can be reduced; therefore, it is very important to reduce soil disturbance as much as possible in order to preserve its organic matter. The migration (source cycle) of soil organic matter contributes substantially to global climate change, since it is significantly influenced by the intensity of soil respiration. The volume of carbon dioxide emission through soil respiration is ten times as high as that of fossil-fuel combustion (Mielnick-Dugas, 2000). Consequently, it can be observed how important it is to provide satisfactory amount of plant nutrients. In particular, agriculture, including deforestation, burning of the biomass and crop cultivation systems are responsible for 5% of carbon dioxide emission (Láng, 2003). Emission can be reduced by fixing carbon in the soil. Bacteria, fungi and worms, which form a more complex organic matter called humus, contribute to this process substantially, by fixing carbon in the soil. Using direct methods of "re-filling" carbon back into the soil – organic fertilisers (plant- or animal-derived matter) – the fixation of carbon is feasible, resulting in 2-20 million tonnes/year carbon remaining in the soil, which is not only important from the point of view of global climate change mitigation measures, but also promotes soil recovery.

In the framework of environment friendly agriculture, it is necessary to achieve reduced energy consumption and reduced volume of mechanical works in order to affect the carbon and carbon dioxide emission from the soil. In the opinion of Hagymássy et al., (2015), precision is an important part of environmental friendly agriculture. If organic matter remains in the soil, it will positively influence the structure of the soil, its stability, buffer

capacity, water storage capacity, biological activity and the nutrient balance (Holland, 2004). Conventional tillage systems have certain consequences which lead to soil and environmental deterioration, resulting in   soil compaction soil structure degradation, decreased level of organic matter, worsening bearing capacity and increasing carbon dioxide emission. The benefits of these soil tillage systems are minor in comparison to their disadvantages (Birkás, 2002). Taking all these aspects into consideration, it is important to replace conventional tillage systems with conservation tillage systems.

Soil tillage is the basis of agricultural land use which contributes to the increase of carbon dioxide emission, thereby causing global climate change. As a matter of course, this is not as intensive as industrial pollution, but comparing with the Earth's surface, in particular with the surface used for agricultural purposes, this emission is significant. When performing tillage, as a result of ploughing, carbon dioxide emission suddenly increases, but it is later restored, mainly due to drier climate and conservation tillage systems (Rochette et al., 1997; Ellert and Janzen, 1999). Accordingly, at the beginning, the intensity of emission mainly depends on the depth of soil tillage (Reicosky and Lindstrom, 1993). In the course of the saturation of soil, carbon dioxide is displaced. In parallel with the drying of the soil, the volume of gases increases (Stefanovits et al., 1999).

**Materials and methods**

In our experiment, we conducted measurements in order to define the influences which soil tillage systems and fertilizers have on the emission of carbon dioxide. In order to achieve the research objective, we examined the tillage and fertilization effects in a polyfactorial long-term field experiment at the trial site of the University of Debrecen (Hajdúság loess plato, 47° 30' N, 21° 36' E, 121 m elevation) on 20 October 2015 and 8 July 2016.

The experiment had a split-split-plot design, with the main plots having three tillage treatments. The investigated tillage treatments were mouldboard ploughing (MP) to a depth of 30 cm, strip tillage (ST) to a depth of 28 cm and chisel ploughing (CP) to a depth of 35 cm, non-fertilized (control). Two nitrogen fertilization levels (80 kg N ha$^{-1}$ and 160 kg N ha$^{-1}$) were applied in a randomized way on the secondary sub-plots in four replications.

The goal of our experiment was to evaluate the influence of soil tillage and nitrogen fertilizer on the volume of carbon dioxide emission from the soil surface. During our experiment, we compared the differences between mouldboard ploughing, strip tillage and chisel ploughing. Mouldboard ploughing is a conventional method of soil tillage, which strongly modifies the structure of the soil. Strip tillage does not cause dramatic changes to the soil structure, with the attempt to maximise the erosion prevention benefits of keeping organic matter and plant residues on the soil surface. Our measurements were carried out five times on each treatment. Carbon dioxide measurements were conducted by using the device CI-340, which is a light portable device to be used both in the field and in the laboratory for measuring the carbon dioxide emission of the soil. A 630 m$^3$ sampling device (reservoir) was used and the changes of carbon dioxide concentration were measured in five minutes. Carbon dioxide emission from the soil was converted into g/m$^2$/h measurement units.

## Results and discussion

It can be concluded that utilising fertilizer carbon dioxide emission significantly increases. The highest amount of carbon dioxide emission was measured in the case of conventional tillage and mouldboard ploughing. The carbon dioxide emission we measured in the case of 80 kg N/ha mouldboard ploughing treatment amounted to 0.1259 $g/m^2/h$. In the case of the 160 kg N/ha chisel ploughing the obtained result was almost the same: 0.1223 $g/m^2/h$. The results of strip tillage in all fertilization treatments were significantly lower than those of the mouldboard ploughing or chisel ploughing.
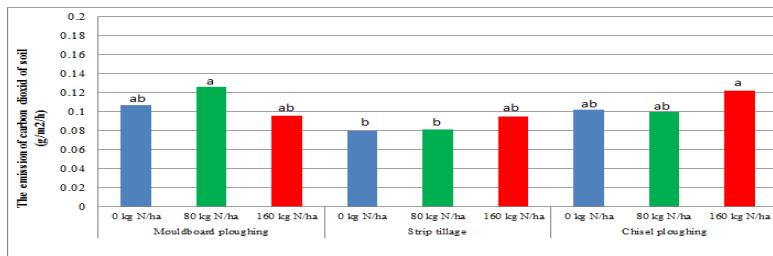


*Figure 1. Effects of soil tillage systems and fertilization on the CO$_2$ emission of chernozem soil. (Debrecen-Látókép, 2015. 10. 30.)*

The results of the second measurements in 2016 have yielded different results in comparison with those of the experiment in 2015. In general, the results of all three tillage methods and nitrogen fertilizer treatments were higher. It was the most remarkable result that the highest emission was measured in the case of all three tillage systems on the plots with 80 kg N/ha. The highest volume was measured on the 80 kg N/ha plots being cultivated by means of chisel ploughing: 1.2828 $g/m^2/h$. The lowest results was measured in the case of strip tillage which was lower on the less disturbed soil. The highest results were obtained in the case of chisel ploughing.
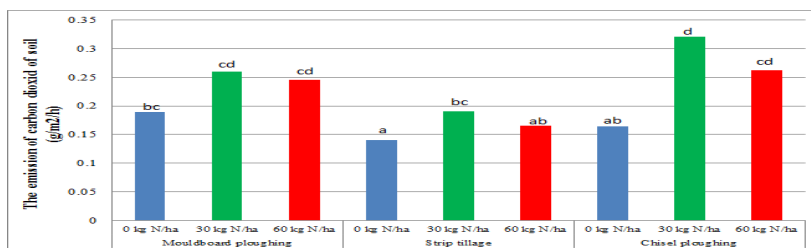


*Figure 2. Effects of soil tillage systems and fertilization on the CO$_2$ emission of chernozem soil. (Debrecen-Látókép, 2016. 07. 08.)*

## Conclusions

The soil, as one of the main sources of carbon dioxide emission, significantly contributes to global climate change, which makes it extremely important to focus on agricultural land use. By conducting our measurements, we analysed the influence of utilising fertilizers in different doses and performing different types of soil tillage. Our experiment shows that the increasingly widespread conservation tillage systems have a positive influence on carbon dioxide emission.

In our experiment, in the case of all three different nitrogen fertilizer doses, the carbon dioxide emission results of strip tillage were lower than those in the case of mouldboard ploughing and chisel ploughing treatments.

The performed measurement proved our hypothesis, i.e. the level of carbon dioxide emission of less disturbed soil is lower due to the fact that it has a better ability to store organic matter. We consider it to be necessary and appropriate to conduct follow-up research and measurements in the future.

### Acknowledgement

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# Gene expression of glutathione-S-transferase in sunflower (*Helianthus annuus* L.) inoculated with arbuscular mycorrhizal fungi under temperature stresses

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Abstract: The association between terrestrial plants and arbuscular mycorrhizal (AM) fungi is one of the most widespread mutualistic plant-fungal interactions in natural and agricultural systems. Several studies suggest that AM symbiosis can help plants to alleviate various biotic and abiotic stresses. Some works show clearly that the amelioration of stress resistance by AM symbiosis is often related to the enhancement of antioxidant levels or activities in mycorrhizal plants. Glutathione-S-transferase is playing an important role in the protection against oxidative membrane damage and necrotic disease symptoms. Plants of different age, inoculated and non-inoculated with arbuscular mycorrhizal fungi were exposed to high temperature- and low temperature stresses. To detect the expression profiles of glutathione-S-transferase gene, total RNA was extracted from sunflower leaves 24hrs after the stress exposition and quantitative real-time PCR was carried out using modified primers. The expression of glutathione S-transferase gene changed under different temperatures influenced by the age of the plants. The highest level of glutathione-S-transferase gene expression was measured in mycorrhizal plants during high temperature stress compared to control plants.
Keywords: plant, mycorrhiza, stress, gene expression

## Introduction

The association between terrestrial plants and arbuscular mycorrhizal (AM) fungi is one of the most widespread mutualistic plant-fungal interaction in natural and agricultural systems. This symbiotic relationship evolved more than 400 million years ago facilitating the colonization of land by plants (Taylor et al. 1995; Smith and Read 1997; Redecker et al. 2000; Smith and Read 2008).

Some biotic and abiotic stress conditions generate reactive oxygen species (ROS) in plant tissues causing damage to proteins, lipids and photosynthetic pigments as well. By ROS-scavenging enzymes (catalase, superoxid dismutase and peroxidase) the plants can detoxify the oxidative ROS (Mittler 2002; Passardi et al. 2004; Matsumura et al. 2007; Kohler et al. 2009). Glutathione S-transferase is considered as an antioxidative enzyme, it plays an important protection role in the plant defense system against oxidative stress, oxidative membrane damage and necrotic disease symptoms (Marrs 1996; Sharma et al. 2004).

The regulation of host antioxidant compounds by AM and the related mechanisms are not well known, and only a few investigations have been carried out at different stages of mycorrhizal colonization. However, an in-depth investigation could help to elucidate the mechanisms of tolerance induced by AM symbiosis and to discriminate the stress-induced processes. Therefore, the main objectives of current research were to estimate changes of the expression of glutathione S-transferase in plant-arbuscular mycorrhizal system under temperatures stresses for evaluating AMF as biocontrol at different stages of mycorrhizal colonization.

## Materials and methods

Seeds of sunflower (*Helianthus annuus* L.) were germinated and the four-day-old pre-

germinated seeds were sown to plastic pots containing substrate (soil:sand 1:1, v/v), previously sterilized in autoclave for 25 minutes at 121°C, 1.2 bar. The seedlings were grown in growth chamber under controlled condition with a 16 hour day length and at a temperature of 24 °C ± 0.5 °C. The moisture of substrate was 60 %.

The commercial product Symbivit® (mixture of *Rhizophagus irregularis* BEG140, *Funneliformis mosseae* BEG95, *Claroideoglomus etunicatum* BEG92, *Claroideoglomus claroideum* BEG96, *G. microaggregatum* BEG56, *Funneliformis geosporum* BEG199) produced by Symbiom Ltd. (Lanskroun, Czech Republic; www.symbiom.cz) was applied at 15 g of inoculum (consisting of 80 propagules $g^{-1}$) per seedling into the planting hole and seedlings were planted immediately.

Healthy 15 and 42 days-old plants (mycorrhizal- and non-mycorrhizal plants) were selected and exposed to two temperature stress conditions. Plants were exposed to 38 °C for 24 h. (High temperature stress, HT) or were incubated for 24 h at 4 °C (Low Temperature stress, chilling stress LT). After 24 h the leaves were collected and analyzed. All treatments replicated in 5 times: non-mycorrhizal and mycorrhizal plants, 15 days old and 42 days old plants and low and high temperature stresses, altogether 40 plants were selected for measurements.

qRT-PCR

Total RNA was extracted from 15 and 42 days-old sunflower leaves using Vantage Total RNA Purification Kit (Origene, USA) according to the manufacturer's instructions, followed by DNase I (Fermentas) digestion to remove residual genomic DNA contamination. For qRT-PCR analysis, first-strand cDNA was synthesized from varieties of all treatments using First-strand cDNA Synthesis for Quantitative RT-PCR kit (Origene, USA).

Modified glutathione S-transferase oligonucleotide primers
GST-f (5'-GAGAAGGCTCAGGCTCGATT-3') and GST-r
(5'-GCAACAGCTTGCTTCTCTCC-3') (Radwan et al. 2005) were used to amplify the glutathione S-transferase gene from sunflower.

PCR cycling program for GST and actin genes consisted of 15 min at 95°C, followed by 40 cycles of 15 sec at 95°C, 30 sec at 57°C and 16 sec at 72°C, with an additional cycle (60 sec at 95°C, 30 sec at 57°C and 30 sec at 95°C) in the end. The real-time PCR experiment was carried out at least three times under identical conditions. The real-time PCR efficiency was determined for each gene (actin gene was used as an internal reference, GST) and each stress condition using the $2^{-\Delta\Delta CT}$ method. QRT-PCR was performed on Mx3000P QPCR System (Agilent). The root colonization (internal fungal structures: hypae, arbuscules, vesicles) were examined using the gridline intersection method (Giovanetti and Mosse 1980).

**Results and discussion**

In the earlier stage (15 days old plants) the high temperature stress caused more intensive gene expression of glutathion S-tranferase in the measurements of the earlier stages (15 days old) of inoculated plants compared to the control (Fig.1.). Lower levels of gene expression were measured in the plants exposed to the low temperature as for the high temperature stress GST expression was higher in plants inoculated compared to those not inoculated.
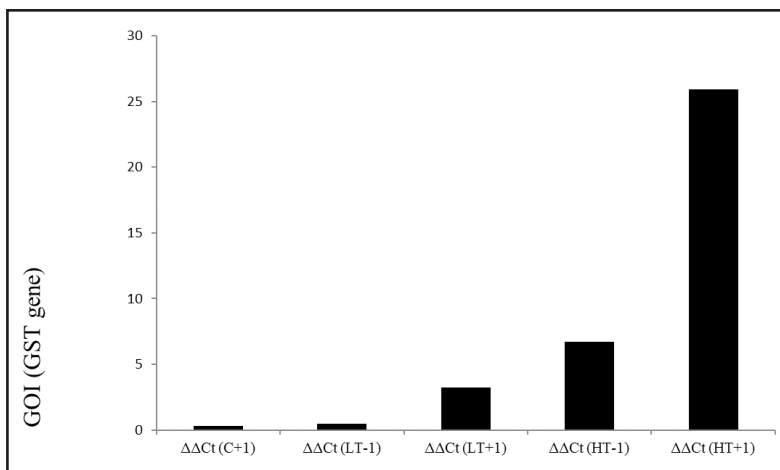
*Figure 1. Glutathione S-transferase gene expression in 15 days old plants*

*y axis:* **GOI**, Gene of Interest (relative amounts of the examined gene (GST) compared to the control sample (C− = Ø)), *x axis:* **C+**, Control, inoculated with mycorrhizal fungi, **LT−**, Low Temperature stress, non-inoculated with mycorrhizal fungi, **LT+**, Low Temperature stress, inoculated with mycorrhizal fungi, **HT−**, High Temperature stress, non-inoculated with mycorrhizal fungi, **HT+**, High Temperature stress, inoculated with mycorrhizal fungi
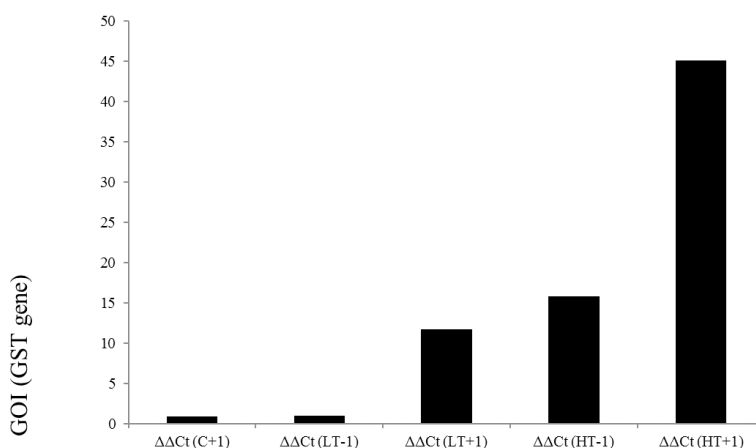


*Figure 2. Glutathione S-transferase gene expression in 42 days old plants*

*y axis:* **GOI**, Gene of Interest (relative amounts of the examined gene (GST) compared to the control sample (C− = Ø)), *x axis:* **C+**, Control, inoculated with mycorrhizal fungi, **LT−**, Low Temperature stress, non-inoculated with mycorrhizal fungi, **LT+**, Low Temperature stress, inoculated with mycorrhizal fungi, **HT−**, High Temperature stress, non-inoculated with mycorrhizal fungi, **HT+**, High Temperature stress, inoculated with mycorrhizal fungi

In the measurements of the older (42 days old) plants compared to the 15 days old plants have clearly showed similar trend of gene expression (Fig.2.). Under high temperature stress conditions the inoculated ones showed especially higher levels of gene expression than the non-inoculated ones. Likewise under low temperature stress conditions the inoculated ones, which had a massive mycorrhizal fungi colonization (inoculation of the plants by arbuscular mycorrhizal fungi were higher than 70%) had showed a higher level of gene expression than the non-inoculated plants and control ones.

## Conclusions

Glutathione S-transferase belongs to the most studied enzyme groups and there are several studies about their function in the cell defend system, like inactivation of the reactive oxygen species (Edwards et al. 2000). Our results demonstrate that mycorrhizal inoculation at different stages stimulates abiotic stress responses in the plants. On the basis of these data we provided a deeper insight into the role of arbuscular mycorrhizal fungi in arresting reactive oxygen species and strengthening antioxidant defense system in the host plants. However, although our results could help the better understanding of various stresses resistance mechanisms, more additional experiments are required to clarify the molecular basis underlying the regulation processes of the enzymes involved. In the future, we plan to extend the experiment to other genes as well. The length of the proceeding book limits the deeper explanation of this work.

## Acknowledgement

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# The comparison of spring to early summer SPAD values of various winter cereals

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Abstract: From the beginning of the history of mankind cereals are the most important crop plants. It can be stated in case of Hungary as well, where normally, during the past centuries, two third of the arable land is used for cereal cropping.

Cereals are produced for their carbohydrate content, a primary product of photosynthetic assimilation. Plants manufacture organic matter from atmospheric $CO_2$ and water taken up by the root system from the soil. Although photosynthesis is driven by abiotic environmental factors, agronomic technologies have remarkable impact on it. Therefore producers should obtain information about its performance.

New technologies give us tools for remote sensing like SPAD that gives reliable value for photosynthetic activity. To use it in an appropriate way, it should be calibrated somehow for each species and varieties.

Our aim in this work was to make reference values for the most critical phenology of different species and varieties of winter cereals. Characteristic differences were found for each of them that can be used for clarifying further technologies.

Keywords: cereal, SPAD value

## Introduction

In Hungary, agricultural production has long been a major issue as almost half of the country's land is arable. In Hungary, just as in the entire world, cereals represent the most plausible source of human alimentation, hence, for a very long time, winter cereals were the most commonly cultivated crop species. There exist numerous factors that have effect on yield (Tarnawa & Klupács, 2006; Horváth, 2014) and among them there are some that could be influenced by the farmer and also some that could not be. The first group is the set of elements of agronomic management the second group is the set of factors of the environment (Várallyay et al., 1985). In the set of environmental factors there are some with more or less effect on yield (Klupács et al., 2010). Even because cropping technologies are not an indoor practice but mostly outdoor; the weather and climate represent a high environmental impact (Láng et al., 2007), and according to former observations, weather plays a significant role (Szöllősi et al., 2004).

The most critical technological points in winter cereal production are agrochemical applications, so that it is essential to reveal and study soundly their impacts (Birkás, 2006). According to the strategy of sustainable agricultural one of the basic principles is to produce safety goods and supply customers with healthy food. Hence we have to deal with chemicals, for example nutrients given as inorganic chemical fertilizers not to overdose. Many inputs and practices used by conventional farmers are also used in sustainable agriculture. The goal is to develop efficient biological systems, which do not need high levels of material inputs. Chemical inputs are seen by the public to be a primary cause of food contamination and environmental pollution arising from agriculture (Jolánkai et al., 2006). Sustainable approaches are those that are the least toxic and least energy intensive, and yet maintain productivity and profitability (Várallyay 2006). As yields still show

lower or higher fluctuation from the long term averages or trends, it should be more than useful to explore how it depends on each element of climate (Pepó, 2010) and technology.

The nitrogen regime of plants has high importance in photosynthetic activity. Strong connection could be found even visually between the color of a crop plant and N supply. That colour, and in parallel the chlorophyll activity can be estimated in an objective way by SPAD analyzer that generates indices on N supply of plants on field. As it measures without destruction, it can be used in different phenological status (Ványiné 2008).

To be able to use the SPAD method for N supply estimation it is necessary to have points to accord. In former studies it was found that even between varieties there can be observed big differences in SPAD values. In this paper our aim is to make reference values for the most critical lifetime of several species and varieties of winter cereals. If characteristic differences could be found for each of them, it can be used for clarifying further technologies.

## Materials and methods

At the Gödöllő campus of Szent István University a set of winter cereals were sown on micro-plots (1 m² each) for demonstrational purposes. Among these items normal wheat (Mv Karéj, Mv Nádor, GK Körös, GK Csillag), hybrid wheat (Hyland), special wheats (Mv Alkor, Mv Menket, Mv Hegyes), durum and spelt wheat (Mv Hundur Franckenkorn), triticale (Tátra, Mv Sámán), rye (Várda), barley (Giga, Korsó, KG Puszta) and winter oat (Mv Hópehely) could be found. Items and the relations between them can be seen in Table 1.

The observation was made in 2015 that was a normal cropyear for winter cereals. The soil of that site is forest brown that is favourable for producing those crops.

Table 1. The items studied in the experiment – name of items and the relations between them.

| glum | | taxonomy | | Name of item | group |
|---|---|---|---|---|---|
| | | genus | species | | |
| nude | Triticum genus | Triticum | aestivum | Mv Karéj | 1 |
| | | | | Mv Nádor | |
| | | | | GK Körös | |
| | | | | GK Csillag | |
| | | | aestivum, hybrid | Hyland | 2 |
| | | | monococcum | Mv Alkor | |
| | | | | Mv Menket | |
| | | | dicoccum | Mv Hegyes | |
| | | | spelta | Franckenkorn | |
| | | | durum | Mv Hundur | |
| | other than Triticum genus | Triticosecale | × | Mv Sámán | 3 |
| | | Secale | cereale | Várda | |
| | | Triticosecale | × | Tátra | |
| glumous | | Hordeum | vulgare | Giga | 4 |
| | | | | Korsó | |
| | | | | KG Puszta | |
| | | Avena | sativa | Mv Hópehely | |

In one of the most critical period, the late spring to early summer, chlorophyll activity was estimated by SPAD-502 PLUS analyzer in different phenological stages. On nine occasion the estimations were made: 23rd April, 27th April, 30th April, 3rd May, 7th May,

22nd May, 28th May, 1st June, 11th June. The SPAD values were measured on them regularly in 10 repetitions for each plot in each time. On the series the statistical analysis was performed by using the MS Excel program package.

## Results and discussion

On figure 1 to 4 the averages of the measurements could be seen. On figures the solid lines refer for each item but dashed lines are for average of a bigger group. As there are so many items, groups were made along the taxonomical distance. Label of groups can be seen in the last column of table 1.

The first group is formed by the four *Triticum aestivum* varieties. The average results can be seen on figure 1. Even the difference between them seems to be big, the shape of curves are similar; the place of local maximum and minimum point are together for most of them.
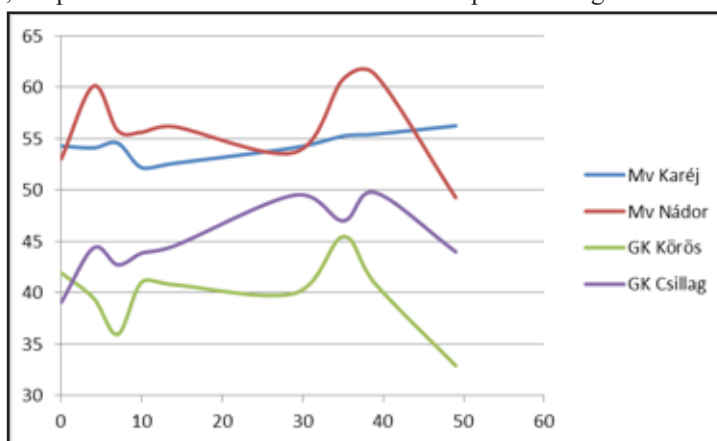


*Figure 1. The average result for Triticum aestivum varieties (group 1)*

Second group includes other varieties from the *Triticum* genus. On figure 2 the average SPAD values for them can be seen with the combined average of group 1. Even that group comprises numerous varieties, lower differences can be found in value but bigger variation in the shape of curves.
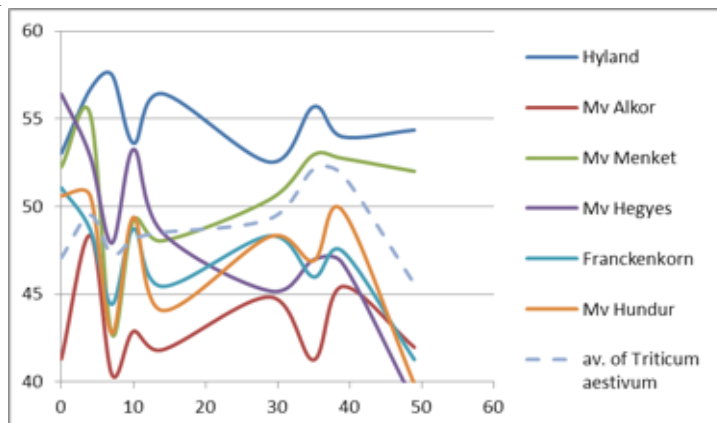


*Figure 2. The average result for Triticum varieties (group 2) with the combined average of Triticum aestivum varieties (group 1)*

Third group is made on nude cereals besides the *Triticum* genus, namely rye and triticale. The rye is comparatively close to wheat as they can be hybridized that results the triticale. As the triticale is closer to wheat, it can be noticed in the similarity to average curves of wheat groups. Rye is differing a bit more (figure 3).
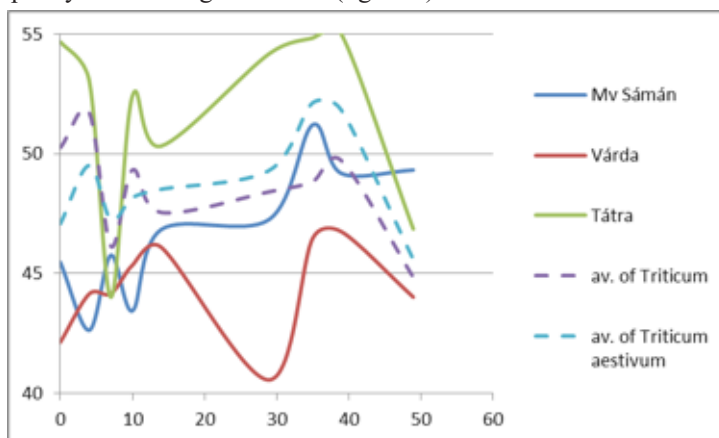


*Figure 3. The average result for non-Triticum nude cereals (group 3) with the combined average of Triticum aestivum varieties (group 1) and Triticum varieties (group 2)*

The last group is a kind of miscellaneous as barley and oat is in it, the base of forming it is that they are all glumous. It can be seen well on figure 4 that the curves of the three barley varieties go similarly but the oat variety (Mv Hópehely) has a diverse performance. As the ripening of barley is earlier, it can be seen that average values are in decline for the last days of measurement.
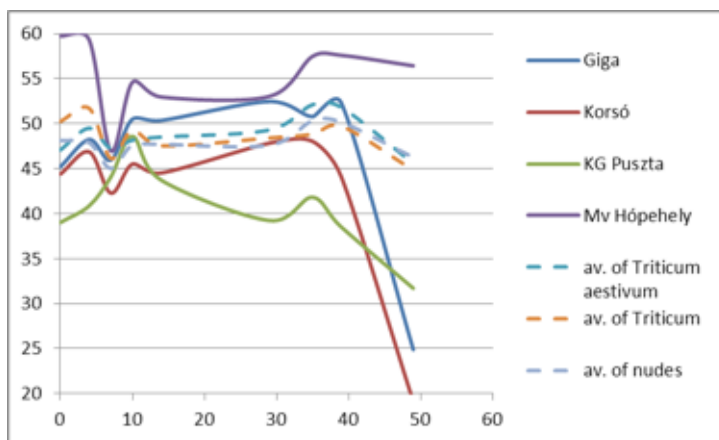


*Figure 4. The average result for glumous cereals (group 4) with the combined average of Triticum aestivum varieties (group 1), Triticum varieties (group 2) and non-Triticum nude cereals (group 3)*

It can be stated that remarkable differences occurred between the averages. Also, the differences show close correlation with taxonomical distances of the species and varieties examined.

## Conclusions

Evaluating the results obtained it can be concluded that it is necessary to have reference values not only for each species but for every variety.

The differences in pattern of averages seem to be correlated with taxonomical distances of the crop varieties examined.

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16<sup>th</sup> Alps-Adria Workshop

Synergism in science

# Effect of salinity on rice (*Oryza sativa* L.) in seedling stage

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Abstract: Salinity is an important limiting factor that decreases the yield of rice (Oryza sativa L.) significantly. The breeding of new varieties for salt tolerance can be the most efficient way of adaptation. Germination, early seedling and reproductive stages are critical growth phases. A hydroponics screen (Yoshida medium set to 12dS/m) developed by the International Rice Research Institute was used to test Hungarian ('Dáma', 'Dunghan Shali', 'Janka', 'M 488', 'Risabell') and international ('Nembo', 'Sprint', 'IE-5593', 'Dular', 'Unggi-9') rice varieties. Parameters of germination, biomass production and special plant physiological characters were investigated. In the early seedling stage (3-4 leaves), biomass production of 'Dunghan Shali' (3.62 g per 20 plants) and 'Dular' (3.32 g per 20 plants) was measured as highest, while 'Nembo' and 'Sprint' produced only 1.53g and 1.44g per 20 plants, respectively. The chlorophyll content of the varieties was observed between 0.71 g/1g fresh weight ('Unggi-9') and 0.41 g/1g fresh weight ('Sprint').

**Keywords:** salt stress, rice, biomass, chlorophyll

## Introduction

Plant growth responses to salinity vary with plant life cycle. Critical stages are germination, seedling phase and flowering (Flowers 2004). Criteria for evaluating and screening salinity tolerance in crop plants vary depending on the level and duration of salt stress and the plant developmental stage (Shannon 1985). In general, tolerance to salt stress is assessed in terms of biomass production or yield compared to non-stress conditions. In conditions of low to moderate salinity, the production capacity of the genotype is often the most pertinent measure, whereas survival ability is often used at relatively high salinity levels (Epstein *et al.* 1980).

Abiotic stress tolerance, especially salinity stress, is a complex trait, because of variation in sensitivity at various stages during the life cycle. Rice is comparatively tolerant to salt stress during germination, active tillering (vegetative growth) and maturity. It has the most sensitive reactions during seedling (3-4 leaf) and reproductive stages (booting). Screening at early growth stage (2–4 weeks) is more advantageous than at flowering. This is due to the fact that it is (1) quick, (2) seedlings take up less space and (3) tolerant seedlings may be recovered for seed production. Therefore (4) seedling tests are more efficient in terms of time and costs.

The major inhibitory effect of salinity on plant growth and yield has been attributed to: 1) osmotic effect 2) ion toxicity 3) nutritional imbalance leading to reduction in photosynthetic efficiency and other physiological disorders. The aim of the present study is to provide information on the effect of salinity on pigment concentration and biomass production.

## Materials and methods

After our previous study, when effects of salt stress at germination phase were examined (Székely *et al.* 2016), physiological parameters under high salinity during vegetative stage were investigated. Therefore, we used the salt tolerance screening manual of the International Rice Research Institute (Los Banos, The Philippines). The method is simple

to use. Ten rice varieties (nine *japonica* varieties ('Dunghan Shali', 'Risabell', 'M 488', 'Janka', 'Dáma', 'Nembo', 'Sprint', 'Unggi-9' and 'IE 5593') and one *indica* ('Dular') were chosen to test salt tolerance. The varieties were grown on Yoshida solution in greenhouse for two weeks. Salt stress was induced by the addition of NaCl to 8 dS/m conductivity (EC) for one week. Final EC was set to 12 dS/m. 20 plants of every variety were used in one replication.

The number of parallels was four. Pigment concentration was estimated on four individual plants after two-week period of stress imposition. A median segment was collected on each youngest leaf. We determined biomass production and content of photosynthetic pigments (chlorophyll a and b, anthocyanins and carotenoids).

According to Krishnan (1996), the best method to extract total chlorophyll is incubation with 80 % acetone; therefore we used Lichtenthaler's method (1987).

The extraction of chlorophyll was made in two phases. We added 1.5 ml 100 % acetone to 25mg piece of leaf and incubated for 24 hours. Then samples were centrifuged (15000 rpm), the supernatant was put aside and 1.5 ml 80 % acetone was added again for 24 hours. At the end of experiment, the extract liquid was made up to a total volume of 6 ml with 80 % acetone.

The absorbance was measured by photometer (Hach DR 4000) at four wavelengths: 470, 534, 643 and 661 nm. The pigment content was calculated by the following formulas:

Anthocyanin = 0.0821*A534 - 0.00687*A643 - 0.002423*A661

Chlorophyll b = 0.02255*A643 - 0.00439*A534 - 0.004488*A661

Chlorophyll a = 0.01261*A661- 0.001023*A534 - 0.00022*A643

Carotenoids = (A470 – 17.1*(Chl a + Chl b) – 9.479*Anthocyanin) / 119.26

## Results and discussion

Morphological parameters such as shoot fresh weight and shoot dry weight are correlated with crop salt tolerance at early growth stages and can be used as an indicator for salt tolerance (Noreen and Ashraf 2008).

The results of salt tolerance screening of rice varieties at the seedling stage are shown on the Table 1. 'Dunghan Shali' and 'Dular' had the highest dry weight regarding shoot development (3.62 g and 3,32 g/20 plants), while 'Nembo' and 'Sprint' produced only 1.53 g and 1.44 g per 20 plants, respectively.

The difference is twice as much. In case of root formation 'Dular' developed the biggest root system while 'Nembo' and 'Sprint' were the most undeveloped ones. The difference is quadruplicate.

This is very interesting, because in our previous study (Székely *et al.* 2016) we confirmed that 'Dular' is the most sensitive variety at germination phase under salinity. Dry matter content was observed in a range of 24.15 % ('Janka') and 29.95 % ('Dáma').

*Table 1. Biomass and pigment content of ten rice varieties under saline condition. Biomass was calculated as an average of 20 plants (g). Fw – fresh weight, DW – dry weight. Anthocyanins, Chlorophyll and Carotenoids are given in µg/g fresh weight*

|  |  | FW | DW | Dry matter | Anthocyanin | Chlorophyll a+b |
|---|---|---|---|---|---|---|
|  |  | Av± SD | Av± SD | Av± SD | Av± SD | Av± SD |
| D.Shali | shoot | 14.90±3.12 | 3.62±0.36 | 24.61±2.73 | 293.82±31.36 | 1004.12±55.59 |
|  | root | 9.60±0.60 | 0.79±0.10 | 8.17±0.57 |  |  |
| M-488 | shoot | 7.89±0.31 | 2.00±0.09 | 25.34±0.09 | 178.06±10.31 | 1126.83±65.38 |
|  | root | 5.28±0.43 | 0.47±0.02 | 8.92±1.11 |  |  |
| Janka | shoot | 9.96±0.57 | 2.41±0.42 | 24.15±2.87 | 165.69±11.35 | 859.74±3.21 |
|  | root | 7.79±2.20 | 0.70±0.13 | 9.17±0.92 |  |  |
| Dáma | shoot | 8.39±0.97 | 2.50±0.02 | 29.95±3.19 | 192.15±5.06 | 1201.75±112.33 |
|  | root | 6.89±0.30 | 0.65±0.02 | 9.46±0.64 |  |  |
| Risabell | shoot | 7.25±0.97 | 1.84±0.31 | 25.38±0.86 | 295.86±37.28 | 1031.67±76.31 |
|  | root | 4.24±1.10 | 0.43±0.09 | 10.12±0.44 |  |  |
| Dular | shoot | 13.74±0.15 | 3.32±0.18 | 24.17±1.02 | 352.14±17.90 | 1226.58±92.39 |
|  | root | 12.42±0.08 | 1.29±0.00 | 10.35±0.10 |  |  |
| Sprint | shoot | 5.37±0.72 | 1.45±0.13 | 27.00±1.21 | 300.87±46.12 | 820.86±33.88 |
|  | root | 3.10±0.32 | 0.32±0.03 | 10.32±0.02 |  |  |
| IE-5593 | shoot | 11.47±1.19 | 2.79±0.12 | 24.37±1.46 | 435.25±20.05 | 948.99±30.04 |
|  | root | 8.15±1.30 | 0.74±0.11 | 9.26±2.82 |  |  |
| Nembo | shoot | 5.20±0.10 | 1.53±0.07 | 29.50±0.86 | 425.96±40.46 | 975.37±83.45 |
|  | root | 3.65±0.59 | 0.36±0.04 | 9.78±0.51 |  |  |
| Unggi-9 | shoot | 8.09±0.74 | 2.20±0.13 | 27.22±0.86 | 326.57±21.89 | 1426.34±84.90 |
|  | root | 5.12±0.79 | 0.50±0.08 | 9.78±0.14 |  |  |
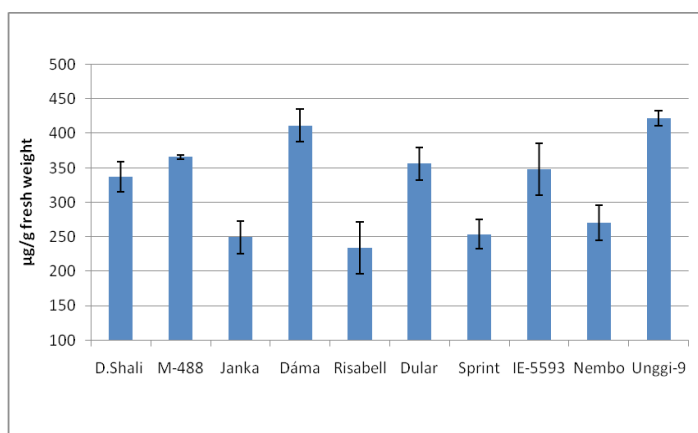


*Figure 1: The carotenoid content of rice varieties under salt stress conditions during the early vegetative stage (µg/g fresh weight).*

Anthocyanins and carotenoids are parts of antioxidant system of plants. The data showed a large range of pigment concentrations. High level of anthocyanins was observed 'IE-5593' (435.25 µg/g fresh weight), while 'Janka' has just 165.69 µg/g fresh weight. Comparison of different genotypes shows that 'Unggi-9' had highest total chlorophyll content and 'Sprint' and 'Janka' had the lowest value. The maximum of carotenoids under saline condition was recorded in 'Unggi-9' and 'Dáma' as 422.27µg/g and 411.59µg/g, respectively (Figure 1). Minimums were calculated in 'Risabell', 'Janka' and 'Sprint'.

## Conclusions

This paper has focused on growth parameters and pigment content of rice seedlings under salt stress conditions and shows that tolerant rice varieties had higher pigment content, while the sensitive ones had lower pigment content. 'Dular' was characterized as the most tolerant variety at seedling stage; 'Sprint' and 'Nembo' were the most sensitive ones. 'Dular', 'Dunghan Shali' and 'IE-5593' produced the highest biomass (shoot and root). 'Dular' had high level of anthocyanin, total chlorophyll and carotenoid content, while 'Dunghan Shali' had only average pigment content. High level of anthocyanin was measured in 'IE-5593' and 'Nembo'. Further testing requires identifying the background of high pigment contents of 'Unggi-9' (high chlorophyll and carotenoid).

## Acknowledgement

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# Performance of rice varieties under aerobic conditions in Hungary

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Abstract: Drought tolerance is an important trait of rice breeding because mainly in rainfed systems, plants must cope with water scarcity from time to time. But it can also be important in the temperate zone. In Hungary, an aerobic rice growing system was developed and the breeding of aerobic rice varieties was started in the 1990's. Successful rice growing under aerobic conditions requires drought tolerant varieties, uniform sowing, weed management and irrigation set to the crop water requirements. Yield and quality of the paddy rice in aerobic systems can be the same as in conventional production. In our experiment, performance of 15 aerobic rice genotypes from the International Rice Research Institute was compared to the Hungarian bred varieties in the Lysimeter Research Station of the NAIK ÖVKI as part of the IRRI INGER nursery program. However, some of the physiological parameters (RWC) of international drought-tolerant varieties were measured superior to the temperate varieties, photoperiodic sensitivity, duration and cold sensitivity limit the direct adaptation under the temperate climate.

Keywords: aerobic rice, Oryza sativa L., drought tolerance, temperate climate

## Introduction

In Hungary, first steps of scientific research on aerobic rice cultivation were begun in the 1940s. The pioneer experiments were carried out to find appropriate varieties for the sandy soils of the Great Hungarian Plain and not to develop water saving technologies. The second phase of aerobic rice research was started in 1984 and a new water-saving rice growing method (Sanoryza) was patented in 1992 (Simon-Kiss 2001). With that method, in favourable years irrigation can be decreased even up to 78 %, but the average amount of saving is also 45-70% compared to the conventional rice production by using of special aerobic genotypes in Hungary (Jancsó 2011). Nowadays, water for agriculture is becoming increasingly scarce and rice is one of the major consumers of irrigation water. The 79 million ha of irrigated rice worldwide is estimated to receive 34-43 % of the total world's irrigation water (Bouman *et al.* 2007). Therefore, water is an important factor for rice production. When water deficit occurs in sensitive periods of plant development, like near the flowering, rice yield is dramatically reduced. Primarily, increased spikelet sterility is a main symptom (Lafitte 2002). However, relative water content (RWC) is an easy way to determine the physiological status of rice plants exposed to different levels of drought; the correlation is strongly influenced by genetic differences in yield potential and maturity (Lafitte 2002). Selection for drought tolerance, especially under the temperate climate is very complex (Courtois *et al.* 2012), because plants are usually exposed to multiple stressors (drought, salinity, low temperature, mechanical damage, etc.) (Sulmon *et al.* 2015). Among all, leaf rolling, leaf drying, harvest index, biomass yield, relative water content, panicle length, grains per panicle, grain yield, root/shoot ratio and root length offer high scope for improvement for drought tolerance by way of simple selection technique (Manickavelu *et al.* 2006). Hungary is one of the northernmost countries for rice growing in Europe; therefore optimal sowing date and cold tolerance are important factors of successful rice production in the relatively short growing season (Gombos and

Simon-Kiss 2008). To develop better selection methods and models, duration of periods from sowing to emergence at different temperature values and base temperatures were determined and a thermal time model was established (Gombos and Simon-Kiss 2005). However, Hungarian varieties e.g. HSC-55 has high tolerance to cold (Ye *et al* 2009), further improvement of the genotypes and testing of international breeding lines are important to maintain rice production under the temperate climate (Simon-Kiss 2001).

## Materials and methods

The experiments on aerobic rice production were set up at the Lysimeter Research Station of the National Agricultural Research and Innovation Centre, which is located in Szarvas (south-east Hungary, latitude 46°86'N, longitude 20°52'E). The Station was built in the year of 1971 when 320 non-weighable backfilled gravitation lysimeters were set up on a 1 hectare experimental field. For the aerobic production, plots between lysimeter blocks were used in 2016. A modified version of Sanorzya aerobic rice production system (Simon-Kiss et al. 1992) was applied for the field management. From the seedling stage, drip irrigation was set up. Source of irrigation water was the oxbow of Körös-river. Meteorological data was automatically collected at the meteorological station of the Szarvas Campus of Szent István University and by a WS-GP1 Compact Weather Station (Delta-T Devices, UK) during the growing season (Table 1). However, rainfall was low in April, May and September. In the period of intensive vegetative growth (from June to the beginning of August), quantity and distribution of natural precipitation was close to the optimal.

*Table 1. Monthly rainfall (mm), air temperature (°C) and soil temperature at 5 cm (°C) in the cropping season in Szarvas in 2016, average temperature data and total precipitation for the season are also shown.*

|  | April | May | June | July | Aug | Sept | Oct | AV | Total |
|---|---|---|---|---|---|---|---|---|---|
| Mean min temp., °C | 7.4 | 10.1 | 15.6 | 16.4 | 15.0 | 12.3 | 6.1 | 11.8 | - |
| Mean max temp. , °C | 19.8 | 23.6 | 27.5 | 29.1 | 27.9 | 26.0 | 14.6 | 24.1 | - |
| Average mean temp. , °C | 13.4 | 16.6 | 21.3 | 22.5 | 21.1 | 18.3 | 9.8 | 17.6 | - |
| Precipitation, mm | 12.3 | 18.8 | 124.4 | 124.4 | 50.5 | 9.8 | 72.7 | - | 412.9 |
| Mean soil temp. at 5cm, °C | 13.8 | 17.4 | 22.6 | 24.0 | 23.2 | 20.0 | 12.4 | 19.0 | - |

New breeding lines and standard varieties (15) for aerobic rice cultivation (Table 2) from the International Rice Research Institute (IRRI, Manila, The Philippines) were compared to the Hungarian bred varieties as part of the IRRI International Network for Genetic Evaluation of Rice (INGER) nursery program (Forty-Second International Upland Rice Observational Nursery, IURON). The experiment was started with the direct dry sowing that was done on the 5th of May 2016. After a preemergent herbicide treatment, first irrigation was started on the 6th of May (20 mm) to accelerate germination. Due to the cold periods in May date of emergence was observed on 20th of May and 25-26th of May for the Hungarian check and the IURON lines, respectively.

The symptoms of cold and drought stress were evaluated after the international standard evaluation system (IRRI 2013). In our experiment (Table 2), tolerant (SES score 2-3 –

tip of leaves slightly dried, folded and light green), moderately tolerant (SES score 4-5 – some seedlings moderately folded and wilted, 30-50% seedlings dried, pale green to yellowish leaves) and sensitive (SES score 6-7 – seedlings severely rolled and dried; reddish-brown leaves) reactions to low temperature were observed after a cold period in May. Relative water content (RWC) of the varieties was measured and calculated as Lafitte (2002) described.

The variation of soil moisture content and soil temperature at 15 cm (Sensor1, Temp1) and at 30 cm (Sensor2, Temp2) below the surface were logged in every 10 minutes of the season (Figure 1) by using of SM300 soil moisture sensors connected to a GP1 data logger (Delta-T Devices, UK). Soil moisture and drought symptoms were regularly checked and irrigation was applied as the crop needs (sensitive checks Beside the agronomic parameters (yield, grain weight, thousand grain weight, plant height) changes in chlorophyll content using SPAD-502 hand-held chlorophyll meter (Minolta Co. Ltd., Osaka, Japan) were also detected.

**Results and discussion**

The rice growing season in 2016 was divisive in Hungary, after a cold period in May (Table 1) the quantity and distribution of natural precipitation was close to the optimal.

*Table 2 Scoring of cold tolerance (after IRRI 2013) and the recovery of the plants described by SPAD values (Szarvas, Hungary, 2016)*

| Breeding line | SES Score of cold tolerance | SPAD readings, 16.06.2016 | Average yield per parcel, g/m² |
|---|---|---|---|
| IR14L594 | 4-5 | 27.1±3.8 | n.p. |
| IR14L176 | 4-5 | 25.3±1.7 | 144.5 |
| IR14L226 | 2-3 | 27.3±1.2 | 4.0 |
| IR14L231 | 2-3 | 26.9±1.8 | 25.0 |
| IR14L537 | 6-7 | 28.4±1.2 | n.p. |
| IR14L540 | 4-5 | 34.0±2.9 | n.p. |
| IR14L546 | 4-5 | 30.2±2.0 | n.p. |
| IR14L560 | 4-5 | 31.8±3.1 | n.p. |
| IR14L562 | 6-7 | 29.7±1.3 | n.p. |
| IR14L572 | 6-7 | 28.2±1.8 | n.p. |
| IR64 | 4-5 | 28.5±2.0 | n.p. |
| IRRI 132 | 4-5 | 22.3±1.7 | n.p. |
| IRRI 148 | 4-5 | 23.1±1.3 | n.p. |
| UPL RI 7 | 4-5 | 20.7±1.9 | n.p. |
| VANDANA | 4-5 | 29.8±2.1 | sterile spikelets |
| JANKA* | 2-3 | 30.4±1.4 | 592.8 |

* Local check, Nucleoryza DH somaclone; n.p. – no heading stage

In the fourth pentad of May, effective temperature was calculated only as 14.8 °C with the minimum temperature of 3.0 °C. However, it has a negative effect on the cold sensitive plants (e.g. rice, sorghum), from the experimental view, on-field cold tolerance could be easily scored. On most of the tested breeding lines, symptoms of moderate tolerance were observed as it was determined in the SES (IRRI 2013). However, 'IR14L537', 'IR14L562' and 'IR14L572' genotypes were set as sensitive lines where seedlings were severely rolled and dried and the leaves had reddish-brown colour right after the cold period. However, the effects of low temperature were observable even after a two-week period of optimal growth temperature on 16th of June (Table 1). The SPAD readings were shown that varieties what were scored as moderately tolerant ('IRRI 132', 'IRRI 148' and 'UPL RI 7') could have long-term damages.
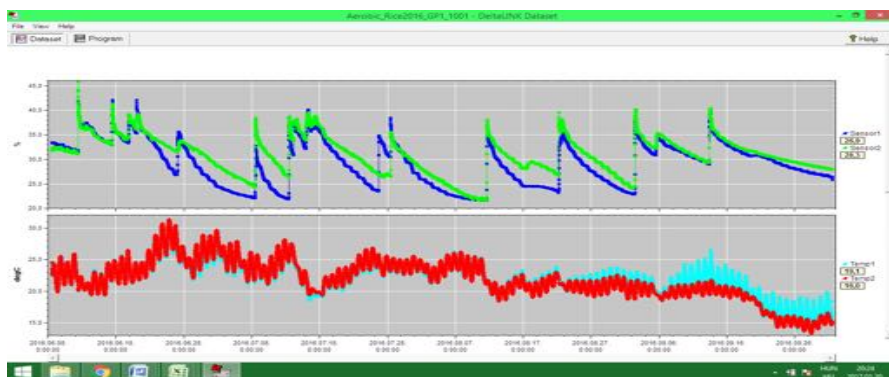
*Figure 1 Variation of soil moisture content and soil temperature at 15 cm (Sensor1, Temp1) and at 30 cm (Sensor2, Temp2) below the surface. (Szarvas, Hungary, 2016)*

Soil moisture content was determined during the season. Irrigation was applied only 5 times as an average of 20 mm (Figure 1). The overall season was favourable for the plants' development (Figure 1). Compared to our previous results (Jancsó 2011), water saving was significant (85%) compared to the paddy fields. Middle of September was the end of the season when soil temperature went below 10 °C.
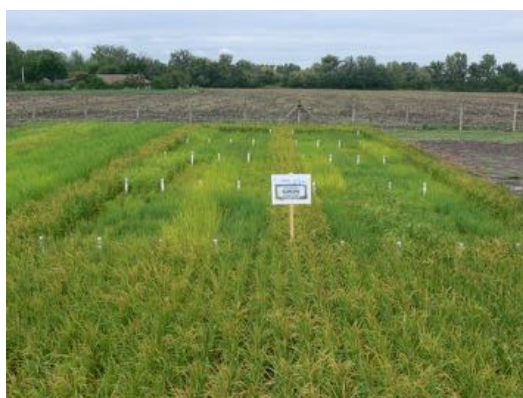


*Figure 2 Aerobic rice experiment after the flowering of 'Janka' on 11th August (Szarvas, Hungary, 2016)*

As part of the experiment, standard varieties were also tested for drought tolerance. Symptoms of drought were observable first on the sensitive plants ('Marilla'). Leaf rolling was significant when soil moisture went below 25-30 % depending on the developmental stage of plants and the air temperature (Figure 1). RWC was determined multiple times during the season. RWC at well-irrigated (29.06) and water-limited (07.07) conditions are also presented (Table 3).

Compared to the results of Lafitte (2002), moderate stress environment was maintained in our nursery field. Sensitive genotypes as the 'Marilla' (87.8±3.5) had lower RWC values, while drought tolerant varieties as 'IRAT 109' (91.2±0.6) and 'Ábel' (91.8±1.0) maintained RWC close to the optimum.

*Table 3 Relative water content (RWC) of Hungarian rice varieties under well-irrigated (29th of June) and under drought prone (7th of July) conditions (Szarvas, Hungary, 2016)*

| Name of varieties | RWC on 29th of June | RWC on 7th of July |
|---|---|---|
| Ábel | 92.4±0.7 | 91.8±1.0 |
| Janka | 94.1±0.5 | 87.5±3.7 |
| Bioryza H | 93.0±0.5 | 89.3±1.7 |
| IRAT 109 | 93.6±1.3 | 91.2±0.6 |
| Marilla | 93.9±0.8 | 87.8±3.5 |

Plots were harvested manually when grains were fully matured. Hungarian varieties were collected at the beginning and middle of September to avoid damage of rodents. International genotypes were harvested when development of plants was stopped due to the low temperature at the end of September. Beside 'Janka', only 'IR14L176', 'IR14L226' and 'IR14L231' produced yield that was very low (Table 2). Our results verified as it was reported by multiple authors (Bouman *et al.* 2007, Courtois *et al.* 2012, Sulmon *et al.* 2015) that in the temperate zone, short growing season (May to September), the long days during most of the growing season, and the low temperatures are also limiting factors. Aerobic rice cultivars need to integrate good tolerance to diverse stressors.

## Conclusions

Drought tolerance is an important trait of rice breeding because mainly in rainfed systems, plants must cope with water scarcity from time to time. But it can also be important under the temperate zone. In our experiment, performance of 15 aerobic rice genotypes from the International Rice Research Institute was compared to the Hungarian bred varieties at the Lysimeter Research Station of the NAIK ÖVKI. Even moderate stress environment could lower the yield performance. Cold resistance is important because in Hungary, low temperature can be occurred in May and in August. In 2016, effective temperature was calculated only as 14.8 °C with the minimum temperature of 3.0 °C in the fourth pentad of May. Therefore, symptoms of cold stress were observable on young plantlets. Moreover, RWC is a good and relative fast method to measure reaction of different plants to drought. However, selection for drought tolerance, especially under the temperate climate is very complex. Photoperiodic sensitivity, duration and cold sensitivity limit the direct adaptation of tropical aerobic varieties under the temperate climate.

## Acknowledgement

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# The marigold's (*Calendula officinalis L.*) drug yield and economic value changes over time and composition of the essential oil active agents under different fertilization settings

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**Abstract:** While we investigated the marigold's (*Calendula officinalis L.*) nutrient requirements in small-plot trial we measured the raw and the dry drug yield, which we harvested in 2015 from 6th July to 17th August and in 2016 from 16th June to 1th August weekly. Thanks to the multiple picking the data we got, we investigated the effect of the different fertilization settings the flower drug's changes over time. The drug crop's change over time could show how many times it is economical to harvest under different fertilization settings. We monitored the harvested marigold's drug's raw and dry mass on a weekly basis. It was concluded, based on the results, in 2015 the biggest raw drug crop in the N30P40K60 fertilization setting and in 2016 in the N75P100K150 setting were measured. Both the raw and the dry mass' measurements of the case, this fertilization settings have the most important effect on the herb's yield. In the measurements of active ingredients we used SPME and GC-MS. We examined the effects of the different fertilization settings for the herb's main active ingredients of essential oil's percentage.

**Keywords:** herb, drug, nutrient supply, marigold, active agent

## Inroduction

The *Calendula officinalis L.* (marigold, gold-bloom, Chinese safflower (WHO, 1999) ) is a mediterranean annual plant (Rápóti and Romváry, 1987). The drug of this medicinal plant is the flower (Dános, 2006). The essential oil is the 0.1 % percent of the total active ingredient content. (Bernáth, 2000). Because of it's high E vitamin content mainly it is used for healing of the skin (Varró, 2011). For horses' the marigold could be used the follow-up care of fractures, bruises and sprains. Internally used to treat stomach ulcers (Marton, 2005).

Research is under the way for a stable LGP (lamellar gel phase) emulsion with using marigold, which could be an alternative to facilitate the healing of wounds (Okuma et al., 2015). Under diabetes, because of the high blood sugar the non-enzymatic glycation of the proteins speed up, and the end products are accumulate int he body. This promotes the typical complications of diabetes, and leading to arterial stiffness that decreases myocardial compliance and aging. The marigold had greater antioxidation potential, which could inhibit these two reactions.(Ahmad et. al, 2012) The diabetic patients limbs' micro traumas are constant possibility of infections, which could lead to amputation. The marigold balm stopped the formation and progression of infections reduced the itching, the redness, the dryness, and the pain. Contributed to the disappearance of scars, while the risk of allergy to the plant is very low (Cioinac, 2016). Under our research we analysed the nutrient requirements and fertilizer reactions of marigold according to the change in the drug yield and the essential oil components distribution, as an effect of the different nutrient dosages. It has been established – under investigations - the different fertilization settings has not got significant influence on the marigold's flavonoids, but the amount of light and the plants' age (Fernandes et al., 2013). The marigold's essential oils' typical

agents – which we investigated - are the terpenes. They have got antifungal antibacterial and antiviral effects (Banai, 2005).

**Materials and methods**

Our experiment took place in the experiment site of the University of Debrecen, Institute of Crop Sciences. The experimental place's soil is chernozem. In the previous year, before our research could be planned, the regular annual nutrient dosages were spread on the land. The nutrient supply necessarily affected the yield. Plot size was 8 $m^2$ and plots were arranged in 4 replicates in randomized blocks, with 6 different fertilizer treatment levels, in 4 rows with 40 cm row space. In 2015 and 2016, sowing took place on the spot on 7th April and on 4th April, in 1 cm depth. The fertilizer doses:

- N0P0K0 (Control)
- N15P20K30
- N30P40K60
- N45P60K90
- N60P80K120
- N75P100K150                    N%, $P_2O_5$%, $K_2O$%

The rainfall on the experimental area in 2015 from 1st January to 30th September was considerably less (286.2 mm) than the 30 year average (445.8 mm). From January till the end of September the average temperature of each month were higher than the 30 year average, except April. in 2016 the rainfall from 1st January to 31th August was considerably more ( 574.9 mm) than the 30 year average. From the 1st January to 31th August in 2016, the measured monthly mean temperature was higher than the 30 year average. We measured the drug yield which, in this case, was the quantity of the raw and the dry drug. Gathering was done 6 times manually between 6th July and 18th August 2015 and 7 times in 2016 between 16 th June and 1 th August. Analysis of the essential oil components was carried out by applying solid phase microextraction (SPME), then gas chromatograph-mass spectrometer (GC-MS). We used HP (Hewlett-Packard) 5890 Series II type gas chromatograph and 5971A type mass spectrometer. Components were identified by applying mass spectrums and Nist98 and Wiley databases. Active agents of the samples taken from each plot were analysed. During processing of the gained data, variance analysis and Pearson's correlation analysis were applied by using MS Excel 2010 and IBM SPSS 22.0 programmes.
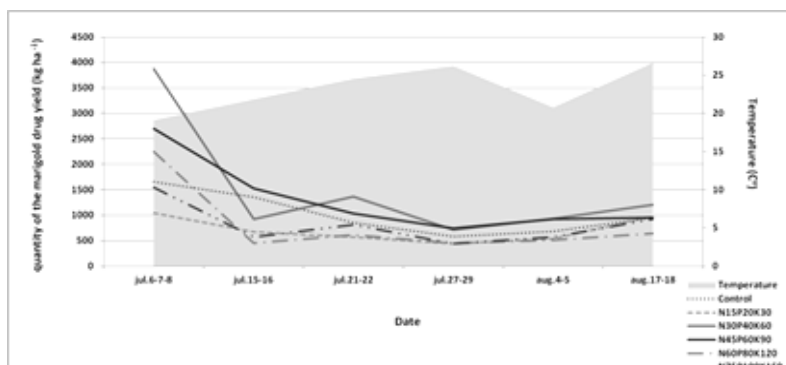


*Figure 1.The connection between the marigold raw drug yield and the average temperature in 2015 (Debrecen, 2015)*

## Results and discussion

*Figure 1.* shows the relationship between the marigold raw drug yield and the average temperature in 2015. Considering the quantity of the raw drug yield, the plots with N30P40K60 had the most favourable nutrient setting, followed by the results of the plots with N45P60K90, then that of the control groups. During the harvest all of the plots' yield decreased. Between 21th and 22th July and between 17th and 18th August we measured a weak growth which was the biggest in N30P40K60 fertilizer treatment. The temperature
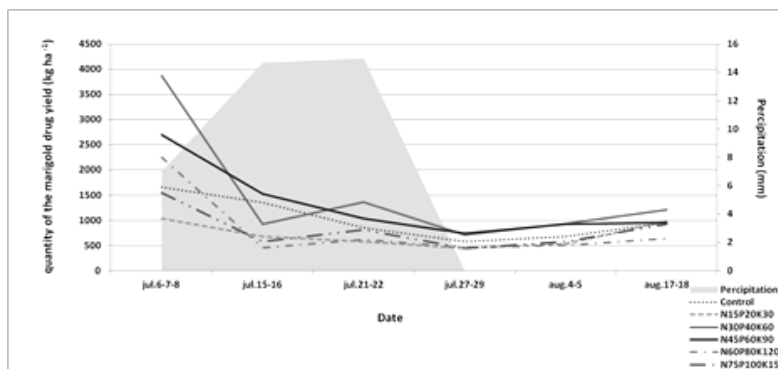


*Figure 2.The connection between the marigold drug yield and the percipitation in 2015 (Debrecen, 2015)*

except in 4th and 5th August grown.We investigated the relationship between the raw drug yield and the temperature with Pearson's correlation test. We measured that, there is a negative medium connection between them (r= - 0.34, P=1%).
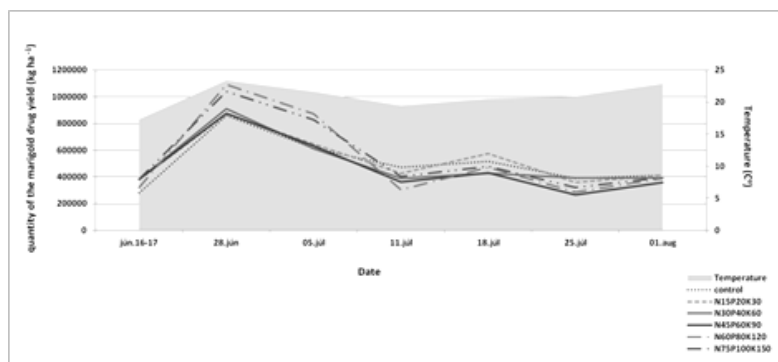


*Figure 3.The connection between the marigold drug yield and the average temperature in 2016 (Debrecen, 2016)*

*Figure 2.* shows the relationship between the marigold raw drug yield and the percipitation in 2015. From the first harvest (6th-7th-8th July) the percipitation approximately doubled. Then the rainfall start to decrease and scheduled for 27th July loss. We measured only a very weak not significant correlation between the the raw drug yield and the precipitation (r=0.106).

*Figure 3.* shows the relationship between the marigold raw drug yield and the average temperature in 2016. In 2016 the N75P100K150, the N60P80K120 and the 15P20K30 fertilization setting has the biggest effect to the raw drug yield, and the N45P60K90 setting has the weakest effect on
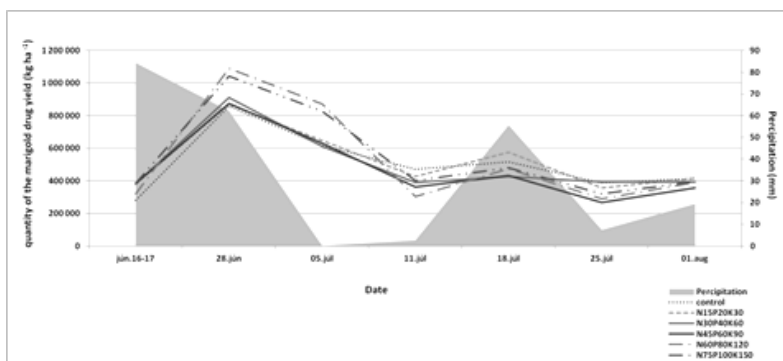
*Figure 4.The connection between the marigold drug yield and the percipitation in 2016 (Debrecen, 2016)*

the full raw yield. During the harvest the plots' yield firstly increased. We measured the highest yiel in the harvest of 28th June. Then the quantity of the yield start to decrease. There were a slight increase again on 18th July, but the yield reduction then continued. Until 11th July the N60P80K120 and the N75P100K150 settings' yields reduced least.We found a positive medium correlation (r=0.39, P=1%) between the raw drug yield and the temperature.

*Figure 4.* shows the relationship between the marigold raw drug yield and the percipitation in 2016. From 5th July to 11th July there was a minimum in rainfall. After this period, the percipitation increased until 18th July. The next minimum int he rainfall was in 25th July and then started a new increase. The Pearson's correlation test showed a very weak, not significant relationship between the raw drug yield and the percipitation (r=0.08).

During the joint analysis of the 2015 and 2016 years datas we measured a medium correlation (r=0.38, P=1%) between the raw drug yield and the percipitation. There were a negative weak relationship (r= - 0.18, P=1%) between the marigold raw yield and the average temperature.
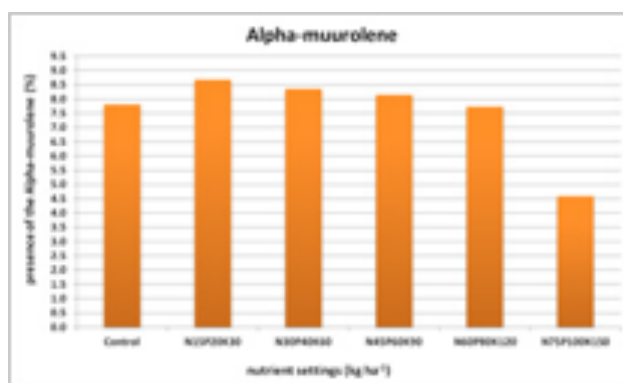


*Figure 5* Presence of the Alpha- muurolene in the drug of marigold depending on the nutrient supply (Debrecen, 2015)

During the harvest, the increase of the number of the harvests, the yield was decrease – the experienced relationship between the number of the harvests and the drug yield is negative but not significant (r= - 0.10). We made a variance analysis between the data of the drug yield and the plots with different fertilizer treatments, but it did not show significant differences.

We analysed the marigold drug's essential oil active agents percentage presence depending on the increases of the applied nutrient doses. These active agents, which we identified are mostly belong to the group of terpenes. We analysed the presence of the Alpha-muurolene and the Gamma-muurolene.

The highest presence of the Alpha-muurolene what we measured was in the N15P20K30 nutrient setting. It was reported a higher percentage from this agent in the N15P20K30,
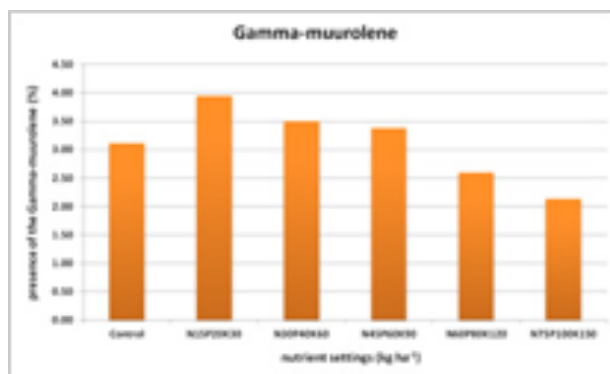


*Figure 6* Presence of the Gamma- muurolene in the drug of marigold depending on the nutrient supply (Debrecen, 2015)

N30P40K60 and the N45P60K90 than in the Control group. Hereinafter the presence of the Alpha-muurolene was decreased with the increasing of the applied nutrient doses (*Figure 5.*). We also used the Pearson's correlation test to analyse the relationship between the presence of the essential oil active agents and the increasing nutrient settings. In the case of the Alpha-muurolene we found strong negative, but not significant correlation (r= - 0,79).

We also measured the highest presence of the Gamma-muurolene in the N15P20K30 nutrient supply. As well a sin the case of the Alpha-muurolene, in the N15P20K30, N30P40K60 and the N45P60K90 were reported higher percentage than in the Control group. Then the following groups have occurred the decrease of the Gamma-muurolene.
The correlation test showed a strong medium negative, not significant relationship between the presence of the Gamma-muurolene and the increasing of the nutrient settings (r= - 0,69).

**Conclusions**

Based on the correlation analysis in 2015 there were a negative relationship between the raw marigold drug yield and the temperature (r= - 0.34, P=1%), and a weak positive between the raw marigold drug yield and the percipitation (r=0.106). In 2016 there were a positive medium correlation between the raw marigold drug yield and the temperature (r=0.39, P=1%), and a very weak positive between the raw marigold drug yield and the percipitation (r=0.08). Analyzing the two years' datas together we find a negative medium correlation between the raw marigold drug yield and the temperature (r=0.38, P=1%), and a weak positive between the raw marigold drug yield and the percipitation (r= - 0.18, P=1%).

Because of these correlation results, and the variance analysis's not showed significance, in our opinion the changes of the temperature and the rainfall are more effective for the marigold's drug yield than the increasing nutrient settings. This phenomenon can influence

the changes of the harvests' number, as well as the possible renewal term of the stand. Based on the results of the Pearson's correlation test between the marigold drug's essential oil active agents and the nutrient supplies, we think, the increasing nutrient supply has a negative effect on the presence of the essential oil active agents in the marigold's drug, but until we can not make more tests, we could not say with certainty, there is relationship between the presence of the essential oil active agents and the different nutrient settings.

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# Aluminum-toxicity responses in *Phaseolus vulgaris* L. genotypes

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Abstract: Al-toxicity in acid soils is one of the major problems in crop production worldwide. The acidification of soils is also a serious problem in Hungary. The liming materials are expensive for most small farmers so to reduce farmer's dependence on lime we need to find significant differences in Al-toxicity resistance in plants.

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for people, and it is the second source of protein. Therefore, the examination of common bean in the case of Al-toxicity has great importance. Five genotypes were investigated in this experiment (Apache, Aztec, Bill Z, Fargo, Grand Mesa). Five-day-old uniform seedlings were transferred to 4.5 L pots with constantly aerated simplified nutrient solution containing 0.5 mM $CaCl_2$, 0.5 mM KCl and 8 μM $H_3BO_3$. This solution allows optimum root elongation for short treatment. After 24 h of root growth, the pH of the solution was decreased from 5.5 to 5.0, after 48 h from 5.0 to 4.5 to avoid pH stress. After 3 days, the first 20 mm of the root apex was marked with permanent marker and plants were treated with 0 and 20 μM $AlCl_3$. The distances from the root apex were measured after 4 h, 8 h, 24 h, 48 h and 72 h after treatment. Moreover, relative chlorophyll content, dry weight of root and shoot and enzymatic assays (lipid peroxidation, SOD=superoxide dismutase, POX=peroxidase, ROS=reactive oxygen species) were measured.

According to the measured parameters of root growth and various enzymatic activities, we found that there is a strong genotype effect regarding the bean plant's response to Al stress. Some bean genotypes were less affected by the Al-toxicity stress than other genotypes.

Keywords: aluminium-toxicity, bean, oxidative stress, root growth

## Introduction

Aluminum (Al) toxicity is a major factor limiting plant growth especially on acid soils.

Common bean (*Phaseolus vulgaris* L.) growing area of about 40 % of Latin America and 30-50 % of central, eastern and southern Africa are affected by Al toxicity resulting in yield reduction from 30 to 60 % (CIAT, 1992). The bean is not the most important legumes in Hungary, it is grown only around 750-800 ha in 2015 and the yield was approx 1500 tonnes (KSH, 2015) but it has important role in human nutrition because of its high protein and nutrient content.

Common bean needs significant improvement in Al resistance to reduce farmer's dependence on lime and fertilizers (Rao, 2001). Genotypic differences in seed yield of 5000 common bean germplasm accessions and breeding lined have been observed in field screening on Al-toxic soils that were amended with or without lime (Rao et al., 2004). Significant genotypic differences in Al resistance in common bean were also reported based on Al-inhibited root elongation in nutrient solution (Rangel et al., 2005; Manriquen et al., 2006).

The primary effect of Al is an inhibition of root growth (Foy, 1988), an effect that can be seen within hours of treatment (Blamey et al., 2004). The major site of Al perception and response in the root apex (Ryan et al., 1993), and particularly, the distal part of the transition zone (1-2 mm) is the most Al-sensitive apical root zone (Kollmeier et al., 2000).

Al-toxicity such as one of the environmental stresses induces the formation of reactive

oxygen species (ROS) in plant cells (Breusegem et al., 2001). Under normal physiological conditions, cell produce ROS by means of the reduction of molecular oxygen (Hippeli et al., 1999), but under conditions of environmental stress this production is increased. All cells possess a defensive system, consisting of various enzymes such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and reductase. These enzymes efficiently reduce SOD under normal circumstances, but if complete reduction does not occur, as under conditions of increased production, the result may be a state of oxidative stress leading to the oxidation of biomolecules (e.g. lipids, proteins etc) (Schieber and Chandel, 2014). The main objective of the study was to prove that there is a strong genotype effect of Al toxicity and sensitivity in five common bean genotypes.

**Materials and methods**

Seeds of common bean genotypes Apache, Aztec, Bill Z, Fargo and Grand Mesa from Pinto line were germinated between filter paper, in an upright position. Five-day-old uniform seedlings were transferred to 4.5 litre pots with constantly aerated simplified nutrient solution containing 0.5 mM $CaCl_2$, 0.5 mM KCl and 8 μM $H_3BO_3$. This solution allows optimum root elongation for three days at least.

After 24 h of root growth, the pH of the solution was lowered to 5.0, after 24 h 4.5 and keeps this pH until at the end of the experiment. The experimental design was a completely randomized design with three pots per treatments, each pot contained 4 plants.

Plants were cultured in a growth chamber with controlled environmental conditions of a 16/8-h light/dark regime, 20/15°C day/night temperature and photon flux density of 574 μmol $m^{-2}s^{-1}$ photosynthetic active radiation at the plant level.

Two hours before Al treatment, tap roots were marked 2 cm behind the root tip using permanent marker, which did not affect root growth during the experimental period. Afterwards, the plants were transferred to simplified nutrient solution containing 0 or 20 μM $AlCl_3$. Root elongation was measured at 4, 8, 24, 48 and 72 h of Al treatment using a 1-mm scale. The shoots and roots of common bean were dried at 60 ° C for 3 days and measured with analytical scale.

The enzymes antioxidants analysed in the roots were superoxide dismutase, peroxidise, lipid peroxidation and the amount of ROS. Activity of superoxide dismutase was determined by the method of Misra and Fridovich (1972).

Assay of peroxidise as proposed by Reddy et al. (1995) was adopted for evaluating the activity of peroxidise. For the measurement of lipid peroxidation in root, the TBA test which determines MDA as an end product of lipid peroxidation was used according to Heath and Packer (1968).

To determine the amount of total ROS (reactive oxygen species) 2,7-dichlofluorescein diacetate was used. Protein was measured by the method of Bradford (1976). Microsoft Office Excel 2003 and Sigma Plot 12.0 version were used to the statistical analysis.

**Results and discussion**

The toxic effect of Al is firstly root related. The dry weight of shoot did not changed significantly when plants were treated with 20 μM Al. The dry weight of root decreased significantly at all

examined genotypes (Table 1). The highest decreasing was observed at Apache.

*Table 1. Effect of Al treatment on the shoot and root dry weight of the common bean genotypes(Apache, Aztec, Bill Z, Fargo, Grand Mesa) grown in simplified nutrient solution containing 0.5 mM CaCl$_2$, 0.5 mM KCl and 8 μM H$_3$BO$_3$ without (0 μM) and with 20 μM Al for up to 72 h at 4.5 pH. Significant difference compared to the 0 μM Al treatment: \*\*p<0.01, \*\*\*p<0.001*

| Genotypes | shoot dry weight (g plant$^{-1}$) | | root dry weight (g plant$^{-1}$) | |
|---|---|---|---|---|
| | 0 μM Al | 20 μM Al | 0 μM Al | 20 μM Al |
| Apache | 0.27± 0.03 | 0.27± 0.05 | 0.09± 0.02 | 0.04± 0.01\*\*\* |
| Aztec | 0.26± 0.02 | 0.23± 0.04 | 0.07± 0.01 | 0.04±0.00\*\*\* |
| Bill Z | 0.23± 0.03 | 0.24± 0.04 | 0.08± 0.01 | 0.06± 0.01\*\* |
| Fargo | 0.27± 0.06 | 0.30± 0.03 | 0.09± 0.02 | 0.06± 0.01\*\*\* |
| Grand Mesa | 0.24± 0.02 | 0.25± 0.01 | 0.08± 0.01 | 0.05± 0.01\*\* |

The effect of Al on root-elongation rate is best shown as Al-induced inhibition of root elongation (Table 2). The genotypes showed highly significant difference in response to Al supply. The genotypes were arbitrarily ranked for Al resistance in four categories, based on the percentage of Al-induced inhibition of root elongation. Accordingly, Aztec genotype was classified as Al-hipersensitive (inhibition > 90 %). Apache and Fargo genotypes were classified as Al-sensitive (inhibition between 50 % and 90 %), Grand Mesa is intermediate (inhibition 30 – 50 %) and Bill Z genotype is Al-resistant (inhibition < 30 %). This classification based on 20 μ M Al treatment for up to 3 days, Rangel et al (2005) with modification.

Reduction of root growth is the most widely recognized symptom of Al toxicity (Foy, 1976). The most frequently genotypic differences in Al resistance entailed measurements of root growth between 24 h and 72 h.

In presence of Al root elongation of both genotypes was several inhibited (30-55 %) 4 h after the beginning of the Al treatment. After 8-h treatment, both genotypes recovered, Aztec and Fargo more than Bill Z and Grand Mesa.

*Table 2. Al-induced inhibition of root elongation of five common bean genotypes (Apache, Aztec, Bill Z, Fargo, Grand Mesa) grown in a solution containing 0.5 mM CaCl$_2$, 0.5 mM KCl and 8 μM H$_3$BO$_3$ for 72 h at 20 μM Al, pH 4.5 (n=12)*

| Genotypes | Al-inhibited root elongation % | | | | |
|---|---|---|---|---|---|
| | 4h | 8h | 24h | 48h | 72h |
| Apache | 54.68 | 66.06 | 75.93 | 83.33 | 64.38 |
| Aztec | 61.54 | 72.73 | 78.92 | 86.68 | 90.32 |
| Bill Z | 30.11 | 32.74 | 26.63 | 18.38 | 26.51 |
| Fargo | 39.76 | 70.07 | 78.13 | 76.65 | 80.59 |
| Grand Mesa | 54.24 | 49.64 | 32.18 | 31.15 | 33.71 |

Cumming et al. (1992) proposed that Al resistance is an inducible trait in common bean requiring a period of stress before a resistance mechanism is switched on. In fact, these authors observed an initial decline in the root elongation of an Al-resistant cultivar

followed by a substantial increase after 24 h of Al exposure, while the Al-sensitive cultivar showed a steady decline in the elongation rate over the experimental time. Resistance to Al might be achieved by chelation or detoxification of Al by organic acids, either within the plant (Al tolerant) or in the rhizosphere by root exudation (Al exclusion) (Foy, 1988).

It is proved that exposure to Al could affect production of reactive oxygen species (ROS) in plants because Al stress causes peroxidation of lipids in the plasma membrane, the effect that could be due to ROS and Al induces the expression of several genes encoding antioxidative enzymes such as glutathione S-transferase, peroxidase and superoxide dismutase (SOD). Metals, including Al, are known to act as catalysts in ROS production and to induce oxidative damage in plants (Yamamoto et al., 2002). The activity of SOD is increased in all genotype, the increasing was significant at Fargo and Grand Mesa. There is no connection between SOD and POX activity effected by Al stress. The POX activity significantly increased in Apache, Aztec and Fargo common bean genotypes (Table 2).

*Table 3. Effect of Al on enzymatic activity (SOD, POX, LP) in the roots of five common bean genotypes (Apache, Aztec, Bill Z, Fargo, Grand Mesa) grown in a solution containing 0.5 mM $CaCl_2$, 0.5 mM KCl and 8 µM $H_3BO_3$ for 72 h at 20 µM Al, pH 4.5 (n=4) Significant difference compared to the 0 µM Al treatment: *p<0.05, **p<0.01*

| Genotypes | SOD ($Ug^{-1}$FW) | | POX ($\Delta A436\ g^{-1}$ FW $min^{-1}$) | | LP (nmol MDA $g^{-1}$FW) | |
|---|---|---|---|---|---|---|
| | 0 µm Al | 20 µm Al | 0 µm Al | 20 µm Al | 0 µm Al | 20 µm Al |
| Apache | 0.15± 0.02 | 0.18± 0.02 | 2.58± 0.58 | 3.52± 0.37* | 5.56± 1.31 | 5.05± 0.83 |
| Aztec | 0.14± 0.03 | 0.22± 0.04 | 2.67± 0.21 | 4.13± 0.75* | 19.90± 4.53 | 10.48± 5.30 |
| Bill Z | 0.12± 0.02 | 0.16± 0.02 | 2.60± 0.25 | 2.65± 0.29 | 7.74± 3.42 | 10.76± 3.81 |
| Fargo | 0.16± 0.01 | 0.29± 0.07* | 2.99± 0.18 | 4.65± 0.92* | 28.49± 9.21 | 28.41± 5.65 |
| Grand Mesa | 0.10± 0.02 | 0.16± 0.01** | 0.79± 0.12* | 0.89± 0.01 | 11.42± 1.44 | 37.41± 13.74* |

*(SOD = superoxide dismutase, ROX = peroxidase, LP = lipid peroxidation, FW = fresh weight, MDA = malondialdehyde)*

Although the thiobarbituric acid (TBA) assay is the most extensively used test for the measurement of lipid peroxidation in cell membranes and isolated lipids (Girotti et al. 1985) limitations of the test have been documented (Halliwell and Gutteridge 1990). Lipid peroxidation is less sensitive to Al than the inhibition of root elongation. Therefore, the close relationship between root elongation rate and lipid peroxidation, independent of the factor responsible for growth inhibition suggests that lipid peroxidation is the consequence rather than the primary cause of Al injury to plant roots (Cakmak and Horst, 1991). The amount of malondialdehyde decreased in Aztec when plants were treated with 20 µM Al. This value was around the control in Apache and Fargo. The amount of malondialdehyde significantly increased at 20 µM Al compared to 0 µM Al treatment (Table 3). According to Souza et al. (2002) a short-term Al exposure has an effect on the protein content and expression in maize root. Their results are demonstrated that the total protein content along the root apex was not affected by Al in the Al-tolerant line, but decreased in the sensitive line. To the contrary, we observed different effect of Al treatment on protein content in common bean root. The amount of protein increased when plant were treated with Al, significant increment was observed in all genotypes. The amount of reactive oxygen species was calculated based on protein content (Table 4). The amount of ROS increased in Apache, Aztec, Bill Z and Grand Mesa genotypes.

*Table 4. Effect of Al toxicity on protein content and ROS in the roots of five common bean genotypes (Apache, Aztec, Bill Z, Fargo, Grand Mesa) grown in a solution containing 0.5 mM CaCl$_2$, 0.5 mM KCl and 8 μM H$_3$BO$_3$ for 72 h at 20 μM Al, pH 4.5 (n=5) Significant difference compared to the 0 μM Al treatment: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001*

| Genotypes | protein (mg protein/FW) | | ROS (RFU μg protein) | |
|---|---|---|---|---|
| | 0 μm Al | 20 μm Al | 0 μm Al | 20 μm Al |
| Apache | 6.36± 0.23 | 7.67± 0.35\*\*\* | 77.43 | 102.66 |
| Aztec | 10.02± 0.88 | 14.58± 2.07\*\* | 4.03 | 43.59 |
| Bill Z | 7.19± 0.30 | 11.02± 0.46\*\* | 36.40 | 56.39 |
| Fargo | 8.57± 0.42 | 12.00± 2.45\* | 59.81 | 38.51 |
| Grand Mesa | 8.71± 0.43 | 9.87± 0.38\*\* | 67.77 | 71.45 |

*(ROS = reactive oxygen species, RFU = relative fluorescence unit)*

## Conclusions

Al$^{3+}$ solubilized in acidic soil is extremely toxic in terms of root elongation, and is believed to be the primary factor inhibiting plant growth. Therefore, intensive research has been conducted in order to ascertain the mechanisms inherent to the Al toxicity and tolerance, on scales from the global to the molecular. Many of the biological activities of the plant are altered via the Al toxicity. So through selection and breeding process strategies, it is possible to develop Al tolerant plant. According to the measured parameters of root growth and various enzymatic activities, we found that there is a strong genotype effect regarding the bean plant's response to Al stress. Some bean genotypes were less affected by the Al-toxicity stress than other genotypes. At this point, we have not determined the critical molecular mechanisms that explain these genotypic differences. This will require some targeted molecular biology examinations. Over the past decades many researches has been done for significant progress towards the goal of developing crops better suited for cultivation with Al toxicity in acid soil.

With further identification of molecular markers linked with Al-tolerance gene it is possible to develop better Al tolerant crop. These measures in the field of research can be able to solve the problem of food scarcity due to abiotic stress and thus give food security to the malnourished population in the developing third world countries.

## Acknowledgement

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# Effect of aluminum and bacteria fertilizer treatment on the *Vigna radiata* root growth and photosynthetic activity on early growth stage

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Abstract: Knowing how aluminum in acidic condition may limit the growth of mung bean (*Vigna radiate)* is important because bean is one of the most important food legumes for people. Aluminum (Al) toxicity is one of the major constraints of crop production in acid soils. Five-day-old uniform seedlings were transferred to 1.7 L pots with constantly aerated simplified nutrient solution containing 0.5 mM $CaCl_2$, 0.5 mM KCl and 8 μM $H_3BO_3$. This solution allows optimum root elongation for short treatment. After 24 h of root growth, the pH of the solution was decreased from 5.5 to 5.0, after 48 h from 5.0 to 4.5 to avoid pH stress. The first 20 mm of the root apex was marked with permanent marker. Subsequently, plants were transferred to simplified nutrient solution containing 0 and 20 μM $AlCl_3$. The distances from the root apex were measured after 4 h, 8 h, 24 h, 48 h and 72 h after treatment. To compensate the Al-stress, bacterium fertilizer was added to the nutrient solution 1) before the Al-treatment and 2) at the same time with Al. The elongation rate was calculated. The value of potential photochemical activity was measured by the chlorophyll fluorescence method with PAM-2001 (WALZ GmbH, Germany). The dry weight of shoot and root were measured with thermo gravimetric method. According the root length elongation, the early time bacterium treatment has positive effect on root length 4 and 8 hrs after Al-treatment, but could not compensate the Al-stress for a longer time. We did not find significant difference in relative chlorophyll content (SPAD) or in the photosynthetic activity (Fv/Fm) among treatment. There results confirm the literature data, the toxic effect of Al is firstly root related.

Keywords: aluminium-toxicity, mung bean, photosynthetic activity, root growth

## Introduction

Aluminum is the most abundant metal element in the earth's crust and bound aluminum will dissolve in acidic soils. In case of neutral pH, aluminum is not soluble and can be found as aluminum oxide and silicate, while phytotoxic form of aluminum will be spread in soil solution and affect root and plant growth when pH decreases. The first effect of aluminum toxicity is its negative effect on root growth (Arsintescu et al., 2001). When soluble $Al^{3+}$ content reaches 10-20 mg/kg or more, it produces severe toxic effects on plants (Kochian et al., 2004). Micromolar concentrations of Al can inhibit root elongation and consequently influence water and nutrient uptake, resulting in poor plant growth (Delhaize and Ryan, 1995). Al not only affects plant roots, but photosynthetic behavior also is subjected to Al toxicity. For Al-sensitive plants, present of Al may reduce stomatal conductance (Mukhopadyay et al, 2012) and chlorophyll content (Mihailovic et al., 2008), change chlorophyll a/b ratio (Ying and Liu, 2005), photosynthetic rate usually declines (Lazarevic et al., 2014).All heavy metals significantly lowered the leaf contents of the photosynthetic pigments (Aldoobie and Beltagi, 2013). The decrease of pigment levels, as a result of heavy metals, has been found in many plants (Van et al., 1990). According to results from broad bean, aluminum toxicity causes the reduction of root respiration and photosynthesis (Arsintescu et al., 2001). The content of photosynthetic pigment is decreased, because of the destruction of chloroplast structure, photosynthetic system and chlorophylls photo oxidation, the destruction of the pre-material of chlorophyll synthesis and the inhibition of chlorophyll biosynthesis.

During different stresses, existing chlorophyll in chloroplast is broken down and thylakoid structure disappeared (Rout et al., 2001). Many plants have different mechanisms in plants have been categorized as: 1. external via the exudation of organic acids from the radical apexes and subsequent chelation of the Al in the rhizosphere (Ma et al., 2001), 2. internal or Al-tolerant as Al chelation is produced inside the cell and then later stored and compartmentalized in cell organelles like the vacuole (Delhaize and Ryan, 1995).

Some plant species have the ability to detoxify Al in the rhizosphere by exuding organic acids. Organic acids play a role in external and internal neutralization of Al. Generally, organic acids secreted by roots are malate, citrate and oxalate. The amount of organic acids released varies between plant species, and the detoxification mechanisms in an internal tolerance (Ma et al., 2001).

It is proven that plant growth promoting bacteria (PGPB) is associated with rhizosphere (Bashan, 1998) and they have the potential to produce a large amount of organic acids (Carson et al., 1991; ), which resulted in P binding by chelation, and may also be a possible mechanism for reducing Al toxicity of roots. These PGPB would enhance the growth of plant grown on soils with high Al content (Tóth et al., 2013; Panhwar et al., 2015).

### Materials and methods

Seeds of mung bean were germinated between filter paper, in an upright position. Five-day-old uniform seedlings were transferred to 1.7 litre pots with constantly aerated simplified nutrient solution containing 0.5 mM $CaCl_2$, 0.5 mM KCl and 8 µM $H_3BO_3$. This solution allows optimum root elongation for three days at least. After 24 h of root growth, the pH of the solution was lowered to 5.0, after 24 h 4.5 and keeps this pH until at the end of the experiment.

The experimental design was a completely randomized design with three pots per treatments, each pot contained 4 plants. Plants were cultured in a growth chamber with controlled environmental conditions of a 16/8-h light/dark regime, $25/20^{\circ}C$ day/night temperature and photon flux density of 300 µmol $m^{-2}s^{-1}$ photosynthetic active radiation at the plant level. Two hours before Al treatment, tap roots were marked 2 cm behind the root tip using permanent marker, which did not affect root growth during the experimental period.

The experimental design was a completely randomized design with three pots per treatments, each pot contained 4 plants.Afterwards, the plants were transferred to simplified nutrient solution containing 0 or 20 µM $AlCl_3$. Root elongation was measured at 4, 8, 24, 48 and 72 h of Al treatment using a 1-mm scale.

The applied bacteria fertilizer contains *Azotobacter chroococcum* and *Bacillus megaterium*. The dose of bacteria fertilizer was 2 ml $dm^{-3}$. The bacteria fertilizer was added to the nutrient solution from the first day of the experiment (Phy+20) and in the same time of Al-treatment on 3rd of the experiment (20+Phy). The nutrient solution was completed with $AlCl_3$ when Al-stress was examined (20µM Al). The experiment was finished on the 6th day of experiment.The shoots and roots of mung bean were dried at 65 ° C for 3 days and measured with analytical scale.

The relative chlorophyll content (SPAD-Units) was measured with SPAD-502 relative

chlorophyll meter (MINOLTA, Japan). The parameters of in vivo chlorophyll fluorescence were detected with a PAM-2001 (Walz, Germany) modulated light fluorometer as described by Schreiber et al. (1986). Samples were dark-adapted for 20 minutes. After dark adaptation, the initial fluorescence ($F_o$) was excited by weak light. The maximal fluorescence ($F_m$) was induced by white saturating flash. Ratio of $F_v/F_m$ was used for characteristics potential photochemical efficiency of PSII. Microsoft Office Excel 2003 and Sigma Plot 12.0 version were used to the statistical analysis.

### Results and discussion

Tóth et al. (2013) established that the dry weight of cucumber shoots and roots were lower in line with the increasing Al concentration. According their results bacteria fertilizer treatment can compensate the Al toxicity effect, thus producing higher dry weight result.

Root growth is the combination of cell division and elongation. Decrease of mitotic activity was reported as a consequence of Al exposure in root tips of several species as wheat (Li et al., 2008) and bean (Marienfeld et al., 2000). Some authors defended that inhibition of cell elongation was the primary mechanism leading to root growth inhibition (Zheng et al., 2005).

The root length was measured after 4h, 8h, 24h, 48 h and 72 h after Al-treatment. The root length decreased when 20 μM Al was applied. The root length was shorter with 50% after 4h, 55 % after 8h, and 89 % after 24 h of Al-treatment. The length of root did not changed after 24h, Al treatment had a very toxic effect on root growth. When bacteria fertilizer was added to the nutrient solution (Phy+20), the root growth was more intensive compared to 20+Phy treatment, when bacteria fertilizer was added at the same time with Al after 4 and 8h. The positive effect of bacteria treatment on root growth could not be detected (results are not shown). The dry weight of shoots and roots of mung bean can be seen in Figure 1 and Figure 2.

There is no significant difference in shoot dry weight. The lowest root dry weight was measured at 20 μM Al treatment compared to control.
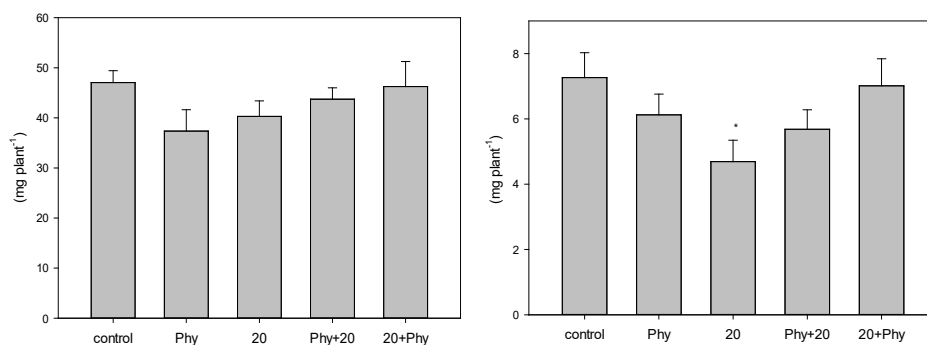


Figure 1: Effect of 20 μM Al and bacteria fertilizers on the dry weight of mung bean shoot (mg plant$^{-1}$) (n=60± S.D.)

Figure 2.: Effect of 20 μM Al and bacteria fertilizers on the dry weight of mung bean root (mg plant$^{-1}$) Significant difference compared to the control: *p<0.05

The Soil-Plant Analyses Development (SPAD) unit of Minolta Camera Co. has developed the SPAD-502 chlorophyll meter (Minolta Camera Co., Japan), a hand-held, self-calibrating, convenient, and non-destructive lightweight device used to calculate the amount of chlorophyll present in plant leaves (Minolta, 1989; Yadava, 1985). This meter records optical density measurements at two wavelengths, converts them into digital signals, and then into a SPAD value (Minolta, 1989). Strong relationships between leaf N concentration and SPAD values were found e.g. in apple (Neilsen et al., 1995), in corn (Chapman and Barreto, 1997) and in faba bean (Abdelhamidg et al., 2003).

The relative chlorophyll content in the first foliar leaf of mung bean was significantly higher when bacteria fertilizer was added at the same the time with Al-treatment (20+Phy) compared to control value. 20 µM Al (20) treatment did not cause significant changes in SPAD-Units (Figure 3).

According to Veres et al. (2006) the chlorophyll content of the plants was higher when bacteria fertilizer was applied from the first day of the experiment than from the fourth day of the experiment.
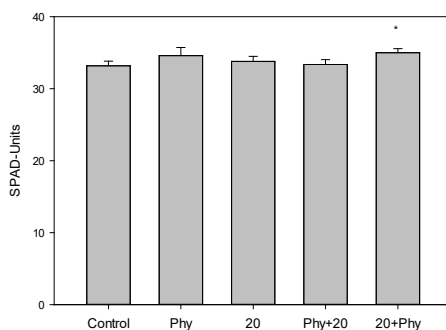


*Figure 3: Effect of 20 µM Al and bacteria fertilizers on the relative chlorophyll content (SPAD-Units) in the first foliar leaf of mung bean (n=60± S.E.) Significant difference compared to the control: \*p<0.05      Treatments: control: 0 µM Al, Phy: bacteria fertilizer, 20: µM Al, Phy+20: bacteria treatment from the first day of the experiment, 20+Phy: bacteria treatment at the same time with Al treatment*
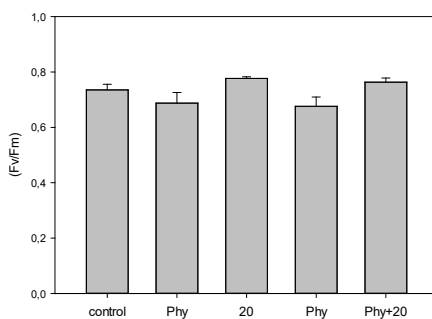


*Figure 4: Effect of 20 µM Al and bacteria fertilizers on the relative chlorophyll content (SPAD-Units) in the first foliar leaf of mung bean (n=60± S.E.) Significant difference compared to the control: \*p<0.05      Treatments: control: 0 µM Al, Phy: bacteria fertilizer, 20: µM Al, Phy+20: bacteria treatment from the first day of the experiment, 20+Phy: bacteria treatment at the same time with Al treatment*

An Fv/Fm value in the range of 0.79 to 0.84 is the approximate optimal value for many plant species, with lowered values indicating plant stress (Maxwell and Johnson, 2000). The Fv/Fm value is lower than the average value, it is between 0.67-0.77. The lowest Fv/Fm value (0.67) was measured at Phy+20 treatments, the highest at 20 µM Al treatment. We assume that Al has not had any negative effect on chlorophyll content or on photosynthetic activity in short-term Al stress experiment. To investigate the decline of photosynthetic activity longer-time Al exposition needed.

## Conclusions

Although some crops (e.g. tea (Matsumoto et al., 1976) are considered tolerant to high levels of exchangeable Al, for most crops it is a serious constraint. Species and genotypes within species greatly differ in their tolerance to Al. For most crops, fertilization and attempts of soil correction (e.g., liming) may not be enough per se to reduce Al toxicity (e.g., as the soil reaction remains strongly acid), and in most target countries these strategies may also be jeopardized by economical constrains. Therefore, it is imperative to fully understand the mechanisms that are used by the Al-tolerant species to cope Al toxicity, as well which genotypes, within the most resistant/tolerant cereal species, are more suitable to grow in acidic soils in order to increase world cereal production. Furthermore, the development of new cultivars (or the reinvestment in ancient genotypes from Al rich regions) with increased Al-tolerance is fundamental and economic solution to increase world food production.

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# The effects of two pesticides on the mortality and reproduction of *Folsomia candida*

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Abstract: The aim of our study was to determine the effects of two pesticides (a fungicide and an insecticide) after the administration as single compounds and in combination on the mortality and reproduction of springtails. The test organism was the *Folsomia candida* (*Collembola, Isopotomidae*), which is widely used for testing the toxicity of soils. Springtails have a significant role in decomposition and mineralization of soils. Testing was carried out using the OECD 232 guideline. The examined insecticide contains lambda-cyhalothrin active ingredients and the fungicide consists of a combination of 3 active ingredients. Five concentrations of both test items have been investigated (insecticide: 0.166, 0.299, 0.538, 0.968, 1.742 mL/L; fungicide: 1.157, 2.083, 3.75, 6.75, 12.15 mL/L). One-way ANOVA test was used for the statistical analysis. In both cases, obviously, the Collembolan species are non-target organisms. However, our results showed that there is significantly higher mortality in 2 concentrations of the insecticide ($2^{nd}$ and $5^{th}$ concentration, $p<0.001$), in 3 concentrations of the fungicide ($2^{nd}$, $3^{rd}$ and $4^{th}$ concentration, $p<0.001$), and in the middle concentration ($p<0.05$) of the combined treatment, compared to the control. The reproduction was found to be significantly lower ($p<0.001$) in all of the examined concentrations compared to the control, except the lowest concentration of the single administrations. These results can indicate that pesticides may also have effect on the non-target organisms.

Keywords: insecticide, herbicide, *Collembola*, *Folsomia candida*, reproduction, mortality

## Introduction

Pesticides have been used with different scopes in significant quantity for decades. Despite of their well-known negative effects and high costs, farmers apply them in increasing amounts. The use and application of pesticides (besides others) are regulated by legal regulations based on the results of variety of previous tests. However, it is clearly known that the residues of pesticides can accumulate in the environment, can get into the ground-water, or the wind can take them far away from the application area. But there are other important and good-to-examine effects, like the effects of the combination of pesticides and the effects on non-target organisms that belong to the ecosystem of the agricultural area or the puffer area.

*Folsomia candida* is a wingless, eyeless, unpigmented and parthenogenetic arthropod, and belongs to *Collembola* subclass. It can be found worldwide, except Africa and India (Hopkin, 1997). It is not so common in natural soils; it prefers humus rich areas (Krogh, 2008). It has a furca, "jumping organ" that helps its active running movement and jumping, when it is suddenly disturbed (OECD, 2009). It is a widely used organism for ecotoxicological soil tests for almost 40 years, it has a short generation time, easy to keep in laboratory and its life cycle is well-known (Fountain and Hopkin, 2005). This springtail is omnivorous, fungal hyphae, bacteria, protozoa and detritus can be found in its food. *Folsomia candida* has an important role in microbial ecology, soil fertility due to the contribution of decomposition processes and the regulation of nutrient cycling (Alves et al., 2014; OECD, 2009; Culik and Zeppelini, 2003). So it can be used as a good indicator of soil pollution (Achazi et al., 2000; Greenslade and Vaughan, 2003; Heupel,

2002).The aim of this study was to assess the effect of different types of pesticides on a non-target soil organism, *Folsomia candida*.

**Materials and methods**

The effect of two pesticides and their combination were tested on mortality and reproduction of *Folsomia candida*.

The Karate Zeon 5 CS (insecticide) contains a synthetic pyrethroid, lambda-cyhalothrin as active ingredient that has broad spectrum and it is used for controlling biting, chewing and sucking insect pests in cereal crops, fruits, vegetables, maize, sugar beets, alfalfa, sunflower etc. The Cherokee (fungicide) contains the combination of 3 active ingredients: chlorothalonil, propiconazole and cyproconazole. It is applied for the control of fungal diseases in winter wheat and both winter and spring barley.

Synchronous (9-12 days) female juveniles of *Folsomia candida* were used for the test. In the breeding period the springtails were kept in plastic Petri dishes on a mixture of plaster of Paris and activated charcoal layer, ensuring the required temperature ($20\pm2$°C) and they were nourished with granulated dry yeast and water was dripped on the surface for the proper humidity once a week.

The 28-day reproduction test was carried out by the standard OECD 232 Guideline (OECD, 2009). The modified OECD artificial soil was composed 5% air-dried sphagnum peat, 20% kaolin clay, approximately 74% air-dried industrial sand and $CaCO_3$. The plastic test vessels contained 30 g moist soil (24.5 g dry soil + 5.5 ml solution). In the case of the simultaneous application of the pesticides 2.75 ml/each concentration was added to the soil. The calculation of concentrations was based on the application procedure of the pesticides, five concentrations in a geometric series (by a factor 1.8) were calculated, and the middle concentration was the application concentration used in practice (*Table 1*). Four replicates for each test concentration treatment and eight controls were set. Ten individuals were placed in each vessel. For nourishing granulated dry yeast was used. The vessels were kept under the same conditions as the culture. At the end of the test, 200 ml distilled water and ink was added to the test vessels and the adults and juveniles were counted.

*Table 1. Concentrations of pesticides used in the experiment (mL/L)*

|   | Concentration of pesticides (ml/l) | |
|---|---|---|
|   | Insecticide | Fungicide |
| I | 0.166 | 1.157 |
| II | 0.299 | 2.083 |
| III | 0.538 | 3.75 |
| IV | 0.968 | 6.75 |
| V | 1.742 | 12.15 |

The normality of the data was determined by Shapiro-Wilk normality test and Q-Q plot. Significant differences between treatments were tested with one-way analysis of variance (ANOVA), and the comparison of treatments with the control was carried out by post-hoc Tukey test, using the R software package (version 3.3.2).

## Results and discussion

According to the OECD 232 Guideline, in a valid experiment the following 3 criteria are required: (1) Mean mortality of adult should not exceed 20% at the end of the test; (2) The average number of juveniles per vessel should be minimum 100 at the end of the test; (3) The coefficient of variation of reproduction should be less than 30% in the control.

In our test, (1) the mean mortality of adults was 5.27±4.44, so less than 20%; (2) the mean number of juveniles was over 100 in every vessel, (3) the CV% was 26.97, which is less than 30%. Overall, the experiment meets the validity criteria (*Table 2*).

*Table 2. Number of individuals in the control group*

| Mark of control vessels | Adults | Juveniles |
|:-:|:-:|:-:|
| C1 | 10 | 437 |
| C2 | 10 | 287 |
| C3 | 10 | 631 |
| C4 | 9 | 411 |
| C5 | 10 | 584 |
| C6 | 10 | 615 |
| C7 | 9 | 295 |
| C8 | 9 | 510 |
| C9 | 9 | 513 |

Only by seeing the mean number of juveniles (*Table 3*), a decreasing trend can be observed in all of the 3 treatments. This can be particularly observed due to the effect of the simultaneous application, where there were no juveniles from the 2nd to the 5th concentration. In the highest concentration of fungicide can be seen, that although in lower number, but juveniles were found. This different result (13 juveniles) was only in one vessel of the four replicates. So this outlier can be explained by an error made during setting the experiment. Because compared with the number of juveniles in the other vessels, this is significantly lower.

*Table 3. Average number of individuals*

| | Mean number of individuals | | | | | |
|:-:|:-:|:-:|:-:|:-:|:-:|:-:|
| | Insecticide | | Fungicide | | Combined | |
| Concentration | Adult | Juvenile | Adult | Juvenile | Adult | Juvenile |
| Control | 9.56 | 475.89 | 9.56 | 475.89 | 9.56 | 475.89 |
| I | 9.00 | 310.75 | 8.75 | 317.25 | 7.25 | 113.00 |
| II | 7.00 | 208.50 | 7.25 | 105.00 | 5.50 | 0.00 |
| III | 9.50 | 135.25 | 5.75 | 0.00 | 4.25 | 0.00 |
| IV | 7.75 | 51.50 | 6.75 | 0.00 | 6.50 | 0.00 |
| V | 3.75 | 13.00 | 8.75 | 3.25 | 6.25 | 0.00 |

Significant differences were detected at the 2nd (p<0.05) and the 5th concentration (p<0.001) of the insecticide, at the 2nd (p<0.1), 3rd (p<0.001) and 4th concentration (p<0.05) of the fungicide, and at the middle concentration of the combined treatment (p<0.05), compared to the control (*Figure 1*). The results of the treatments were significantly higher compared to the control group



*Figure 1. Significant differences in mortality between the treatments and compared with the control according to the Tukey test in the case of insecticide (1.), fungicide (2.) and the combined treatment (3.)*

It can be stated, that the mortality due to the single administrations and the combined treatment was not dose-dependent apart from the significant differences.

In the case of reproduction, all of the examined concentrations showed significant difference compared with the control, except the lowest concentration of the single administrations (*Figure 2*).

The results of the 4 significantly different treatments of the insecticide (2nd concentration (p<0.01), 3rd, 4th and 5th (p<0.001)) and of the fungicide (2nd, 3rd, 4th and 5th (p<0.001)) and all of the treatments of the combined concentrations (p<0.001) were lower compared with the control.

*Figure 2. Significant differences in reproduction between the treatments and compared to the control according to the Tukey test in the case of insecticide (1.), fungicide (2.) and the combined treatment (3.)*

## Conclusions

The mean number of juveniles compared with the control was reduced with 72% in case of fungicide and with 100% in case of the insecticide and the simultaneous treatment at the practically application rate. Decreasing (or a stagnating) trend can be observed with the increasing of the concentration. Moreover, the 2nd concentration of the combined treatment caused already 100% reduction in the reproduction. However, the same effect cannot be seen in the mortality. So based on our results it can be stated that the reproduction of the *F. candida* has a higher sensitivity to pesticides than the mortality and gives us more information. Similar conclusions have been drawn by Krogh and Petersen (1995) and Crouau et al. (1999).

These results can indicate that these pesticides may also have effect on the non-target organisms; however, more information and experiments are needed for examining the effect of different pesticides on non-target organisms.

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# Herbicidal activity and inhibitory potency of two essential oils on weeds under natural condition

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Abstract: The unsuitable use of synthetic herbicides in agriculture along with their accumulation in soil and groundwater caused adverse effects on the environment and human health. For this reason, a particular attention have been paid over the world, to reduce the chemicals used in agriculture production by introducing biological and ecological methods. Therefore, using of alternative means, such as products-based essential oils to control herbaceous layer, is needed because of their high potential as natural herbicides more friendly to the ecosystems. This work focused on studying the inhibitory effect of two formulated essential oils; One based on cypress (Cupressaceae) collected from the region of Blida (north Algeria) and the other one based on Thuja (Cupressaceae) of the region of Tipaza (northern Algeria). The treatments were carried out in the field, dilutions prepared from a 10% concentrated stock solution to obtain respective doses: D1 = 0.1 g / l; D2 = 0.2 g / l and D3 = 0.3 g / l. The results showed that the bioproducts based on essential oil of the two plants had different actions on the germination of the herbaceous stratum according to the dose and the time. Indeed, for the cypress-based products, the strongest inhibition was obtained with the lowest dose (D1) at a rate of 33.87% whereas for that based on thuja, it was the maximum dose (D3) with the highest rate of inhibition (14%).

Keywords: Bioherbicide, cupress, essential oils, inhibition, thuja.

## Introduction

The use of pesticides, herbicides and fungicides influences the environment. In order to minimize the use of synthetic products. New sciences have emerged in use natural molecules and biological means. It is therefore fundamental to design an integrated culture since building of protection strategy require a good biological understanding of disease or weed problems and effectiveness of different control methods. Depending on the choice of the subsequent control plan; the producer, as well as the company, ought to recognize the benefits and risks of each product.

The competition between weeds and crops also affects yield (Le Bourgeois et al., 1995) causing losses from 20 to 30%. Such a phenomenon leads to a very large monetary deficit, especially in cereal crops (Hussain et al., 2007). Thus, the sustainable development and the valorization of plants with phytosanitary characteristics of Algeria, is of a great interest. The main objective of this study is to test the vivo herbicidal activity of formulated bioproducts based on essential oils extracted from plants of the cupressaceae family.

## Materials and methods

This work tested the biological activity of bioproducts at different doses in which their active ingredients were essential oils extracted from the Cupressaceae family, the experiment was carried out in vivo at the experimental station of biotechnology faculty, department of nature and life science. The extraction of the essential oils was done by hydrodistillation from cypress and hydro-vapo-distillation from thuja. Three repetition were perfomed in a square of 25cm of sides. The distance between each repetition was 50 cm and between the doses was 75 cm. The concentration of the stock solution of the bioproducts was 10%, the protocol established three doses D1 = 0.1 g / l; D2 = 0.2 g / l and

D3 = 0.3 g / l with three replicates for each dose. The treatments are carried out at the level of the plot using a backpack sprayer with a capacity of 10 liters. The doses and the control, as well, were sprayed in 5 liters/square. This quantity of 5 liters, allowed to moisten the soil to a depth of 10 cm, a daily monitoring was made during 16 days.

## Results and discussion
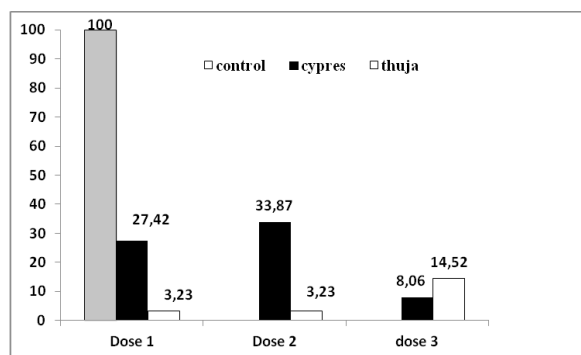


*Figure 1: Overall germination rate of different treatments*
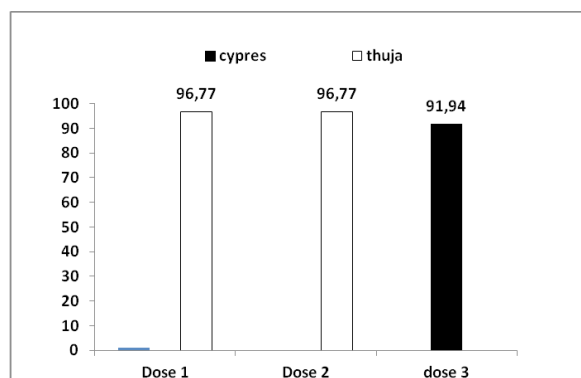


*Figure 2 : Comparison of Effective Doses of Inhibition*

From the results of Figure 1, it was observed an absence of the weeds under the effect of bioproducts based on various essential oils. Dose 2 of Blida cypress represented 33.87% of germination followed by dose 2 at 33.87%, then reduced to 8.06% with the dose 3 while the dose 1 and dose 2 from thuja reported 3.23% versus 14.52% for dose 3. The lowest percentage of germination was obtained in the cedar-based bioproduct since the lowest doses and the main doses have the same rate (3.23%). On the other hand; the cypress (with the highest percentage of inhibition) was obtained with a high dose. This phenomenon of allelopathy offers promising prospects for weed management. Allelopathy can be direct, through the cultivation of live plants or indirectly by the release of products during the decomposition of plants. The natural compounds presented in certain plants could be used successfully as bioherbicides (Da Mastro and al., 2006). By visualizing the overall percentage of germination. The present results marked the varabiabilty in the germination of the weeds according to the formulated bioproducts based on essential oils. All essential oils have demonstrated a potential to suppress the germination except for the

bioproduct based on rosemary essential oil. The oils from cypress and rosemary had an overall intermediate repression whereas Thuja oil demonstrated more severe toxicity, so allelopathy effect is present and its impact on germination depends on the plant used. It was noted that dose 1 and dose 2 of cedar affected significantly on germination. On the other hand; for cypress, the efficiency was observed when the highest dose applied. The allopathic impact of aromatic plants was observed on bioproducts made from cypress, cedar with a very important anti-germinative power. Based on the hypotheses put forward, our results are consistent with the work already carried out or in progress. It is accepted that under natural conditions, seed germination is a biochemical and physiological process where, from the first contact of the seed with the stimulus exogenous (water), an amylase enzyme is synthesized and secreted to degrade starch (albumins) to provide the embryo with the energy needed for germination (Regnault-Roger et al., 2008).

Once secreted, embryonic growth begins and then intervenes by another physiological process, where the actors are the vegetable growth hormones including auxin (Lesuffleurs, 2007). According to De martino et al, (2010) some oxygenated monoterpenes showed strong inhibitory activity on germination and radicle lengthening of radish and garden watercress seeds: It is well-known that these compounds have phytotoxic effects which can cause anatomical and physiological changes in seedlings: reduction of certain organelles such as mitochondria, accumulation of lipid globules in the cytoplasm may be due to inhibition of synthesis of DNA or rupture of membranes.

Amri et al. (2014) demonstrated that *Cupressus arizonica* inhibited germination and seedling growth of *Lolium perenne* L. and *Poa pratensis* L., causing anatomical changes in seedlings and modification of the structure of the plant. Zeghada (2009) reports that some plants have an inhibitory effect on germination such as *T. articulata* (species with the strongest effect), *G. alypum*, *P. lentiscus* and *R. pentapylla* both on the *Lactuca sativa* and of *Rhaphanus sativus*. According to Asghar (2012) the results showed significant differences between the two species of grasses studied for percentage germination, germination index, germination inhibition rate, seedling inhibition rate, length seedling, root, shoot, and seedling dry weight. The means of comparison of *Lolium perenne* and *Poa pratensis* revealed that the percentage of germination, germination index, length of seedlings, root, stems and weight of dry seedlings of *Lolium perenne* were higher than those of *Poa pratensis*. However, the rate of inhibition of germination and seedling rate of inhibition *Poa pratensis* was greater.

**Conclusions**

Bioproducts based on essential oils could be exploited against weed germination. All the bioproducts of the two plants showed an inhibition of germinative power for seven days to fifteen days after treatments. The treatments with Thuja led to the lowest germination percentage at the lowest dose compared to that with cypress which obtained the lowest rate of germination with the highest dose.

According to a rather conclusive results established by complementary studies, it would be interesting to characterize the essential oils used chemically to study the impact of its bioproducts on soil pedofauna and the selectivity of its bioproducts on weeds. Based on rather conclusive results, it would be interesting to investigate further in this area. The

chemical characterization of the essential oils used, the impact of its bioproducts on soil pedofauna and the selectivity of its bioproducts towards the herbaceous stratum in order to promote sustainable development and the preservation of ecosystems.

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# Biocidal effect of two formulated essential oils compared with a synthetic product on a green aphid *Chaitophorus leucomelas* (KOCH, 1854) of black poplar *Populus nigra* (L, 1753)

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Abstract: The issues related to insect outbreaks in forest ecosystems become nowadays of topicalty, due to their adverse effects.. To overcome these issues, the use of essential oils, as natural molecules of ecological and economic interest with insecticidal properties, is proposed as a promising solution. The study carried out focused on the temporal evaluation of the biocidal activity of two essential oils extracted by a hydrodistillation process of two plants, namely: Thym (Thymus vulgaris L, 1753) and the other of Bitter Orange (Citrus aurantium L, 1753). The latter were formulated and tested at two concentrations (C= 1.5 g/l) and (DC = 3 g / l) and compared to a synthetic insecticide whose chemical composition is the combination of two active ingredients (141g/l Thiamethoxam + 106g/l Lambda cyhalothrin) (AC = 1 g / l), on poplar aphid populations. The results showed a significant reduction in residual aphid populations for all applied concentrations compared to the control and a direct relationship between mortality rates, dose concentration and aphid exposure duration. A precocity of action of Thyme's essential oil and the synthetic product was observed from the 3rd day and a higher toxicity of the Bitter Orange's essential oil, with a better efficiency of the double dose was recorded (10% <PR<20%), which toxicity increases up to the 12th day. However, the latter reported a moderate resumption of aphidian populations compared to that caused by the homologous synthetic product (20% <PR<30%) from the 9th day of treatment.

Key words: Essential oils, Citrus aurantium L, Thymus vulgaris L, Aphid, Popla

## Introduction

Poplar is a fast growing tree species, which has economically significant importance, for production, so that is thanks to their increasing global interest. Indeed, it can contribute to overcome the global wood deficit. (Breton, 2000). Besides its generally high sensitivity to pathogens (bacteria, viruses, fungi) (Villard, 1998), the poplar has one of the most diverse phytophagous insect processes compared to other hardwood species (Whitham et al., 1996). *Chaitophorus leucomelas* K is an aphid that attacks mainly black poplar. It feeds on young shoots of *Populus sp.* In the spring, under the leaves, it is especially present in the leaves glued by larvae of the ringworm, or leaf galls left vacant by other insects (Reid et al., 2015). To date, infestations are largely controlled using synthetic insecticides. which remain responsible of many problems, both for the environment and for human health. Moreover, their massive use is the reason of the selection of populations of resistant insects (Nauen et al., 2003).

This observation leads scientists to seek new approaches to struggles that would effectively control these pests while limiting their negative impacts (Regnault-Roger et al., 2005). Currently considered is the formulation of new insecticides called bio-insecticides based on bioactive molecules disrupting certain biochemical functions essential to the survival of the target insect pest. They guarantee a high level of selectivity. The use of natural insecticidal molecules thus proves to be a good alternative (Giroux, 1994; Roger et al., 1995). Essential oils are used as biopesticides for their biological activities on phytophagous insects, which are exerted on several levels and limit the renewal of the generations (Catherine et al., 2008.

The present study focused on the temporal evaluation of the biocidal activity of two formulated essential oils of thyme and the bitter orange applied at different concentrations and compared to a synthetic compound "Thiamethoxam-Lambda cyhalothrin", on the residual populations of the green aphid *Chaitophorus Leucomelas K* of Poplar Black *Populus nigra L.*

## Materials and methods

The aphid populations of *Chaitophorus leucomelas K* on the leaves and petioles of the *Populus nigra* L. black poplar have been selected for the present study. The thyme (whole plant of *Thymus vulgaris* L.) and the sweet orange (*Citrus aurantium* leaves) were harvested during the winter period in the areas of Ben-Chikaou and Chebli respectively, then dried at room temperature and protected from light. Extraction of the essential oils was carried out by hydro-distillation using Clevenger equipment. The extracts of essential oils obtained from the two plants were formulated according to the procedure of Moussaoui et al. (2014) or in the form of a gel at a concentration of 10% of active ingredients, which has been preserved for the preparation of the dilutions (Table 1).

*Table 1:* The different treatments used for the evaluation of toxicity on Chaitophorus leucomeas

| | | Active ingredients | Type of formulation | Concentration Used (Dilution) | Action plan |
|---|---|---|---|---|---|
| Treatment applied | Biopesticides | EO of *Thymus vulgaris L* | gel | C=1.5g/l | |
| | | EO of *Citrus aurantium L* | | DC=3g/l | |
| | Chemical Pesticide | Thiamethoxam/ lambda cyhalothrin | liquid | AC= 1g /l | Systemic Contact |

The experimental protocol consists of 18 trees of *Populus nigra L*, divided into 6 blocks of 3 trees of 50m apart and on each tree four branches corresponding to the four cardinal corners. They were taken at a 3 day interval over a period of 12 days of the period of investigation. The 20 cm branches are placed in labeled paper bags (date of collection, number of tree, direction, number of the block) and then stored in a refrigerator for possible counts. The application of the treatments carried out using a manual sprayer

Aphidian populations were counted using a binocular microscope (G × 8) to follow the structures of winged and apterous females and their larval stages. The effectiveness of the different products applied was revealed by the estimation of the residual populations of the aphids. The biocidal effect of the bioproducts and the synthetic insecticide was estimated from the day 3 after treatment by taking measurements every three days, as compared with the initial infestation status, thus determining the percentage of residual populations (PR) according to the DUNNETT Test (Magali, 2009) with PR = Number of mobile form (NFM) per treatment x 100 / Number of mobile form (NFM) by control (water) stipulating that PR <30% (toxic), 30% <PR <60% (moderately toxic molecule), PR> 60% (neutral or weakly toxic molecule). The data collected of this study were analyzed statistically by Principal Component Analysis (PCA) and one-way ANOVA analysis, using the PAST1.37 software (Hammer et al., 2001).

## Results and discussion

The variance analysis (Table 2);reported no significant differences between the different concentrations of the treatments applied for all life stages, larvae, winged adults and adults with respective probabilities of p = 0.9834, p = 0.9459, p = 0, 6044.

*Table 2:* One-way ANOVA applied to the residual populations of larvae, apterous adults and adult's alates regarding the effect of the conentration of the different treatments applied.

| | | Sum of squares | DDL | Average of squares | F-ratio | p |
|---|---|---|---|---|---|---|
| Larvae | Between concentration | 784.09 | 4 | 196.02 | 0.093 | 0.983[NS] |
| | Inter group | 41915.1 | 20 | 2095.76 | | |
| | Total | 42699.2 | 24 | | | |
| Adults Apterous | Between concentration | 573.6 | 4 | 143.40 | 0.162 | 0.945[NS] |
| | Inter group | 17648.9 | 2 | 882.44 | - | - |
| | Total | 18222.5 | 24 | - | - | - |
| Adults alates | Between concentration | 940.49 | 4 | 235.12 | 0.694 | 0.604[NS] |
| | Inter group | 6769.48 | 2 | 338.47 | - | - |
| | Total | 7709.98 | 24 | - | - | - |

N.S.: p>5%

The temporal evolution of residual populations under the effect of the different treatments applied to the green poplar aphid (Figure 1) shows that during the first period after treatment (day 3), the concentrations (C and DC) Of the two essential oils, bitter orange and thyme, which showed neutral (PR> 60%) and medium toxicity (40% <PR< 60%), respectively, were switched to high toxicity (10% <PR<20%) with a better efficiency for (DC), which is emphasized until the end of the next step. On the other hand, the (AC) of the synthetic product displays not only a precocity of action (from day 3 to day 9) compared to the rest of the treatments but also a very high toxicity, depicting a relatively zero PR up today 9, when a recovery is reported. Significant toxicity of bioformulations has been reported in larval and apterous adult populations and less toxicity in alates adult populations.

This high toxicity of the essential oils could be explained by the presence of a major compound (Thymol) (41.39%), reportedly very toxic. Dorman et al. (2000) emphasized that thymol has the broadest spectrum of activity against 25 kinds of bacteria tested. Also, the presence of linalyl acetate (56.80%) (Haubruge et al., 1989), plays a decisive role in the biocidal activity of the essential oil of Bitter orange. This was confirmed by Regnault rauger and Hamraoui (1995) who reported a high toxic effect of linalole on the bean hive. However, all aphid larval, apterous adults and alates adults stages of the green aphid are very sensitive to the different treatments with the same moderate action of (C) of the thyme's essential oil and the same recovery of the aphidian populations reached by the homologous dose (AC).This fact leads us to believe that certain chemical compounds of E.O are more active than others. According to Chiasson, Belauges et al., (2004) the main factor modifying the insecticidal activity of E.O is the type and molecular structure of the active components present. Bioproducts formulated with the E.O of Thyme and Bitter orange. produced a greater toxic effect on larval and adult apterous populations than on

winged adults of black poplar green aphid.
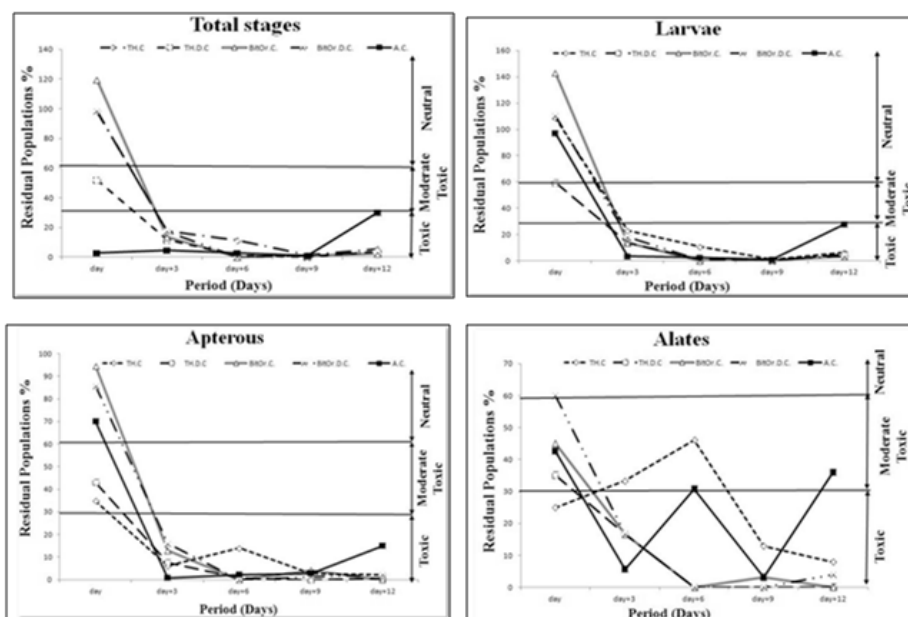


*Figure* 1. Temporal evolution of the residual populations of Chaitophorus leucomelas K under the effect of the formulated essential oils of Thyme and Bitter orange and the synthetic pesticide. Th.C.: Thym Concentration; Bitor.C: Bitter orange Concentration; A.C: Approved Concentration; Th.D.C: Thym Double Concentration; Bitor.D.C: Bitter orange Double Concentration

Our results are in agreement with those reported by Brengues and Coosemans (1977), considering age to be an important factor of variation and as a rule older individuals are less sensitive than younger individuals. The repressive and permanent effect of bioformulations could be explained by the nature of constituents that helped to maintain the integrity of the two E.O, due to their chemical and physical stability, consequently reinforce their modes of action, possibly by contact and / or ingestion. The action of pesticides on crop pests can cause various internal changes once the chemical is in the body by directly altering the endocrine system or indirectly by the attribution of energy, which affects the reproductive capacity of the individual, generating individual and inter-individual perturbations (Mayer et al., 1992). For the difference in the rate of action of the chemical relative to the biological products, this would be affected by the rate of metabolism, Soderlund et al. (1983) stated that arthropods metabolize most of the active ingredients of synthetic pesticides within few hours but no studies have yet been done on the metabolism of the molecules of E.O.

## Conclusions

From the present study, it emerges that formulations based on essential oils of thyme and Bitter orange, have shown a repressive effect and a certain insecticidal power on targeted aphidian populations, compared to that of chemical treatment. Also, all of the applied concentration depicted a reduction in residual populations of *Caitophorus leucomelas* K over time and showed a direct relationship between aphid mortality rates, product concentration and exposure duration.

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# Three different genotypes of maize hybrids yield response to sowing date and plant density changes

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Abstract: The small plot-field experiment was set up on chernozem soil at the Látókép research area of the University of Debrecen in four replications. The aim of the experiment was to set-up different genotypes of maize hybrids to study the effect of sowing date and plant density. The experimental plots were planted by a Gaspardo planters machine. We harvested the experiment with a SAMPO plot harvester that can determine their exact plot yield weight. We determined the moisture content and corrected the yield to 14% moisture. The results were processed using the SPSS 19.0 and Microsoft Excel programs. Single-factor analysis of variance and Pearson's correlation analysis was performed. We examined the following hybrids SY Arioso (FAO 300), P9486 (FAO 360), DKC4943 (FAO 410). The experiment was set up in three different plant densities. These were 60,-76,-90 thousand plant ha$^{-1}$. We used three different sowing date early, average and late. We observed that DKC 4943 hybrid had the highest amount of yield (13,6 tons ha$^{-1}$)at the second sowing date (April 1). The second biggest yield was produced by the early sowing time (April 1). It was close the same amount of yield in the 76 thousand plant ha$^{-1}$. It was (13,5 tons ha$^{-1}$). The late sowing date (May 5) has lowest amount of yield. In our experiment SY Arioso had the lowest amount of yield in every sowing date in the 60 plant ha$^{-1}$ plant density.

Keywords: maize, sowing date, plant density,

## Introduction

The great variability of the climate is the biggest risk factors in the crop production (Nagy, 2006). Maize is the most important crop in the agriculture of Hungary. The corn is the most profitable plant in the arable production. Therefore it is very important to deal with the tasks of the versatile maize production in the future (Pepó and Sárvári, 2004). As an effect of global climate change the sowing date of some important plant can be changed. The optimal sowing date is very important work process in crop production. That's because the adaptability of maize is limited. The maize produce much less yield in dry crop year than in average or wet weather condition. (Bene et al, 2014). The sowing is the most important work process of a successful growing season. The mistakes what made in the sowing cannot be fixed in the following (Fűzy, 2005). To determine the optimal sowing we know several process. The big growers take many factors into account but the average opinion is the same. The early planting of corn is the best because the high risk of the changing weather (Menyhért 1985), (Sárvári és Futó 2001). Maize needs 300 liter of water to produce 1 kg of dry material. Therefore it is very important to determine the optimal plant density (Pepó 2012), (Szabó, 2012). The yield is lower if the density is less or more than optimal. In the 20th century agricultural workers thought that lower plant density was better because each plant grown bigger. This attitude has changed for now. (Pálovics, 2006.). Now it is necessary to determine the optimal sowing date and plant density of each type of maize hybrids. That's because these are very modern hybrids and the different genetic background need different growing condition (Molnár és Sárvári 2005).

## Materials and methods

The field experiment was carried out at Látókép research area of the University of Debrecen on chernozem soil. Soil of the research area is of good agricultural condition, medium

hard soil with medium humus content and good neutral level. Water supplies of the soil are favorable. We examined three important hybrids of Hungary what were SY ARIOSO (FAO 300), P9486 (FAO 360), DKC4943 (FAO 410). The experiment was set in three different plant densities 60, -76, -90 thousand plants ha$^{-1}$. The Experiment was set in three sowing dates. Early sowing date: April 1, average: April 21, late sowing: May 5 number of replication was 4. The results were presented in the average of the replications. The fore crop was winter wheat. The experiment was set in one nutrient level. The fertilizer was dispensed in spring. The amount of N was 108 kg ha$^{-1}$ (in Pétisó). We planted with a modern Gaspardo corn planter with GPS technology and harvested the plot with a special SAMPO plot harvester. The results of the experiment was analyzed by SPSS 19.0 and Microsoft Excel. Data was analyzed with single-factor analysis of variance and Pearson's correlation analysis. The meteorological factors are shown in *Table 1*.

*Table 1.* The amount of meteorological parameters in the examined crop years (Debrecen, 2015)

| | Months | April | May | June | July | August | September | October | Total/Average |
|---|---|---|---|---|---|---|---|---|---|
| Precipitation (mm) | 30 year's average | 42.4 | 58.8 | 79.5 | 65.7 | 60.7 | 38.0 | 31.8 | **376.9** |
| | 2015 | 21.9 | 52.9 | 60.5 | 35.6 | 84.0 | 48.9 | 86.6 | **390.4** |
| | Difference | -20.5 | -5.9 | -19.0 | -30.1 | 23.3 | 10.9 | 54.8 | **13.5** |
| Temperature (°C) | 30 year's average | 10.7 | 15.8 | 18.8 | 20.3 | 19.6 | 15.8 | 10.3 | **15.9** |
| | 2015 | 10.1 | 15.8 | 19.9 | 22.9 | 23.3 | 17.8 | 11.2 | **17.3** |
| | Difference | -0.6 | 0.0 | 1.1 | 2.6 | 3.7 | 2.0 | 0.9 | **1.4** |

**Results and discussion**

On the *Figue 1.* we show the amount of yield in the average of replications. Based on the amount of yield we found that the highest yield was produced by the DKC 4943 hybrid in the three sowing date. The second highest yield was produced by the P9486 in early, average and late sowing date. The SY Arioso produced the less yield in the late sowing date. In our experiment in the early sowing date the hybrids have higher yield in 90 thousand ha$^{-1}$ plant density. Here increasing the plant density increased the yield. In later sowing plant like much more the less plant density. In the early sowing date the plants in the 90 thousand ha$^{-1}$.plot reach the highest amount of yield. In the average sowing date every hybrid reach close the same high amount of yield in the 75 thousand ha$^{-1}$ plant density. The plant density growing is decreasing the amount of yield at the late sowing date. Here the plants produced the highest yield in 60 thousand ha$^{-1}$ plant density and produced the less in 90 thousand ha$^{-1}$.

Analyzing the data's with the single factor variance, we found the significant difference between the early and late sowing date and between the average and late sowing date *(Figure1.)*. This result confirm the Pearson correlation result. During the experiment the difference between each plant density shows great different therefore we all cases can be justified statistically. We analyzed each sowing date differently because this way we get realistic result.
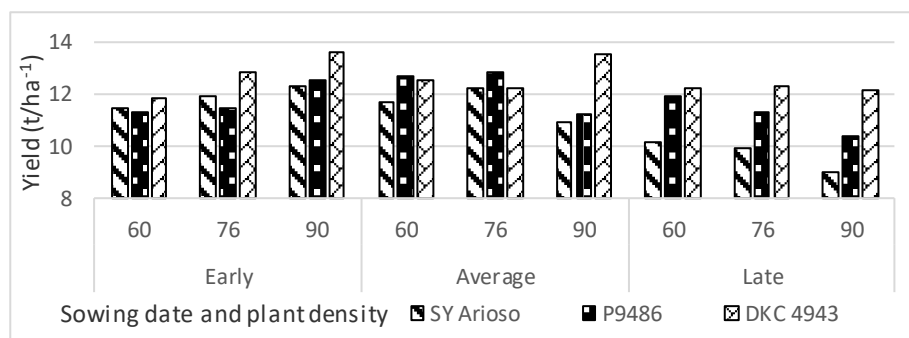
| | Yield |
|---|---|
| $LSD_{5\%}$ Sowing date | 0,71 |
| $LSD_{5\%}$ Plant density | 0,67 |
| | V1 | 0,94 |
| $LSD_{5\%}$ Hybrid | V2 | 1,17 |
| | V3 | 1,62 |

*Figure 1.* The effect of the sowing date and plant density in different genotype on maize hybrid grain yield (2015)

In analyzing the Pearson correlation values below *(Table 2)*, 0,3 characterized weak correlations, values between 0,3 to 0,5 mean medium, in between 0,5 to 0,7 the correlations was strong, while in the case of the correlation over 0,7 mean very strong correlation. Analyzing the data's we experienced negative moderately strong connection between the sowing time and yield *(Table2)*. So delaying the sowing we can measure yield decreasing. There is no statistical different between the plant density and yield.

*Table 2.* Correlation between the analyzed parameters (2015)

| | Yield |
|---|---|
| **Sowing date** | -0,388[**] |
| **Plant density** | -0,007[NS] |

(*) Correlation is significant at the $SzD_{5\%}$- level  (**)Correlation is significant at the $SzD_{1\%}$- level  (NS) Non significant

In the experiment the highest yield was produced by the DKC4943 in the early and average sowing date in the 90 thousand ha^{-1} plant density (13.6 t/ha[-1]). The second best hybrid was the P9486. This hybrid reach their maximum yield in the average sowing date in 76 thousand plant ha^{-1} plant density (12.9 t/ha[-1]). The third hybrid was the SY Arioso which reach their top yield in the early sowing date in 90 thousand plant ha^{-1}.

**Conclusions**

In our experiment there was three different sowing date and three plant density. We use three different hybrid from three genotype. The highest yield was produced by the DKC 4943 in all sowing dates. The second best was the P9486 and the third was the SY

Arioso. Analyzing the data we found difference between the three sowing date. We have significant difference between the early and late and average and late sowing. The sowing date significantly decreased the yield of maize. We measure difference between the three plant densities but the statistics can't confirm that. In the first sowing date the 90 thousand plant ha^{-1} plant density is better than the others. In our experiment the 76 thousand plant ha^{-1} plant density was the best in average and late sowing. In the late sowing the lower plant density was better. That's because the plants haven't got enough water in the soil at the case of late sowing. Therefore they have to drain the water from each other. Earlier sowing produce yield as good as average sowing. Therefore, early sowing can be recommended to prevent atmospheric summer's drought in Hungary. This way we can achieve greater crop safety.

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# Runoff and infiltration – Case study of a Cambisol

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Abstract: The soil erosion is a complex phenomenon, which depends on influencing factors. It is also a potential hazard of agricultural fields of Hungary. The runoff is one of the driving forces in soil loss, hence it is important to have knowledge of hydraulic characteristics of a soil. The runoff/infiltration ratio depends on several factors. The soil crust, the soil moisture content, the slope and the intensity creates a complex dependency matrix where the determination of the most influencing factor on the runoff is complicated. The main goal was to compare the runoff ratios of the same soil under constant high intensity but different surface conditions (moisture content; crust) and slope steepness. An intensively tilled Cambisol was studied with laboratory rainfall simulator, and the results were validated with field simulations. According to the runoff results, the runoff/infiltration ratio shows similar values in the laboratory and in the field, although it is higher in the field. Generally, the 12% slope triggers the highest values in the runoff and sediment concentration but the moisture content or a developed crust can influence the results, which means in erosion estimation "the soil surface status" factor has to get more attention.

Keywords: infiltration; runoff; rainfall simulation

## Introduction

The accelerated soil erosion is a serious problem in Hungarian agricultural areas, the rainfall simulator experiments, concentrates on soil erodibility in Hungary was summarised by Centeri et al. (2011). With the climate change, high intensity rainfalls occur more and more often and causes accelerated soil loss. Measuring the effect of combined factors on soil erosion regards to their interrelated influences are having increasing importance. A laboratory-scale rainfall simulator is a useful tool for examining the influencing factors of soil erosion one by one or as a system. The literature of simulated soil erosion experiments suggests several approaches due to the complex process of erosion. Kerényi (1986) studied the splash erosion, Defersha – Melesse (2012) examined the effect of the rainfall intensity, the initial moisture content and slope steepness on erosion, while Gómez – Nearing (2005) concentrate on the effect of the surface roughness on soil loss and runoff. Kinnell (2016) reviewed the main use and limitations of the rainfall simulators but a general standard of simulator design or simulation method still does not exist. In this study, we examined the response of a Cambisol on erosion under different slope angles and initial soil moisture contents with a lab rainfall simulator and field validation. The main questions were: 1) How does the runoff and infiltration ratio change with the slope steepness and soil moisture content? 2) In a pairwise comparison of the simulations, which parameter is the most influencing related to the runoff ratio and sediment concentration values?

## Materials and methods

The rainfall simulations were conducted at the Eötvös Loránd University with a redesigned Zámbó-Weidinger type simulator (Zámbó – Weidinger, 2006). During the field validation

a redesigned Pannon-R2 simulator (Jakab – Szalai 2005) was used. The main differences between the simulators were the experimental area: in the laboratory 0.5m$^2$ while in the field 6m$^2$ were used, respectively.

The sample was taken from an intensively tilled field near Ceglédbercel, Hungary (N 47.249765°, E 19.678761°, 150 m asl.). Previously, field scale erosional studies were done by Szalai et al. (2016) and detailed aggregate related laboratory scale soil loss was also studied by Szabó et al. (2015). The current study concentrates on erosional response of the soil in case of the slopes (2; 5; 12% steepness) and surface status (Table 1.) changes. The surface status combines the initial moisture content and the roughness together because of the effort to model frequent surface statuses.

During the high intensity experiments, all the runoff was collected. The time was recorded after every 1-litre runoff in the laboratory and 1.5-2l in the field due to the experimental design. Weights of dried soil losses per litre of runoff were recorded at the nearest 0.01 g and summed per simulations.

*Table 1: Applied surface characteristics during the experiments (moisture content and roughness variations)*

| Code | Description |
|------|-------------|
| 1 | tilled surface and field capacity moisture content |
| 2 | crusted surface and saturated soil |
| 3 | inland inundation and saturated soil |
| 4 | drought simulation with big rifts, dry, crusted surface |

The simulations were planned to collect predetermined amount of runoff hence the time period and the amount of precipitations vary in relatively wide range (525–2531 s and 13.58–52.03 mm) (Table 2.). The effect of length and amount of the precipitation on runoff was disregarded because we assumed, this effect is not significant in this case.

*Table 2: Main characteristics of the simulations (L-laboratory; F-Field; slope angle (2, 5 or 12%); surface characteristic (see Table 1.) code)*

| Experiment code | Precipitation (s) | Precipitation (mm) | Intensity (mm/h) |
|-----------------|-------------------|--------------------|------------------|
| L/5/1 | 2531 | 54.84 | 78 |
| L/5/2 | 1963 | 47.98 | 88 |
| L/12/1 | 2014 | 52.03 | 93 |
| L/12/2 | 525 | 13.85 | 95 |
| L/2/3 | 1309 | 36.36 | 100 |
| L/2/4 | 1762 | 50.41 | 103 |
| F/5/2 | 626 | 16.87 | 97 |
| F/12/2 | 635 | 18.17 | 103 |

### Results and discussion

Figure 1. illustrates the runoff and infiltration ratio and sediment concentration of the experiments arrayed by precipitation intensity to show, the differences of the intensity has not caused significant impact neither on runoff ratio neither on sediment concentration. The runoff rate varies between 35–55 % in the laboratory and is around 25% on the field (Figure 1.). This difference corresponds to the scale effect (Iserloh et al. 2012) hence during the comparison, the field experiments count as a control, and underline, the scale is matter. The sediment concentration increased with the slope steepness on both locations and it was higher on the tilled surface compared to the crusty one which indicates detachment limited erosion on crusty surface. The runoff rate was the lowest in case of the drought simulation (2%) where the big rifts were draining the water, and as it was expected, the

highest on 12% slope. The infiltration rate is not related to the crusts clearly although Assouline – Ben-Hur (2006) found strong relationship between surface sealing and runoff production.
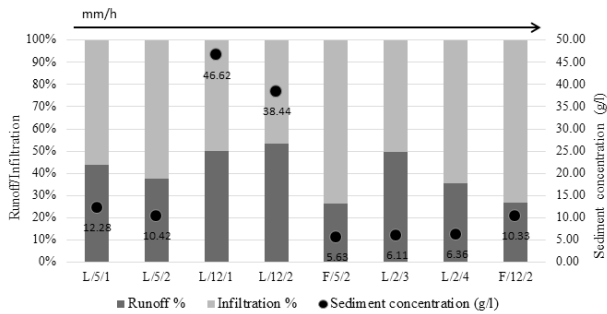


*Figure 1. Runoff, infiltration rate and sediment concentration arrayed by precipitation intensity (Table 2.)*

Figure 2. shows a pairwise comparison of the runoff rate ([%]; upper part of the matrix) and sediment concentration ([g/l]; lower part of the matrix) among the experiments to examine the effect of the slope and surface. Simulation code in the matrix cells reveals the higher value from the comparison. Beside the 12% slope dominancy on both runoff rate and soil loss concentration, the marked (asterisk) cells –primarily extreme moisture content simulation – show the pairs, where the surface status was dominant against the slope. During the inland inundation, simulation (L/2/3) the runoff rate was higher than in laboratory 5% slope steepness, and field experiments (Figure 1, 2.). Defersha, – Melesse (2012) studied the erosion of three different soil types (one of them was a Cambisol) also in laboratory, but in smaller plot. They also found that slope was a dominant factor during the erosion but the runoff rate and sediment concentration varied with initial moisture contents.

|  | L/5/1 | L/5/2 | L/12/1 | L/12/2 | L/2/3 | L/2/4 | F/5/2 | F/12/2 |
|---|---|---|---|---|---|---|---|---|
| **L/5/1** |  | L/5/1 | L/12/1 | L/12/2 | L/2/3* | L/5/1 | L/5/1 | L/5/1* |
| **L/5/2** | L/5/1 |  | L/12/1 | L/12/2 | L/2/3* | L/5/2 | L/5/2 | L/5/2* |
| **L/12/1** | L/12/1 | L/12/1 |  | L/12/2 | L/12/1 | L/12/1 | L/12/1 | L/12/1 |
| **L/12/2** | L/12/1 | L/12/1 | L/12/1 |  | L/12/1 | L/12/1 | L/12/1 | L/12/1 |
| **L/2/3** | L/5/1 | L/5/2 | L/12/1 | L/12/2 |  | L/2/3 | L/2/3* | L/2/3* |
| **L/2/4** | L/5/1 | L/5/2 | L/12/1 | L/12/2 | L/2/4 |  | L/2/4* | L/2/4* |
| **F/5/2** | L/5/1 | L/5/2 | L/12/1 | L/12/2 | L/2/3* | L/2/4* |  | F/12/2 |
| **F/12/2** | L/5/1* | L/5/2* | L/12/1 | L/12/2 | F/12/2 | F/12/2 | F/12/2 |  |

*Figure 2. Matrix summarizes the pairwise comparison of the runoff ratio and sediment concentration among the experiments. Different colours indicate different slope angles.\* indicates the results which are not follow the slope trend*

## Conclusions

Based on the gained data runoff and sediment concentration were correlated better with slope but its actual effect on the runoff and sediment concentration could vary with the moisture content and surface roughness. Inland inundation occurs mostly in flat terrain, where local topology has a small relief. Our results imply, in saturated soil condition the sediment transport is significant in local scale, which could encourage the crust development and change hydraulic conditions as well. Hence, more experiments that are

complex are needed to reveal the dependences of the factors. It was also found that the field validation is essential for checking the goodness of the laboratory experiments to avoid extrapolation errors.

## Acknowledgement

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# The role of climate strategies and green infrastructure in the adaptation to climate change

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Abstract: Climate change is one of the greatest challenge for the economy, society and settlements. Today it is an expectation to mitigate the expected negative consequences and to make adaptation efforts. To achieve this, municipal climate strategies are needed which must include successfully used possibilities in adaptation. In our paper, we overview the specialities of the current Hungarian climate strategies, expectations for the future climate strategies and the role of green infrastructure as a possible method for adaptation. We investigated the climate strategies, and also conducted additional experts' interviews. To confirm the effectiveness of green infrastructure, we analysed the inside and outside temperatures in a green-walled building for a two-month period. As a result of the analysis and the comparison of Hungarian and international climate strategies, we can conclude that these documents put great emphasis on the use of renewable energy sources. Our suggestion is that the application of energy-saving green walls should get a greater role among adaptation possibilities in the future. First results of our measurement show that in the case of green-walled buildings the difference in the temperature between walls with and without shadow can reach 20 °C, and shading can reduce indoor minimum temperature with 4-5 °C. Besides that, application of green walls has a great importance from the perspective of reducing $CO_2$ emission, and improving of air quality thanks to the $O_2$ production.

Keywords: adaptation, climate change, climate strategies, green infrastructure

## Introduction

Adaptation to the expected impacts of climate change becomes a central question which can be seen in different research projects, documents and institutions (Birch, 2014; EEA Report No 12/2016; Massona, et al. 2014; Rosenzweig et al., 2011). Similarly to many countries of the word, Hungary recognized the importance of mitigation and adaptation to climate change. The National Climate Change Strategy, or other documents and initiatives, like Climate Friendly Municipalities Association or Energy Efficient Settlements Association help to achieve this. In our research we focused on the local answers for the challenges of climate change with analyses of climate strategies of European and Hungarian cities.

Green infrastructure can play an important role in practical implementation on local level (Gill, et al. 2007). Adaptation and mitigation mean a challenge which can be approached from a regulatory aspect, where the focus is on energy savings and energy efficiency, or actions taken for example during heat waves.

Our aim was to explore the potential of a Hungarian medium sized town (Kecskemét) in green walls application.

Also our aim is to investigate the temperature inside green-walled buildings, which indicates the potential yearly energy savings. Above all, we try to estimate the amount of oxygen emitted by plants and the absorbed carbon dioxide, which can contribute to improve urban air quality.

We would like to draw attention for the potential in the application of green walls, which

could be important in the reduction of greenhouse effect and energy consumption, and can improve the life quality of urban population.

**Materials and methods**

In case of climate strategies, we reviewed the significant international results of climate protection, the main principles of climate policy in the European Union and Hungary. Besides these, we analysed the climate strategies of the Hungarian medium size cities. After that, we prepared experts interviews with the prominent persons who participated in the preparation of strategies or working in local climate associations. They have appropriate insight on the effectiveness of the local climate protection programs.

On the base of climate strategies green infrastructure is one of the successful practical solutions. In addition to „classic" green areas it is also worth to investigate other parts of green infrastructure because more green areas do not necessarily lead to local improvement in condition of settlements, and only constitute a part of the overall concept of urban climate (Gaffin, et al. 2012; Matthewsa, et al. 2015).

Ivy is one of the most popular plants in developing green walls because it tolerates urban conditions and its shielding ability is adequate. Researchers of Hochschule für Technik und Wirtschaft in Dresden investigated a 1000 m² ivy green wall with facing south. They calculated the oxygen emission and carbon sequestration, and the results for 1 m² green wall (Hedera helix) are 2,3 kg $CO_2$ sequestration and 1,7 kg $O_2$ emission per year (Schröder, 2009).

In our examination we focused on public buildings of Kecskemét. The list from Urban Development Ltd. of Kecskemét consists of 103 public buildings. We purified this database, so we assessed 62 buildings all together. It meant the estimation of front surface; the size of doors and windows were deducted from the wall surface. In relation to oxygen emission and carbon sequestration we made calculation on the base of ivy green walls.

We measured the temperature in order to monitor the impact of green walls on the buildings. This measurement has done in Clarion Hungary Kft., Nagykáta. This factory has a green wall by Zöldfalkert Horticultural Ltd., and it provided the suitable conditions for the measurement. The period of measurement was between 03. 08. 2015. – 23. 09. 2015.

The measurement was made simultaneously at four points:

- outdoor surface of bare building wall
- indoor room behind the bare building wall
- green walls wrapped wall
- indoor room behind green walls (periods with and without air condition)

**Results and discussion**

Until this time, only 10 medium size cities prepared publicly available climate strategy, but some of these documents are only „water management climate strategies" (such as in the case of Vác and Pomáz). These documents only focus on water management issues like flood protection and rainwater management. Some of the other strategies put the focus on energy management and efficiency.

Nowadays we have found several background materials, scientific and policy documents – like the Climate-guide edited by Fülöp (2009) – that can be useful for the Hungarian settlements to prepare an elaborate climate strategy. According to this Climate-guide, the first (and most important) steps are revolving around energy, like founding local energy committees, creating an energetic database and preparing local energy-conceptions. Almost all of the climate concepts contain the land-, water-, and forest-management, flood protection, heat and UV action plans, but the recommendations of the Climate-guide also point out that in Hungary, local energy management and the increased use of renewable energy is considered as the most effective way to mitigate the effects of climate change. We carried out an analysis which shows the targets of these documents (Fig. 1.):
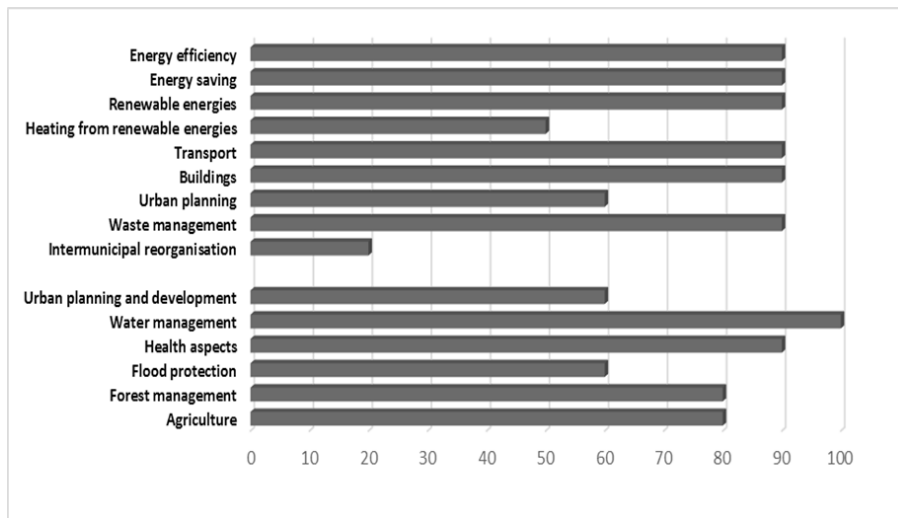


*Figure 1.: Topics of mitigation and adaptation (%) in the examined Hungarian climate strategies*

It can be concluded that most of the local climate strategies contain almost every topics with the exception of the need for restructuring of the public services (inter-municipal reorganization). It shows, domestic strategies consider every topics important in theory, but the realization is weak in practice in many cases. The analysed climate strategies pay particular attention to the question of energetics which supports our hypothesis that energy management is one of the most important topics for the settlements.

We have selected six settlements, where we analysed the local climate strategies and conducted interviews with the prominent experts on the subject. The experts also agreed on that energetics will have the most important role in climate protection in the future. Despite some disagreements all experts agree on that „not used energy is the safest, cheapest and most environmentally friendly energy".

In addition to mitigation topics in European cities (Reckien et al., 2014), Hungarian possibilities mentioned in the city strategies are the following:

- reducing emissions, $CO_2$ sequestration
- development of monitoring network (e.g. air pollution)
- increasing green areas and green roofs
- landscape rehabilitation
- awareness for mitigation

Based on the above mentioned topics, two things are worth highlighting: importance of energy savings and increasing of green areas. These two themes can go hand in hand; green areas play a role in reducing emissions and $CO_2$ sequestration, furthermore green roofs or green walls have an insulating role which may help in energy savings. It means, green infrastructure can be a central element in climate strategies.

In order to examine this issue we analysed 62 public buildings in Kecskemét from the point of view of green wall application, possible energy savings and oxygen emission/ carbon sequestration. The estimated area of walls without windows are approximately 32 000 $m^2$. If these walls were all wrapped with green walls – for simplicity it could happen with ivy – the oxygen emission approximately would be 54 400 kg, carbon sequestration would be 73 600 kg per year. On the base of literature data we can calculate a 15 000 kg $O_2$ emission and 13 500 kg $CO_2$ sequestration for 1 hectare per year in case of forests. On the basis of this values oxygen emission are similar to results of urban green walls, but $CO_2$ sequestration is less than our calculation. It is clear from the comparison that the green walls on public buildings of Kecskemét could greatly contribute to improving urban air quality or reduction the amount of greenhouse gas emission.

Kecskemét Municipality assessed the expected savings from energetic modernisation in case of the owned buildings. On the basis of three different possible modernized fields the total savings would be 620 777 Euro in 62 buildings we surveyed. The majority – 509 677 Euro – of this saving comes from architectural modernization, which means wall insulation mainly. The amount needed for architectural modernization – based on the calculation of government data – is approximately 8 926 000 Euro. A significant part of this considerable amount could be reserved with the application of green walls besides wall insulation.

On the basis of the temperature measurement we can conclude that the difference in air temperature between a wall without shadow and a shaded wall is significant. Temperature in front of the wall without shadow was more than 60 °C in many times in August (Fig. 2.), meanwhile maximum temperature in front of the wall with shadow (green wall) was
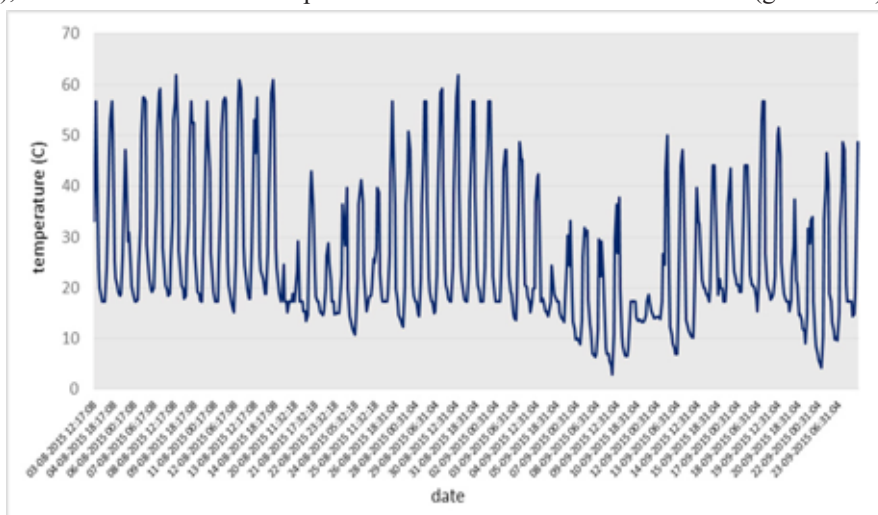


*Figure 2.: Outside air temperature in front of the building without green wall (°C)*

about 40 °C (Fig. 3.). Temperature of the room without shadow is about the same with the outdoor air temperature in shadow. Temperature of the room with shadow in the period without air condition is similar with the other room's temperature but there was a 4 days difference between indoor and outdoor values.

**Conclusions**

Sustainability, climate protection, energy management, renewable energy sources and
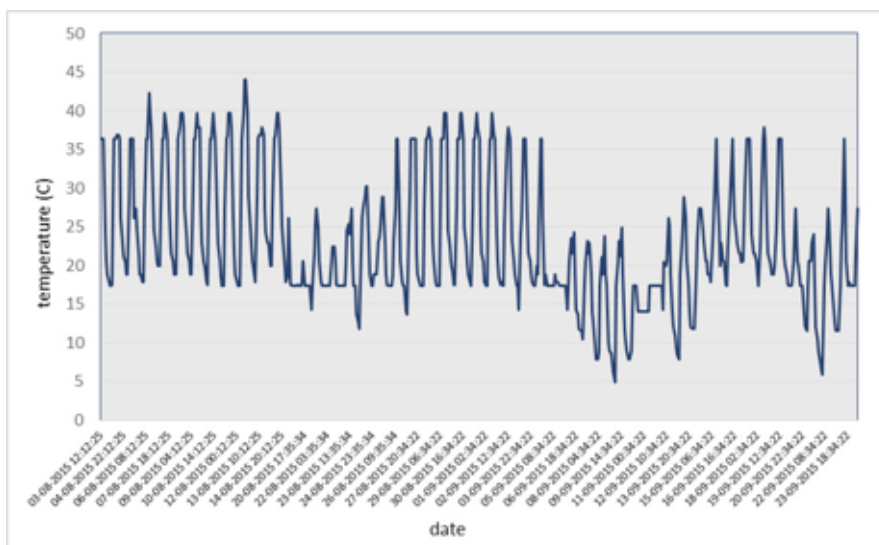


*Figure 3.: Outside air temperature in front of the building with green wall (°C)*

green infrastructure are closely related concepts. Renewable energies have an important role in climate strategies. But the current reality is that renewable energy use is ranked behind energy savings and efficiency in the examined settlements (and in the similar cities). Renewables require significant investments which hinders their spread. If we concentrate to energy savings and efficiency, green infrastructure – such as green walls – could come into the front.

The potential in the application of green walls in Kecskemét is significant, with approximately 32 000 $m^2$ walls of public buildings. Green infrastructure in cities can largely contribute to the reduction of $CO_2$ emission (which is important from the point of view of climate protection) and improving of air quality thanks to the $O_2$ production.

Reduction in energy consumption is imaginable with the replace estimated costs of insulation to green walls in case of public buildings, and reduction of heating and cooling costs during operation of buildings. We have data about costs of insulation at this moment. On the basis of this, it can be seen that the possible financial savings can reach hundred thousand Euro.

Our results show that in the case of greenwalled buildings the difference in the temperature between walls with and without shadow can reach 20 °C, and shading can reduce indoor minimum temperature with 4-5 °C.

One of the main challenge of urban ecology nowadays, how it can contribute to the liveable urban environment with own researches, by extension, to the reduction of negative effects of climate change. One of the obvious answer for it is application of plants (green infrastructure), as part of climate strategies of the cities. Urban vegetation means not only parks, roadside alleys or green roofs, but also vertical greens– with increasing number of greenwalled buildings.

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# Climate consciousness and adaptation from the viewpoint of farmers

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Abstract: Adaptation to climate change and finding solutions to the environmental consequences are urgent tasks. The role of farmers in the adaptation is essential, that is why beliefs of farmers in this theme are important. In our work we give an overview about the opinion, knowledge and attitude of farmers in relation to climate change. We revealed their knowledge and individual insights with questionnaires. 50 farmers from the area of Kiskunság participated in it. Our aim with the questionnaire was to assess the farming community's information about the climate change, explore the changes in their natural environment and actions against unfavourable trends. In addition, we tried to get answers about what kind of assistance the producers require to overcome these problems in the future. Beside the questionnaire we interviewed large agricultural entrepreneurs, agricultural public administration employees and agricultural intellectuals about the effects of climate change in the agriculture. We found that farmers are mostly waiting for outside help for resolving their problems, in the form of financial support and a solution for the problem of irrigation. It is highlighted that up-to-date knowledge and flexibility are needed in order to prevent damages. It means adequate production technology and farming practices instead of only increasing the monetary aid.

Keywords: adaptation, agriculture, climate awareness

## Introduction

Nowadays, it is an established fact – not only in Hungary, but in the world –, that the climate sensitivity of many regions is high, and big territories are vulnerable to global warming and climate extremes. One of the most sensitive field is the agriculture. That is way vulnerability and adaptation are in the focus of the international literature recently (Tripathi – Mishra, 2016; Ayanlade et al. 2016; Khatri-Chhetri et al. 2017). The topic receives a special attention also in Hungary (Li et al. 2016; Birkás 2010; Soltész et al. 2011). On the base of the climate models, Hungary is in water deficiency more and more – summers become drier, the length and frequency of droughts are increasing. The expected changes are only the most anticipated processes. Besides these countless other unexpected effects may also arise, which can disrupt the delicate environmental balance of our landscapes. Involvement in agriculture is significant, therefore one of the most important questions of the future: how can the Hungarian agriculture and forestry adapt to the constantly changing microclimate in the next decades? How the entrepreneurs, interested in agricultural production, will be able to climate conscious thinking and how can they eliminate threats? In summary, what can the farmers do in the changing environment in order to maintain the values of agriculture and rural life – so what new alternatives and methods will be needed to preserve for successful land management? The role of farmers in the adaptation is significant; therefore their beliefs in this theme are important. In our work we give an overview about the opinion, knowledge and attitude of farmers in relation with climate change.

## Materials and methods

We revealed the knowledge of farmers and their individual insights with questionnaires. 50 farmers from the area of Kiskunság participated in the survey, which is not considered

representative, because a significant portion of the respondents are biofarmers, or family farmers with climate conscious production practices. Consequently they are more informed and more sensitive about changes in production circumstances in their work.

Our aim with the questionnaire was to assess the farmer community's information about the climate change, explore the changes in their natural environment and their actions against unfavourable trends. In addition, we tried to get answers about what kind of assistance the producers require to overcome these problems in the future.

Besides the questionnaire we interviewed large agricultural entrepreneurs, agricultural public administration employees and agricultural intellectuals about the effects of climate change in the primary sector. Altogether 14 conversations have been processed and we tried to achieve large national territorial coverage (from Baranya to Hajdú-Bihar counties).

The results described in this paper were made within the "Climate change and rural development – the effect of global warming on land use changing" scientific project, which was part of the "Rural research 2012-2013" programme, financed by Hungarian National Rural Network (MNVH), National Agricultural Advisory, Education and Rural Development Institute (NAKVI) and Hungarian Academy of Sciences (MTA).

**Results and discussion**

In our paper we highlight the most notable answers from the results of questionnaires. The interviewed farmers are working in the Homokhátság territory. Without exception all of them stated, that the condition of the landscape on Danube-Tisza Interfluve is rapidly deteriorating and the effects of climate change are well detectable. The experienced changes in their environment are the following: weather extremes, drought and uneven distribution of the precipitation.Respondents pointed to the declining yields especially, which is the most palpable consequence of the climate change during their farming (Fig. 1.).
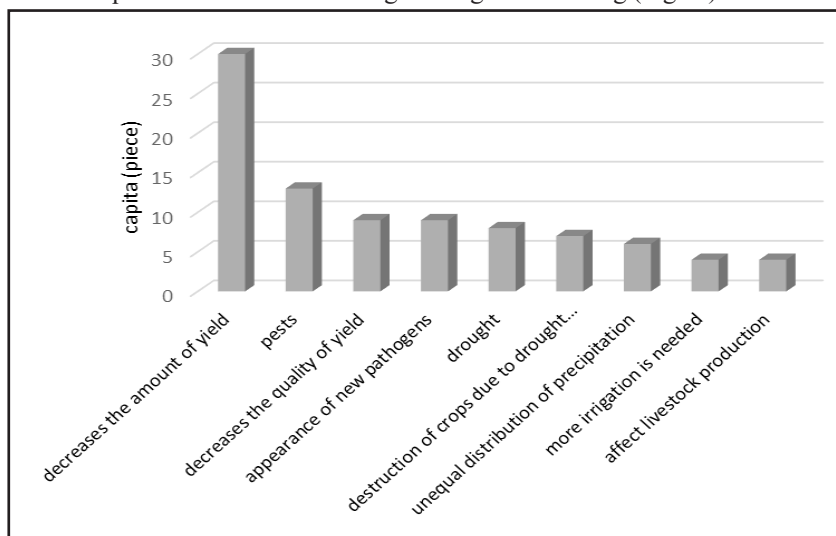


*Figure 1.: Detected effects in the respondents' own farm*

According to the farmers in the Kiskunság area, lower yield unfortunately also means lower quality. The unanimous response is: extremes of recent years influenced farming practices in the wrong direction. Respondents highlighted among the expected negative aspects that year after year more chewing and sucking pests can be found, pathogens proliferate, weeds spread intensively and more and more new species appear.

The actions, which have been already taken by the farmers to counterbalance the unfavourable changes, are important indicators of the future adaptation prospects. On the basis of their answers, they try to compensate the negative effects with changing the method and quantity of the irrigation (Fig 2.).
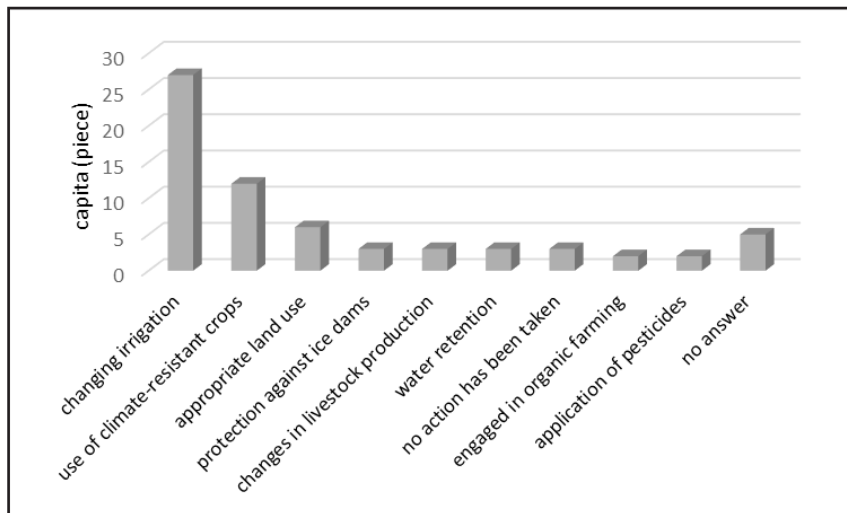


*Figure 2.: Actions taken against the effects of the climate change*

The interviews responses suggest that the large agricultural entrepreneurs try to change agricultural technology and to mainly use drought tolerant species. Many interviewed mentioned that they increased their irrigated area, or built ice nets in orchards. They use polymerized, encapsulated, or organomineral fertilizers and pesticides, which gradually migrate into the soil. Many farmers apply the mulch technology because the mixed crop residues on the soil retain water content.

It is definitely positive, that some of the respondents – in addition to irrigation and change of species – are also trying to adapt to the weather extremes with using appropriate land use, cultivation or soil coverage. They realised that it is in their interest to improve the air and water supply of the soil, or the reception and preservation of precipitation.

It suggests that farmers in the Danube-Tisza Interfluve now recognized the importance of rational management of the available water resources. At the same time, the construction of irrigation systems also enjoys a prime location among the actions planned in the future. It shows that the farmers still see irrigation as a key solution to water scarcity.

Those farmers, who do not dare to spend too much money for a bigger development, trying to focus more on selection of fertile materials. Because of this, they plan the acquisition of drought tolerant species. There were some farmers, who partially plan the change-over for new plant species, because they cannot finance replacing all the crops at once. There

are some farmers, who feel that they do not have enough information for the cultivation of new, drought tolerant species. Agricultural public administration employees primarily highlighted the role of water retention and water management.

Over half of the interviewed farmers did not ask for help from anywhere so far, in order to solve the problems of global climate change. Most farmers informed themselves individually (mainly from the media) and they try to overcome obstacles with their own power. Those who used some support turned for information and advice to the consultants of National Association of Agricultural Economics.

In response to the question "what kind of support they expect in the interest of successful adaptation in the future", respondents highlighted technological development (which mostly means irrigation technology) and support for accessing new plant species (Fig. 3.).

The answers of the farmers show the recognition of the problems related to the climate
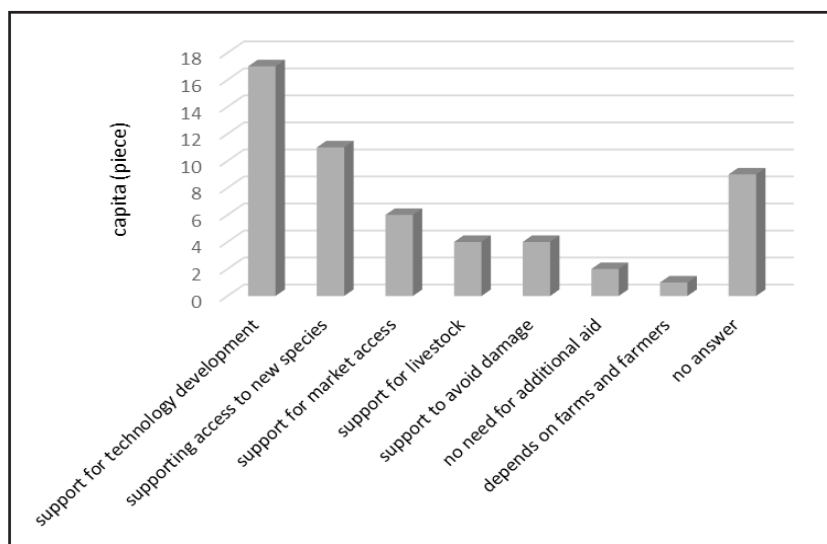


*Figure 3: Importance of subsidies according to farmers*

change but also show they do not have enough information about the various solution possibilities. From our results we can clearly see that the farmers get their information mainly from the media because there are no other easily available information sources despite of the demand.

Besides the demand for consultancy and information services the farmers think a new water governance system should be introduced in the Danube-Tisza Interfluve to maintain and secure the production output of the primary sector. In their opinion this new water governance should include new irrigation channels and maintenance of the existing ones which highlight the fact they would like to solve the water scarcity and aridity problems with new water supplies (e.g. from Danube). In accordance with the EU strategy for the 2014-2020 period we can recommend the following actions for a successful adaptation process:

- introduction of a new land use system which is in line with the environmental conditions,

- new research programmes to identify the long-term social and economic effects of the new planned land use system,

- new insurance and risk management system to counteract the negative effects of climate change for the producers and the rural population too,

- introduction of new innovative technologies and raising the proportion of renewable energy consumption in the primary sector,

- strengthening of agro-environmental and climate protection targets and subsidies within the CAP.

In addition to the above mentioned recommendations, to make the adaptation process successful, the public administration and the farmers need information and instructions what to do at a local or personal level. These can form the base or the starting point of a broader change in the production and the land use system too.

In the area of Homokhátság significant investments are needed because of the special environmental, economic and social conditions. The complex elements of this development process can be the following: introducing a water retention system with new water governance which involves infrastructural and institutional investments, improving internal and external accessibility of the region, promoting the use of renewable energy resources and utilization of its bioenergetics potential.

**Conclusions**

Agriculture is one of the key areas for the successful adaptation to climate change. This success is mostly depends on the farmers and on their knowledge and actions. That's why it is important especially in the case of Hungary to provide the producers with information and possible adaptation strategies which they could use in the everyday production.

The producers wait for the help of the state in this question, and it is important to know they demand more subsidies and solutions for the water scarcity. Also they would like to get the support of the municipalities and the local societies especially in the question of irrigation water. This means they would like to reduce the price of irrigation water and to build and maintain new irrigation channels. This situation would be optimal for the producers but it will not encourage activities related to water retention or reduce water demand and the overall sustainability of agriculture will not improve. The producers have to consider new methods and technologies because of aridity before they demand new water supplies for the area.

These facts show that the first step to a successful adaptation is to change the attitude of the farmers and raise their climate awareness. During this learning process we think they will understand the subsidies will not solve their problems in the long term. The consumers can also contribute to this above mentioned transition. It is obvious nowadays that consumers looking for healthy products, but they can also take into account other aspects such as whether a product is climate friendly or not. So the technological changes in the production should be ensured not only with regulations and subsidies but also with building demand for products which are produced in a sustainable way. This can generate a market pressure on the farmers which will help to change their attitudes.

The producers have to be interested in the damage prevention related to the effects of the climate change such as extreme weather conditions. This is important from the aspect of mutual responsibility and insurance costs in the primary sector, which can rise rapidly in the future. The farmers can reduce these costs by changing their activities based on new scientific results for example if they switch to the production of drought tolerant crops.

The National Rural Strategy for 2012-2020 also states that the conservation of natural resources and values are the most important strategic task of a sustainable agricultural and rural development policy. The agricultural output can be increased in the future but the producers must take the environmental conditions into account and have to apply sustainable land use management techniques in their activities.

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# Nitrate dispersion-diffusion coefficients in agricultural soil profile of Žitný ostrov locality (Slovakia)

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Abstract: Agricultural activities are probably the most significant anthropogenic sources of nitrate contamination. Nitrates ($NO_3^-$) are the one of the principal N forms taken up by plants. This fact has motivated the intensive use of nitrogen-based fertilizers to boost up the productivity of crops. Nevertheless, when nitrogen-rich fertilizer application exceeds the plant demand and the denitrification capacity of the soil, nitrogen can leach to groundwater usually in the form of nitrate which is highly mobile with little sorption. The non-point sources of pollution include fertilizer and manure applications, dissolved nitrogen in precipitation, irrigation flows, and dry atmospheric deposition. Numerous studies have suggested that leaching of $NO_3^-$ following high input rates of chemical fertilizer and due to mineralization of organic N already present in the soils can cause degradation of surface and groundwater quality. The experiments studying transport of nitrate ions were conducted by soil column leaching tests for unsaturated steady-state flow, defined geometry of the soil columns, defined volumetric water content, dry mass density. Measured values were depicted as a function $c/(c)o = f(t)$. The experiments were conducted for various average pore velocities $v_1 = 3,5.10^{-6}$ m.s$^{-1}$, $v_2 = 5,8.10^{-7}$ m.s$^{-1}$, $v_3 = 8,3.10^{-8}$ m.s$^{-1}$ and four initial ion concentrations : $c_o = 100, 200, 300, 500$ mg.l$^{-1}$. By interpreting of $c/co = f(t)$ dependences (breakthrough curves) the significant values of $(c/co)_{max}$ and $t(c/co)_{max}$ for nitrate ions were obtained and the dispersion-diffusion coefficients $D_i$ were estimated.

Keywords: water quality, nitrate ions, transport parameters, dispersion-diffusion coefficients

## Introduction

Nitrogen (N) is a critical nutrient needed by all plants for growth. Nitrate (NO3-) is the one the principal N forms taken up by plants. As such, inorganic N is widely used in agriculture and numerous studies have suggested that high input rates of fertilizers can cause degradation of surface and groundwater quality (Almasri et al., 2007).

The studied soil samples were take away from the Žitný ostrov area (Slovakia). The Žitný ostrov is one of the most productive agricultural areas of Slovakia, situated on the Danube Lowland. Under its surface is the richest water reservoir of Slovakia. For this reason it is very important to deal with quantity and quality of water resources in this region. In terms of geomorphology, this territory can be characterised as a young riverside plain with a very low denivellisation of the surface with an overall inclination from north-west to south-east. The territory is break up in transversal as well as in longitudinal directions into different geomorphological areas. In the sense of transversal division, we recognize the upper, the middle and the lower parts of the Žitný ostrov. We distinguish the Pleistocene core, late Holocene aggradation dikes (walls) and early Holocene depressions. The early Holocene depressions are characterised by a relatively high groundwater table. In terms of climatic situations, the Žitný ostrov belongs to a very warm and very dry agroclimatic area with mild winter. In terms of hydrological conditions, the Quaternary formation of sandy gravels created conditions for accumulation of groundwaters (Koczka Bara et al., 2014).

Anthropogenic activities realized in river basins may result in a deterioration of water quality with detrimental effects on the ecosystems. Nitrate leaching from agricultural land is usually considered a non-point source pollution problem, making the specific polluter hard to identify (Čelková, 2014, Dulovičová-Velísková, 2014, Wick et al., 2012). Problems

of determination of the hydrodynamic dispersion in river with presence of transient storage were solved by Sokáč-Velísková, 2016. Nitrate ($NO_3^-$) is the one of the principal N forms taken up by plants. Numerous studies have suggested that leaching of $NO_3^-$ following high input rates of chemical fertilizer and due to mineralization of organic N already present in the soils can cause degradation of surface and groundwater quality.

**Materials and methods**

A theoretical analysis of the movement of nitrogen conpounds in unsaturated soil with zero-order denitrification is presented in many studies. Analytical solutions of equations describing miscable displacement for steady-flow conditions and uniform water contents are known for many boundary conditions. The differential equation in the one-dimensional form used for consideration of advective-dispersive transport assume isothermal conditions, difference, no-volume change conditions is written (van Genuchten et al., 1982, Yong et al., 1992) :

$$\frac{\partial(\theta c)}{\partial t} = \frac{\partial}{\partial x}\left[D\,\frac{\partial(\theta c)}{\partial x}\right] - \frac{\partial(q\;)}{\partial x} \tag{1}$$

where c – the solute mass per unit volume of solut. ($ML^{-3}$), D – the diffusion-dispersion coefficient ($L^2T^{-1}$), θ- the volumetric water content ($L^3L^{-3}$), , q – the Darcian Flux of the soil water ($LT^{-1}$), t – time (T), x – distance(x). Equation (2) describing transport of water and chemicals in porous media, including sorption and microbial transformation terms is (de Smedt-Wierenga, 1978, van Genuchten et al., 1982) :

$$\frac{\partial c}{\partial t} = D\,\frac{\partial^2 c}{\partial x^2} - v\,\frac{\partial c}{\partial x} - \frac{\rho}{\theta}\,\frac{\partial S}{\partial t} \pm \Phi \tag{2}$$

where δS / δt represent sorption term and Φ - represent kinetic change of chemicals, ν - average pore-vater velocity. The governing differential eqaation in the one-dimensional form used for consideration of advective-dispersive transport assume isothermal conditions, absence of significant density difference, no-volume change conditions, without transformations for i-ion can be written (2):

$$\frac{\partial c_i}{\partial t} = D_i\,\frac{\partial^2 c_i}{\partial x^2} - v_i\,\frac{\partial c_i}{\partial x} - \frac{\rho}{\theta}\,\frac{\partial S_i}{\partial t} \tag{3}$$

where $D_i$ – the diffusion-dispersion coefficient ($L^2T^{-1}$), ρ – the dry mass density ($ML^{-3}$), $v_i$- average pore-vater velocity ($LT^{-1}$), S – the adsorbed amount of ion ($MM^{-1}$).

According to Gupta and Greenkorn (1974) :

$$D_i = \frac{v_i.x}{4\pi\left(\dfrac{\partial c_i}{\partial \beta}\right)^2_{\beta=1}}$$

(4)

where β - is the pore volume, $\beta = v_i.t/x$.

The methods for estimation of $D_i$ numerically were introduced (van Genuchten - Wierenga, 1986, Almasri et al., 2007, Kanvar et al., 1980, de Smedt et al.,1978, Grattoni et. al, 1993).

Experiments studying transport of nitrate ions were conducted by soil column leaching tests in four soil column separately. Defined geometry of the soil columns (radius r = 0.03 m, cross-section area $2,826.10^{-3}$ m², length h = 0.3 m) and unsaturated, steady state flow of liquid phase was used. The columns were filled up by the soil continuously. Soil samples were mechanically adapt (dried, crushed and 2 mm size sieved) and chemically (wash by LiCl) prepare. Laboratory experiments were conducted for three pore velocity : $v_1 = 3,5.10^{-6}$ m.s⁻¹, $v_2 = 5,8.10^{-7}$ m.s⁻¹, $v_3 = 8,3.10^{-8}$ m.s⁻¹ and four initial ion concentrations : $c_o = 100, 200, 300, 500$ mg.l⁻¹. Volumetric water content of the soil, determined gravimetric, take values in range 0,32 – 0,38 cm⁻³.cm⁻³. Dry mass density values were in range 1,32 – 1,40 g.cm⁻³ . The columns were washed by defined volume V = 500 cm³. The time changes of nitrogen ion concentrations were observed. Nitrates were determined by spectrofotometer DR 2800 fy Hach Lange. Measured values were graphicaly depict as function $ci / (ci)o = f(t)$. The experiments were conducted in pH 6,5 and t = 25°C conditions.

## Results and discussion

Measured values were graphically plotted as function $c_i / (c_i)_o = f(t)$. The dispersion-diffusion coefficients $D_i$ were estimated from the equation suggested by Gupta and Greenkorn (Eq. 4). For laboratory experiments were used soil samples from locality Lehnice (Calcaro-haplic Phaeozem), characterful for Žitný ostrov. The soil texture, physical and chemical properties were determined (Tab.1-3).

Table 1: Soil textue (grain composition)

| locality | Soil unit | Sample depth (m) | Sand (%) >0,25  0,25-0,05 (mm) | | Silt (%) 0,05-0,01  0,01-0,001 (mm) | | Clay % <0 001 (mm) |
|---|---|---|---|---|---|---|---|
| Lehnice | Calcaro-haplic Phaeozem | 0.1 – 0.3 | 7 | 18 | 38 | 37 | 15.6 |
| | | 0.4 – 0.7 | 7 | 16 | 42 | 35 | 16.1 |
| | | 0.7 – 1.0 | 11 | 19 | 41 | 29 | 14.3 |

*Table 2: Physical properties*

| locality | Sample depth | $\rho_s$ | $\rho_d$ | P | $\theta_{FC}$ | $\theta_{PDA}$ | $\theta_{WP}$ | AC |
|---|---|---|---|---|---|---|---|---|
| | (m) | g.cm⁻³ | g.cm⁻³ | % | pF 2,3 | pF 3,4 | pF 4,2 | % |
| Lehnice | 0.1 – 0.3 | 2.70 | 1.62 | 40 | 34.1 | 21.5 | 15.4 | 10.8 |
| | 0.4 – 0.7 | 2.72 | 1.47 | 45.9 | 33.8 | 19.8 | 16.6 | 7.9 |
| | 0.7 – 1.0 | 2.73 | 1.54 | 43.5 | 29.6 | 17.3 | 15.3 | 5.6 |

$\rho_s$,$\rho_d$ – particle/bulk density,P–tot. porosity,$\theta_{FC}$ –field cap., $\theta_{PDA}$ –point of dec. avail., $\theta_{WP}$ –wilting point, AC–min. air capacity

*Table 3: Chemical attributes*

| locality | Depth | pH | | $CaCO_3$ | $C_{ox}$ | Humus | EC | ESP |
|---|---|---|---|---|---|---|---|---|
| | (m) | $H_2O$ | KCl | % | % | % | mS.m⁻¹ | % |
| Lehnice | 0.1 – 0.3 | 7.9 | 7.6 | 29 | 1.8 | 3.5 | 95 | 3.15 |
| | 0.4 – 0.7 | 8.2 | 7.8 | 32 | 1.9 | 3.7 | 82 | 2.75 |
| | 0.7 – 1.0 | 8.4 | 7.9 | 30 | 1.2 | 2.8 | 113 | 2.51 |
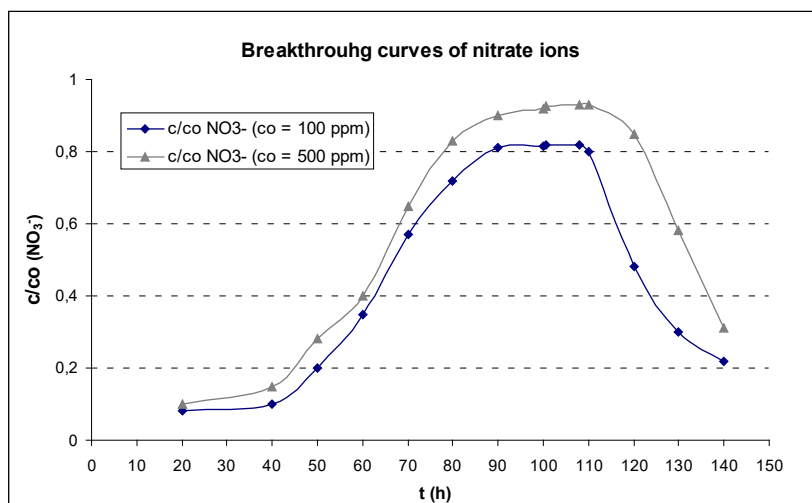


*Figure 1. Measured breakthrough curves for $NO_3^-$ ions stemming from a pulse of $NO_3^-$ (100 and 500 mg.l⁻¹) leached through the short soil column at 25°C ( pore velocity $v_1 = 3,5.10^{-6}$ m.s⁻¹)*

$CaCO_3$ – cont. of carbonates, Cox – organic carbon, EC – electrical conductivity, ESP – exchangeable sodium percentage

Estimated dispersion-diffusion coefficients $D_i$ are sumarised in Tab. 4.

*Table 4: Dynamic parameters of the columns*

| $c_o$ mg/l $NO_3^-$ | 100 | 200 | 300 | 500 |
|---|---|---|---|---|
| $\theta$ [cm³/cm³] | 0.32 | 0.35 | 0.33 | 0.35 |
| $\rho$ [g/cm³] | 1.32 | 1.38 | 1.40 | 1.35 |
| $v_1 = 3.5 \times 10^{-6}$ m/s | | | | |
| $(c/c_o)_{max}$ | 0.82 | 0.85 | 0.88 | 0.93 |
| $t_{(c/co)max}$ [h] | 100.5 | 102.3 | 105.0 | 108.3 |
| D [m²/s] | $2.1 \times 10^{-9}$ | $2.3 \times 10^{-9}$ | $1.2 \times 10^{-9}$ | $9.9 \times 10^{-9}$ |
| $v_2 = 5.8 \times 10^{-7}$ m/s | | | | |
| $(c/c_o)_{max}$ | 0.85 | 0.87 | 0.92 | 0.95 |
| $t_{(c/co)max}$ [h] | 140.3 | 143.2 | 145.2 | 148.4 |
| D [m²/s] | $3.7 \times 10^{-10}$ | $4.6 \times 10^{-10}$ | $3.2 \times 10^{-10}$ | $2.5 \times 10^{-10}$ |
| $v_3 = 8.3 \times 10^{-8}$ m/s | | | | |
| $(c/c_o)_{max}$ | 0.85 | 0.90 | 0.94 | 0.95 |
| $t_{(c/co)max}$ [h] | 210.0 | 218.5 | 225.0 | 230.5 |
| D [m²/s] | $5.2 \times 10^{-11}$ | $7.2 \times 10^{-11}$ | $9.1 \times 10^{-11}$ | $1.1 \times 10^{-12}$ |

$\theta$ - volumetric water content, $\rho$ - dry mass density, $v_1$, $v_2$, $v_3$ - average pore water velocity, D – dispersion-

diffusion coefficient

## Conclusions

The resulting process of the nitrates migration may be characterized with the solution of partial precesses of the dispersion type, adsorption type, transformations type etc. Estimation of transport, adsorption and transformation nitrogen compounds parameters is need for modeling the movement in an unsaturated soil.

In this paper the main role was directed on the behaviour out the nitrate ions in dynamical conditions of the electrolyte flow. The concentration profiles were estimated and was investigated the influence of the flow electrolyte velocity on the change of nitrates ions concentration in the soil columns at defined conditions. The quantitative parameters of the transport equation were investigated.

From measuring values results that included with decreasing the flow velocity and increasing the initial concentration, the dispersion-diffusion coefficient is decreasing. The dependences $c/co = f(t)$ (breakthrough curves) and the values of $(c/co)_{max}$, $t(c/co)_{max}$ are significant in achievement steady-state in exact time (Fig. 1). The dispersion-diffusion coefficients $D_i$ were estimated for three pore velocity $v_1$, $v_2$, $v_3$ by Gupta and Greenkorn equation as input parameters to the mathematical models (Tab. 4).

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# Effects of carbon dioxide concentration on chlorophyll fluorescence of peas „*Pisum sativum* L."

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Abstract: The atmospheric concentration of carbon dioxide increases from decade to decade in increasing pace. In 1957, atmospheric carbon dioxide levels were around 315 ppm, while in 2012 it amounted to 394.49 ppm concentration. The atmospheric concentration of carbon dioxide is expected to reach 550 mol$^{-1}$ to mid-century. In parallel, the global temperature is rising, which is projected to average 1.5-4.5°C. These global environmental changes, directly or indirectly affect plant growth, development, yield and quality of the crop. During the research, in climate chambers, "Irina" pea was sowed, which were tested near 700 and 400 ppm carbon dioxide concentration. Chlorophyll fluorescence was measured both in dark-adapted ($F_v/F_m$ test) and in light-adapted leaf samples (Yield test; Y(II)).

Keywords: carbon dioxide, concentration, pea, chlorophyll fluorescence

## Introduction

The rising concentration of atmospheric carbon dioxide ($CO_2$) contributes to global warming, and thus the changes affect both precipitation and evaporation quantity. Moreover, the concentration of carbon dioxide directly affects the productivity and physiology of plants (Kruijt, 2008). The atmospheric concentration of carbon dioxide is expected to reach 550 ppm in the middle of the century (Carter et al., 2007). In parallel, the global temperature is rising, which is projected to average 1.5-4.5°C. It could be more frequent occurrences of extreme weather events such as heat waves and/or drought (Carter et al., 2007). These global environmental changes, either directly or indirectly affect plant growth and development, yield and quality of the crop (Ainsworth and Rogers, 2007, Seneweera et al., 2005). The carbon dioxide concentration is a key factor that in interaction with the light, affects the plant's photosynthesis. If a particular crop is being tested, the $CO_2$ concentration may be influenced by the contents of soil organic matter (soil respiration), the type of plants, air movement, etc. The crop itself is an opened ecosystem, which has continous, constant and dynamic interaction with the biotic and abiotic environmental factors. The rate of photosynthesis is affected by a number of external (environmental) factors such as light intensity, $CO_2$ concentration, temperature, water and nutrient supply, and internal factors such as the plant's age, medical condition, particularly to the leaves. Among the various factors significant interactions prevail: environmental factors affect the growth and development of plants, leaf area and composition, the functioning of the photosynthetic apparatus, the duration and length of growing season.

## Materials and methods

Chlorophyll fluorescence was measured in dark-adapted and in light-adapted samples using $F_v/F_m$ (dark-adapted) test to determinate the maximum quantum yield and light adapted yield of photosynthetic efficiency of PSII for the determination of effective quantum photochemical yield. The maximum quantum yield of PSII in the samples was measured

after 30 min-long dark adaptation of the leaves by using $F_v/F_m$ protocol. $F_v/F_m$ ratio is used for estimate of the largest proportion of absorbed quanta used in PSII reaction centres. Dark adaptation allows the reoxidation of PSII and to relax nonphotochemical quenching. Minimum and maximum fluorescence ($F_0$ and $F_m$) of dark-adapted leaves were measured in the same leaves after 0.8 second of saturation pulse (35W halogen lamp with 690 nm short pass filter) on previously dark-adapted samples. Variable fluorescence ($F_v=F_m-F_0$) and maximum quantum yield of PSII ($F_v/F_m$) were calculated by the Fluorometer software, and maximal efficiency of the photochemical process in PSII ($F_v/F_0$) could be counted. Actual quantum yield of PSII (Y(II)) in the samples was measured using Yield protocol which is a light adapted steady-state test of photosynthesis, measure the ratio of light amount used in photochemistry in PSII, the light amount adsorbed by chlorophylls of PSII. Leaves were tested by steady-state photosynthetic conditions. This protocol shows the achieved efficiency of PSII in addition specific light condition. Steady-state fluorescence ($F_s$) and maximum fluorescence ($F_{ms}$) of light-adapted leaves were measured, actual quantum yield of PSII (Y(II)=($F_{ms}-F_s)/F_{ms}$)) and estimated relative electron transport rate (ETR) were calculated by the Fluorometer software. The tests were occured in precision and hermetically sealed air chambers. The soil emissions of carbon dioxide continuously and easily can be measured. In addition to monitoring the reactions of plants, chlorophyll fluorescence measurements were made. We can do statistical comparison of the research and compare the results. Datas were analyzed statistically by Independent-Samples T test for all pair wise comparisons using SPSS for Windows (SPSS®, version 21.0) at $p \leq 0.05$. Experiment details are the following; the plant was Irina pea (3x3 crops / climate chamber), the soil contained N>0.3 w/w%, $P_2O_5$>0.1 w/w%, $K_2O$>0.3 w/w%, pH 6.8, the drilling depth was 5 cm, 14 hours of light condition, 21-23°C internal temperature, varying humidity, measurements schedule happened in 4-6 leaf and flowering phenophases.

## Results and discussion

Yield Protocol: In terms of steady-state fluorescence there is significant difference between the two concentrations. At higher concentration, higher value can be observed. The efficiency of PSII system was superior to 700 ppm concentration. In case of actual quantum yield of PSII, the difference is significant, as it is with ETR, by the way, the actual quantum yield of PSII was better on lower concentration (Table 1).

*Table 1: Differences between 400 and 700 ppm $CO_2$ concentrations in 4-6 leaf phenophase by Yield protocol*

|  | CO₂ ppm | N | Mean | Std. Deviation | F | Sig. | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| **Fs** | 700 | 4 | 1384 | 66.79321 | 2.585 | 0.159 | 0.001 |
|  | 400 | 4 | 808 | 189.910 |  |  | 0.006 |
| **Fms** | 700 | 4 | 3874,75 | 3.86221 | 7.026 | 0.038 | 0.235 |
|  | 400 | 4 | 3410,25 | 704.583 |  |  | 0.279 |
| **Y** | 700 | 4 | 0,6425 | 0.01748 | 0.896 | 0.38 | 0.000 |
|  | 400 | 4 | 0,7638 | 0.0096 |  |  | 0.000 |
| **ETR** | 700 | 4 | 32,35 | 0.85829 | 0.736 | 0.424 | 0.000 |
|  | 400 | 4 | 38,475 | 0.49244 |  |  | 0.000 |

In phenophase of flowering, steady-state fluorescence shows no significant difference between the two concentrations. Among the PSII efficiency of the systems, there were no significant differences and there is no significant difference in maximum fluorescence too. In case of actual quantum yield of PSII the difference is significant, as it is with ETR, but it is striking that in the case of flowering the actual quantum yield of PSII was better on higher concentration (Table 2).

*Table 2: Differences between 400 and 700 ppm $CO_2$ concentrations in flowering phenophase by Yield protocol*

|  | $CO_2$ ppm | N | Mean | Std. Deviation | F | Sig. | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| **Fs** | 700 | 4 | 1241,25 | 55,61999 | 22,44 | 0,00 | 0,038 |
|  | 400 | 4 | 2164,5 | 694,79325 |  |  | 0,076 |
| **Fms** | 700 | 4 | 3876,75 | 2,06155 | 9,66 | 0,02 | 0,179 |
|  | 400 | 4 | 3591,5 | 374,89421 |  |  | 0,225 |
| **Y** | 700 | 4 | 0,679 | 0,01431 | 23,03 | 0,00 | 0,019 |
|  | 400 | 4 | 0,4008 | 0,17373 |  |  | 0,049 |
| **ETR** | 700 | 4 | 34,175 | 0,73655 | 22,56 | 0,00 | 0,019 |
|  | 400 | 4 | 20,15 | 8,76907 |  |  | 0,049 |

$F_s$: Steady-state fluorescence, $F_{ms}$: maximum fluorescence, Y: actual quantum yield of PSII, ETR: relative electron transport rate

$F_v/F_m$ protocol: In 4-6 leaf phenophase, minimum fluorescence according to a significant difference but no significant difference was measured by maximum fluorescence. Variable fluorescence shows significant differences too. In case of $F_v/F_m$ ratio, significant differences were observed as a result, and further measurements as well $F_v/F_0$ (Table 3). In case of atmospheric concentration, in 4-6 leaf stage, the values are higher, and they are significantly different.In flowering phenophase, minimum fluorescence according to a significant difference but no significant difference by maximum fluorescence. Variable fluorescence shows significant differences as it was in 4-6 leaf stage. Here too in $F_v/F_m$ ratio, significant differences were measured as a result, and $F_v/F_0$ as well. The measured values are higher near 400 ppm concentration in this phenophase too (Table 4).

Table 3: *Differences between 400 and 700 ppm $CO_2$ concentrations in 4-6 leaf phenophase by $F_v/F_m$ protocol*

|  | CO2 | N | Mean | Std. Deviation | F | Sig. | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| **Fo** | 700 | 4 | 1257.00 | 61.04 | 0.26 | 0.63 | 0.00 |
|  | 400 | 4 | 973.75 | 51.14 |  |  | 0.00 |
| **Fm** | 700 | 4 | 3871.25 | 3.50 | 3.00 | 0.13 | 0.47 |
|  | 400 | 4 | 3872.75 | 1.71 |  |  | 0.48 |
| **Fv** | 700 | 4 | 2614.25 | 60.16 | 0.24 | 0.64 | 0.00 |
|  | 400 | 4 | 2899.00 | 49.91 |  |  | 0.00 |
| **FvFm** | 700 | 4 | 0.68 | 0.02 | 0.25 | 0.64 | 0.00 |
|  | 400 | 4 | 0.75 | 0.01 |  |  | 0.00 |
| **FvFo** | 700 | 4 | 2.09 | 0.15 | 0.26 | 0.63 | 0.00 |
|  | 400 | 4 | 2.99 | 0.20 |  |  | 0.00 |

Table 4: *Differences between 400 and 700 ppm $CO_2$ concentrations in flowering phenophase by $F_v/F_m$ protocol*

|  | CO2 | N | Mean | Std. Deviation | F | Sig. | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| **Fo** | 700 | 3 | 1293.67 | 53.72 | 0.32 | 0.60 | 0.00 |
|  | 400 | 4 | 843.00 | 43.40 |  |  | 0.00 |
| **Fm** | 700 | 3 | 3873.00 | 5.29 | 6.04 | 0.06 | 0.46 |
|  | 400 | 4 | 3789.25 | 176.85 |  |  | 0.41 |
| **Fv** | 700 | 3 | 2579.33 | 54.50 | 2.07 | 0.21 | 0.01 |
|  | 400 | 4 | 2946.25 | 148.90 |  |  | 0.01 |
| **FvFm** | 700 | 3 | 0.67 | 0.01 | 1.54 | 0.27 | 0.00 |
|  | 400 | 4 | 0.78 | 0.01 |  |  | 0.00 |
| **FvFo** | 700 | 3 | 2.00 | 0.12 | 1.01 | 0.36 | 0.00 |
|  | 400 | 4 | 3.50 | 0.16 |  |  | 0.00 |

$F_0$, $F_m$: minimum and maximum fluorescence, $F_v$: variable fluorescence, $F_v/F_m$: maximum quantum yield of PSII, $F_v/F_0$: maximal efficiency of the photochemical process in PSII

## Conclusions

The photochemical efficiency was measured by fluorescent parameters ($F_0$, $F_v$, $F_m$ - minimum, variable and maximum chlorophyll fluorescence) with comparing these ratios. The $F_v/F_m$ ratio is also informative to us, in terms of efficiency. In our experiments, significant differences can be seen in several cases. It causes by interaction between the plant and the environmental factors ($CO_2$). In Yield protocol, the fluorescence maximum values are higher near 700 ppm $CO_2$ concentration in the case. In case of $F_v/F_m$ protocol informative indicators were higher in all cases near lower concentrations. It is also important to note that at 700 ppm concentration, the vegetation period is shorter, the plants thrived after 35 days, the 4-6 leaf stage achieved within 16 days. Despite the higher concentration, the green mass of plants grown significantly more than in control plants. Overall, the increased green mass, the shortened growing season are due to the increasing level of $CO_2$ concentration. I am planning tests under different concentrations, which will be compared with the current results and further examination of plant varieties.

## Acknowledgement

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# Infiltration and runoff measurements on arable land with different slopes and rainfall intensities

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Abstract: Nowadays the fertile soil and the water retention have a great importance, therefore more and more studies deal with the subbject of surface runoff decrease and infiltration capacity increase. In the present study *in situ* rainfall simulation experiments were carried out on different plots in order to characterize the soils' infiltration rate under different circumstances. On the field three intensities (30–60 and 90 mm/h) and two slope steepness (5-12%) were investigated. The main objective was that analyses of the effect of different conditions on soil permeability, conductivity and infiltration capacity. During the measurements, the wetting, the ponding and runoff periods and rates were registered, so the soil saturation process was permanently followed. The results show that the infiltration rate decreased, while runoff rate increased with time and higher intensities. Proportionally with slope increasing the infiltration rates were decreasing. At 30 mm/h rainfall intensity the infiltration rate mostly exceeds the runoff rate, while at higher intensities the runoff starts earlier. On average the infiltration intensity not increased with increasing rain intensities.

Keywords: Rainfall simulation, runoff, infiltration rate,

## Introduction

Soil protection plays a crutial role in our future, especially with the threats of climate change, increasing brownfield investments (Martinát et al. 2016), and furthermore, urbanization processes. Soil infiltration capacity is one of the most important factors with respect to soil water erosion. The surface runoff occurs when the rainfall intensity exceeds this infiltration capacity (Dunne and Leopold, 1978), therefore the challenge of recent days is the water retention within landscape by increasing the infiltration capacity. Infiltration capacity depends on several soil properties as soil texture, soil organic matter, soil pore system configuration etc. In case of soil erodibility, the soil hydraulic conductivity has a determining importance (Várallyay, 2011). Infiltration process is affected by rainfall intensity, soil surface state (crusted, freshly tilled), antecedent soil moisture content, surface roughness, slope steepness, as reported from studies under simulated rainfall conditions (Janeau et al., 2003; Rodrigo Comino et al., 2016; Van den Putte et al., 2013; Ribolzi et al., 2011; Truman et al., 2011; Rimal and Lal, 2009; Kato et al. 2008).

During the rainfall, the most important events are the start of ponding and runoff. The surface ponding indicates the surface saturation or sealing and crusting formation. At this stage the infiltration rate is less, than the rainfall rate, resulting in surface runoff. In some cases the hydraulic conductivity increasing, as it was noticed in case of higher rainfall intensities (Nassif & Wilson 1975). This phenomenon can be explained with the higher kinetic energy which can destroy and disrupt the surface seals and crusts, which could obstruct the infiltration (Bowyer-Bower, 1993). Similar observations were found by Jakab and Szalai (2005) who determined that the soil permeability increase proportionally with higher rainfall intensity load which is partly due to the increasing pressure of the water and partly due to influential effect of slope gradient.

Within this study our main objective was to examine the runoff and the soil infiltration rate under different rainfall intensities and slope gradients. The main research questions were as follows: 1. which parameter has greater impact on the infiltration rate; and 2. how the rainfall intensity affects the infiltration rate?

**Materials and methods**

The experiment was carried out in July 2015, in Gerézdpuszta (Somogy County, Hungary) with Shower Power-02 rainfall simulator, which was constructed by the Geographical Institute, Research Centre for Astronomy and Earth Sciences, Hungarian Academy of Sciences. In field 30–60 and 90 mm/h rainfall intensities and two slope sections with 5 and 12% inclinations were applied on fenced ground with 6 m² plot size (3x2 m). The device is equipped with two 80100 Veejet alternating nozzles. The rainfall intensity can be adjusted with the number of nozzle-swing during a given time. The drainage water is collected by two metal triangles with drain-pipe at the bottom of the plot. The runoff volume was registered through these triangles, and the time with the amount of runoff was read when one of the two measuring barrels reached the 2 liter limit.

The experimental area can be characterized with eroded Raman's brown forest soil and Regosol. At the infiltration intensity determination, the applied model was the Horton (1933) type, where the illustration of intensity in the mirror of time can give a curve which can characterize the whole process. The relationship has the following format:

$$Y = P0 * (x – P1) – (P0 / P2) * (1 – \exp(-P2 * (x-P1)))$$

where Y: Cumulative runoff (l); x time of the process (min); P0 maximal, constant runoff intensity (l/min); P1 the starting time of runoff (min); P2 Runoff changing indicator (1/min). In knowledge of P0 parameter we can represent the soil permeability in mm/h.

**Results and discussion**

Generally, the infiltration rate decrease is typical with time and when the runoff starts it shows near constant values. The runoff intensity proportionally increased with ascending rainfall intensity and slope gradient. Based on the charts (Figure 1-2.) we can reveal that under 20 mm/h rainfall intensity runoff doesn't occur, while at about 50 mm/h, the rate of infiltration and runoff is almost equal and above that the runoff rate exceeds the infiltration rate.
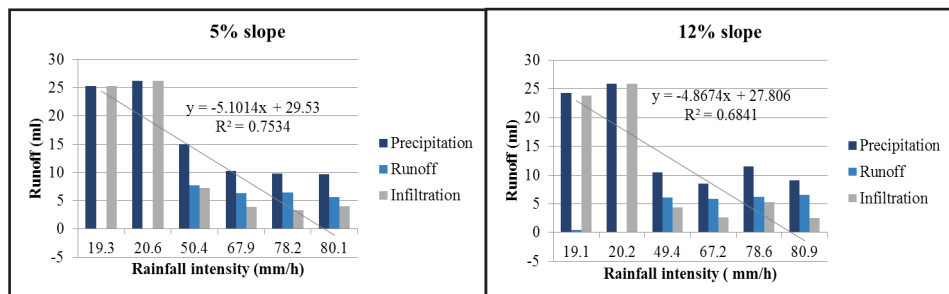


*Figure 1.: Relationship between rainfall intensity and infiltration rate in case of 5% and 12% slope*

Infiltration intensities were further investigated with respect to rainfall intensities and

two different slope inclinations (Figure 2.), where at 5% slope the infiltration intensity increased slightly with the rainfall intensity, what has not proved in case of 12% slope.
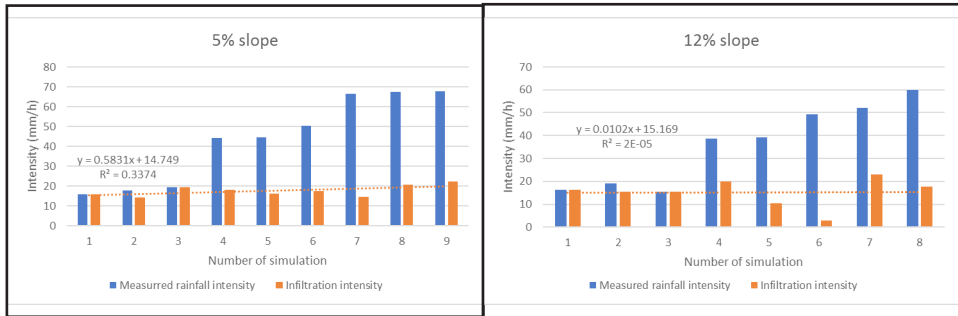


*Figure 2.: Relationship between rainfall infiltration intensity in case of 5% and 12% slope*

Based on the measurements, the relationship between the rainfall intensity and runoff is high (Figure 3.), therefore in this case we can tell that while the infiltration rate doesn't the amount of runoff more depend on the rainfall intensity.
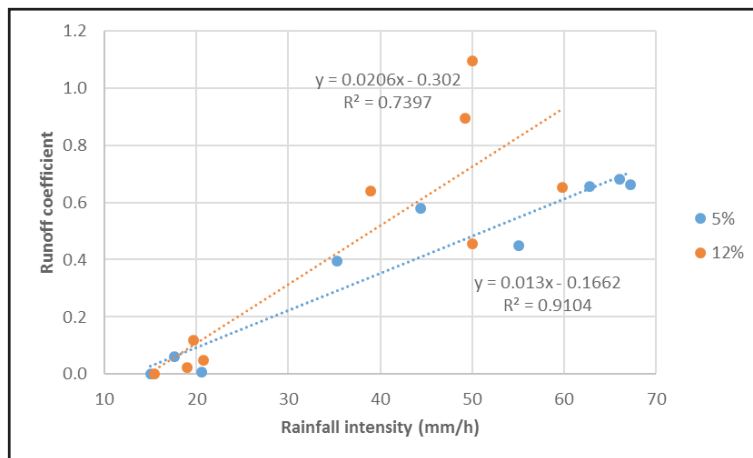


*Figure 3.: Relationship between rainfall intensity and runoff coefficient in case of 5% and 12% slope*

**Conclusions**

Based on the results the slope category and the rainfall intensity do not determine the infiltration rate, although on lower slope section slight (statistically insignificant) increasing was observed with higher rainfall intensities. Stronger relationship was observed between the rainfall intensity and the runoff coefficient.

**Acknowledgement**

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# Impact of crop year and nitrogen topdressing on the quantity and quality of wheat yield

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Abstract Impacts of N topdressing applications were studied in a field experiment to determine water availability grain yield and protein formation interrelations. Five winter wheat varieties and six nitrogen application levels were applied in two crop years representing different precipitation and temperature patterns to evaluate yield, yield components and quality manifestation. The results obtained suggest, that precipitation patterns in relation with the wheat development phenophases had profound influence on the grain yield and the protein formation of wheat crop. Varietal differences were determined regarding yield, protein values in relation with plant nutrition and crop year impacts. There were no, or minor differences only between varieties, however plant nutrition treatments induced significant differences in both crop years.

Keywords: N topdressing, wheat, grain yield, protein yield, crop year

## Introduction

Grain yield and yield quality of winter wheat *Triticum aestivum* L. is highly influenced by the meteorological conditions of the given crop year, especially the amount and distribution of precipitation and the actual temperature (Grimwade et al 1996, Győri 2008, Pepó 2010). Weather conditions are evaluated and labelled favourable or non-favourable in relation with the optimum requirements of the crops' phenophases (Lásztity 1999; Ványiné and Nagy 2012). Concerning precipitation, the most vulnerable periods during growth and development of winter wheat are the phenophases of heading and flowering (Feekes 10-10,5; Zadoks 51-70). In relation with temperature, two critical periods can be detected. One is the vernalisation, and the other is the ripening stage (Feekes 1-2 and 11; Zadoks 10-13 and 71-99), (Pollhamer 1981, Kismányoky and Ragasits 2003). Crop yield and grain quality can also be influenced by agronomic applications. Plant nutrition in general and N topdressing in particular should be considered as the most effective treatments within the technologies of winter wheat production. The amount of nitrogen and the timing and distribution of the application have an impact on wheat quality, especially on the protein production of the crop (Győri 2006, Pepó 2010, Vida et al 1996).

## Materials and methods

A wide range of high milling and baking quality winter wheat *Triticum aestivum* L. varieties were examined under identical agronomic conditions in a long term field trial. The small plot trials were run at the Nagygombos experimental field of the Szent István University, Crop Production Institute, Hungary. Soil type of the experimental field is chernozem (calciustoll).Annual precipitation of the experimental site belongs to the 550-600 mm belt of the Northern edges of the Hungarian Great Plain.  Experiments were conducted in a split-plot design with four replications. The size of each plot was 10 m². Plots were sown and harvested by plot machines (standard Wintersteiger cereal specific experimental plot machinery series). Various identical agronomic treatments were applied to plots. Plant nutrition applications were done in single and combined treatments. N topdressing variants were applied by single and repeated topdressings representing 6 levels:

0, 80, 80+40, 120, 120+40 and 160 kg/ha N in single and split applications. All plots were sown with identical series of wheat varieties for studying their performance in relation with agronomic impacts. The recent study presents the performance and evaluations of six winter wheat varieties (Alföld-90, Mv Magdaléna, Mv Suba, Mv Toborzó and Mv Toldi) of the 2013 and 2014 crop years. Wheat grain quality parameters: protein, and wet gluten contents were determined from grain samples, as well as quality characteristics at the Research Laboratory of the SIU Crop Production Institute, and RET Regional Knowledge Centre laboratories according to Hungarian and EU standards (MSZ 1998; EK 2000). The protein figures were correlated with the treatments applied, and analyses were done by Microsoft Office 2003 statistical programmes (Horváth 2014). Figure 1 demonstrates the phenophases of winter wheat by the grading of two internationally used systems. Phenological phases have been evaluated in accordance with the monthly precipitation and temperature figures of the respective crop years by the methods of Pollhamer (1981) and Kismányoky and Ragasits (2003).
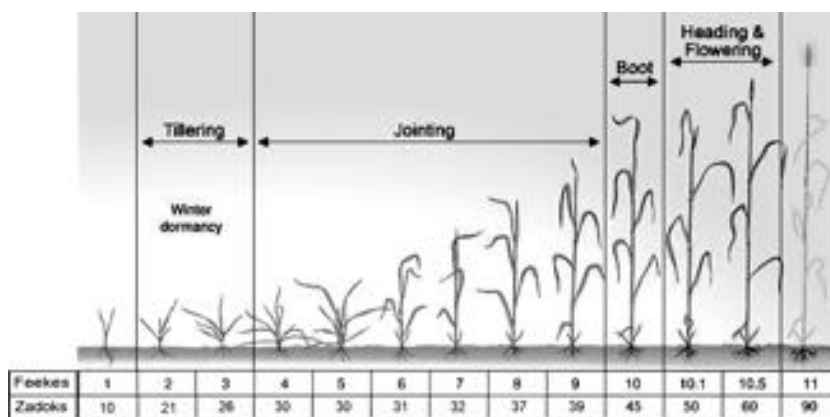


*Figure 1. Growth stages of winter wheat  - Feekes and Zadoks values. (Source Kismányoky and Ragasits 2003).*

Crop year conditions were evaluated in accordance with the monthly values of temperature and precipitation in favourable (2013) and non-favourable (2014) crop years during the vegetation period. The monthly periods are considered in accordance with the magnitude of deviation in relation with the long term mean temperature and precipitation values. A plus or minus 20 % of precipitation and 1 ºC of temperature were applied as threshold values.

### Results and discussion

Yield results of the trial are summarized in Figure 2 and 3. The total amount of grain yield (kg/ha) is indicated for the two respective crops years for all the wheat varieties examined.

The results obtained suggest, that the two crop years examined had different levels of grain yield regardless to varieties. In 2013 grain yield amounts ranged from 2,9 to 7,5 t/ha with definite differences between N applications, while in 2014 this turned to be 4,8 to 7,3 showing less variations between plant nutrition treatments. In both crop years minor varietal differences were detected only.

Quality information is provided by in Figure 4 and 5. The total amount of protein yield

(kg/ha) is indicated for the two respective crops years by all the wheat varieties examined. The results obtained highlight three factors. The first of them is the difference between the
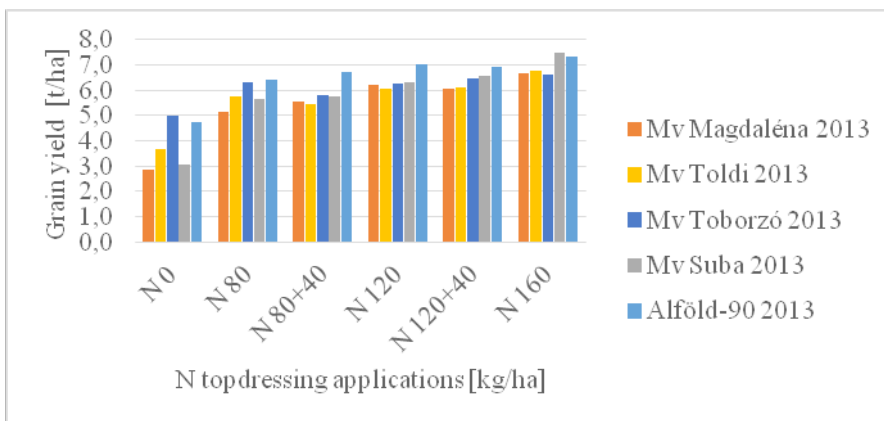


*Figure 2. Total grain yields in favourable crop year. Nagygombos 2013*
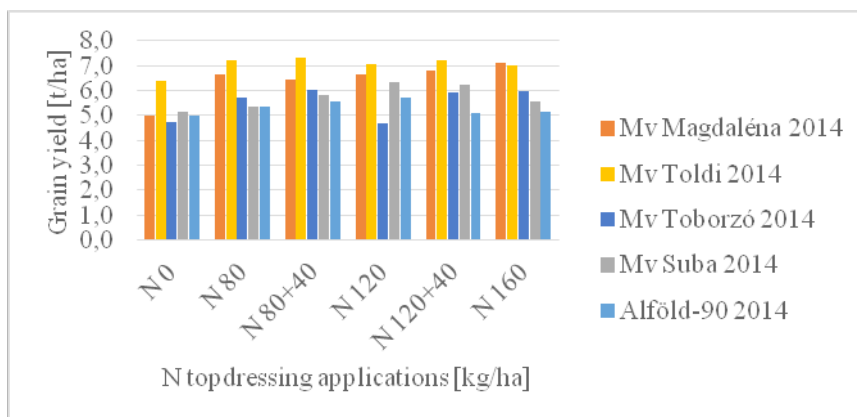


*Figure 3. Total grain yields in non-favourable crop year. Nagygombos 2014*
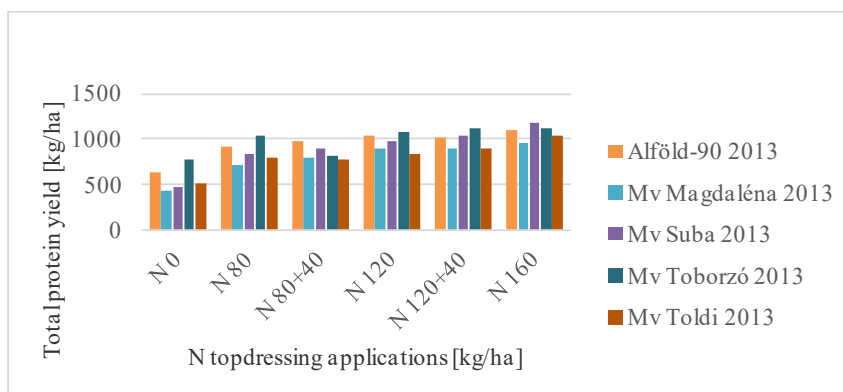


*Figure 4. Total protein yields in the favourable crop year. Nagygombos 2013*

amounts of protein yield. In 2013 the range of total amount of protein was between 412 and 1187 kg/ha. In 2014, a non favourable crop year resulted in 513 and 988 kg/ha protein yield values. The second is the consequent differences between the impacts of N application levels. These differences were significantly bigger in the favourable crop year in comparison with those of the non-favourable vintage. The reason of such deviation was due to the amount of precipitation during the phenophases of flowering and grain filling of the respective crop years.

The third factor detected was the performance of varieties. From among the five varieties examined three cultivars – Mv Suba, Mv Toborzó and Mv Toldi proved to be the most efficient regarding the amount of total protein yield production. The highest protein yields were obtained by Mv Toborzó in 2013, while in 2014 the Mv Toldi cultivar produced
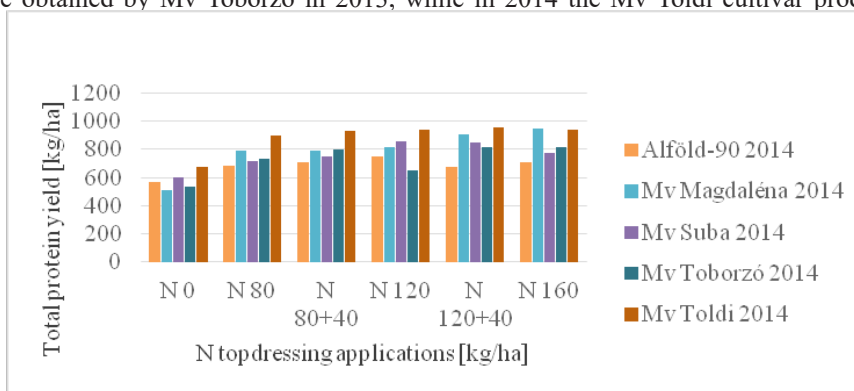


*Figure 5. Total protein yields in the non-favourable crop year. Nagygombos 2014*

superior figures. Tables 1 and 2 present correlation figures of experimental variants for both crop years. For better understanding, the tables show data on further interrelations not discussed in this paper, but which may provide information on the protein formation performance. These are the following apart from protein values: grain yield, hectolitre weight, thousand grain weight, gluten and Zeleny figures. The results obtained suggest that the strongest correlation was detected between the total amount of protein and the experimental treatments, regardless to the impact of crop years' weather in accordance with the findings of Győri (2008) and Pepó (2010).

*Table 1. Correlation between plant nutrition (control vs treatments), yield and protein yield by respective varieties. Nagygombos 2013*

| Wheat varieties | Yield [t/ha] | Hecto-litre weight [kg/hl] | Thousand grain weight [g] | NIR analysis data | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | Protein content [%] | Total amount of protein [kg/ha] | Gluten [%] | Zeleny number [ml] |
| r (Alföld-90) | 0.9837 | 0.9979 | 0.5640 | 0.9962 | 0.9909 | 0.9931 | 0.9806 |
| r (Mv Magdaléna) | 0.9883 | 0.9750 | 0.9563 | 0.4003 | 0.9901 | 0.2015 | 0.8071 |
| r (Mv Suba) | 0.9936 | 0.9144 | 0.9432 | 0.1305 | 0.9990 | 0.1414 | 0.8023 |
| r (Mv Toborzó) | 0.9452 | 0.7715 | 0.4595 | 0.9509 | 0.9661 | 0.9220 | 0.9124 |
| r (Mv Toldi) | 0.9802 | 0.9556 | 0.7422 | 0.6782 | 0.9863 | 0.6306 | 0.7531 |

Yield figures of the cultivars were in close correlation with plant nutrition with a few exceptions only. However this correlation proved to be stronger and at the same time more balanced in the favourable crop year.

*Table 2.. Correlation between plant nutrition (control vs treatments), yield and protein yield by respective varieties. Nagygombos 2014*

| Wheat varieties | Yield [t/ha] | Hecto-litre weight [kg/hl] | Thousand grain weight [g] | NIR analysis data | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | Protein content [%] | Total amount of protein [kg/ha] | Gluten [%] | Zeleny number [ml] |
| r (Alföld-90) | 0.4578 | 0.7055 | 0.3952 | 0.9877 | 0.8826 | 0.9924 | 0.9974 |
| r (Mv Magdaléna) | 0.9463 | 0.9174 | 0.0151 | 0.9954 | 0.9828 | 0.9917 | 0.9753 |
| r (Mv Suba) | 0.5977 | 0.8952 | 0.7827 | 0.9463 | 0.8592 | 0.9405 | 0.9513 |
| r (Mv Toborzó) | 0.5716 | 0.6887 | 0.8961 | 0.9536 | 0.8645 | 0.9453 | 0.9662 |
| r (Mv Toldi) | 0.7506 | 0.7831 | 0.8343 | 0.9729 | 0.9308 | 0.9757 | 0.9838 |

The correlations of crop yield components were much weaker in both crop years in comparison with those of yield and protein values. The most vulnerable phenological periods of winter wheat were the stages of heading and flowering in relation with precipitation and vernalisation and ripening concerning temperature performance in accordance with the results of Pollhamer (1981) and that of Kismányoky and Ragasits (2003).

**Conclusions**

Precipitation and temperature data were studied in a long term field experiment to determine water availability and plant nutrition impacts on yield quantity and quality. The aim of the study was to evaluate favourable and non-favourable crop year conditions for winter wheat *Triticum aestivum* L. Five winter wheat varieties and six nitrogen topdressing application levels were applied in two consecutive crop years representing different precipitation and temperature patterns to evaluate yield, yield components and quality manifestation. The results of the experiment suggest that precipitation patterns in relation with the wheat development phenophases had profound influence on the yield and the protein formation of the crop. From among phenophases flowering and grain filling periods proved to be the most influential stages. The two crop years resulted in different amounts of protein yield. The favourable one significantly increased the total amount of protein in comparison with that of the non-favourable vintage. There were detectable differences in the protein yield of the wheat varieties studied. However the efficiency of the respective varieties also differed in the two crop years. Strong correlation was detected between the total amount of protein and the experimental treatments in both years. Yield figures of the wheat varieties were in close correlation with plant nutrition in general. Correlations of crop yield components were lower in both crop years in comparison with those of yield and protein values.

## Acknowledgement

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# Investigation of the phytotoxic effect of herbicide 2,4-D with hormonal function on winter wheat

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Abstract: The wheat is one of the most important grain crop in Hungary. It is grown in every year on 1.1 million hectare in Hungary. The Hungarian grain production sector has a lot of technologies to reduce the weed infection on grain fields. One of the most important work to protect against the broad leaves weeds.

We have some active agents, which can solve this problem, among these are the herbicides with hormonal function, like MCPA and 2,4-D. These herbicide we can spray until tillering, because of late application can cause a hard side effect. Recently some studies indicated that there is an opportunity to spray these herbicides after the stem elongation without serious plant deformation.

Our purpose was investigate the phytotoxic side effect on a field experiment. We settled 4 treatments beside the control. We used a normal and a double dosage from the herbicide 2,4-D in usual application time at tillering, and at late time in full flowering. The double dosage was required because of the potential double coverage.

Our experiment shows, that in time spraying has less negative effects than the late, independently from the double dosage. The treatments causes minimal effect on the plant height, but causes considerable damage on the ear length, the number of grains in the ear and the thousand seed weight. But we observed that the treated plots had a bigger dominancy of crop, because the control plots had a lot of weeds in it, so the competition was strong between wheat and weeds.

Keywords: wheat, 2,4-D, herbicide, phytotoxicity, grain

## Introduction

In our country, it is the highest proportion of crops, which represents approximately 1.121 million hectares of cultivated land. This ratio is due Hungary favourable conditions, as well as multi-annual yields prove. With these capabilities, knowledge and work can be added to the growing reputation of Hungarian wheat to produce remarkable results (Koháry, 2003).

In order to maintain our competitiveness in the market as in other countries, high-quality bread wheat cultivation target should be set, possibly with a smaller cost.

Our thesis aims – a small-plot field experiment- to examine the effect of active ingredient 2,4-D-containing hormonal herbicide for wheat production. The 2,4-D is used in cereal grains from the age of 4-6 leaves until the end of tillering. If we spread over the early spring, it is effective against broad leaf weeds. Today, due to the extreme weather conditions, the optimum time for application on weeds, as well as the crop is getting more difficult to meet. Hormonal agents have an undesired characteristic that the delayed treatment negatively influence the further development of the plant, whereas on crops can be phytotoxic. However, the advantage is, that the effect can be obtained appropriate and favourable price. In our thesis we analyzed that if it causes damage can we measure, and how to act economically. The winter wheat is used in our country especially bread and other food grain as feedstock (including a variety of pasta, bakery products, cereals, puffs, biscuits etc) and a significant amount will became animal food (Koltay - Balla, 1975). Its importance increases to adaptability - as well as our country's "qualities" - almost the entire country allows the cultivation. Our country has always been significant wheat export in our history wheat (Lelley - Rajháthy, 1955).

In recent years, domestic wheat acreage was formed around 1.1-1.2 million hectares, although the more profitable crops grown increasingly pushes down this area. It is important that a crop, grown on such a large area need special treatment, and needs the many year cultivation experiments

Considering the expected population growth of 1.14% forecast for the year, further increasing the production of cereals will be needed in the future and thus increasing the per hectare yield level could be the basis (Láng - Bedő, 2012).

**Materials and Methods**

The experiment took place in the fall 2015th on a field near Sümegprága. The size of the area was 3 ha. The forecrop was sunflower. The varieties for the experiment were selected from among the currently cultivated varieties.

We chosed the winter wheat named Amerigo, which variety has outstanding productivity and stable milling quality, and also has a high-yielding, very good yield stability, middle-late maturing, shredded wheat.

The plant is medium height, leg strength, their stamina is good, and it has an average tendency to shatter. Above-average winter-hardiness. Has high gluten content, protein content, falling number of good quality, and has a flour mill I. quality.

The object of the experiment was based on the 2,4-D herbicide impact assessment. The 2,4-D is a selective hormonal herbicide, used against the dicotyledonous, broad-leaved weed species (Erdei, 2011). During the experiment, we selected twenty parcels, and we studied the effect and phytotoxic symptoms of the U-46D Fluid SL agent, which contains 2,4-D (Tab.1). We used on the parcels several treatments, including normal and double dosage in suggested application time (in the stage of 3-5 leaves, in the second decade of march) and normal and double dosage in overdue application (just before the flovering, in the end of april). With the double treatment we studied the effect of herbicide on occasional double-sprayed parts of the fields. And also, the experiment contained control parcels too. Normal dosage was 1,4 l/ha$^{-1}$, double dosage was 2,8 l/ha $^{-1}$.

*Table 1.: Distribution of treatments*

| Treated parcels | | | | | |
|---|---|---|---|---|---|
| 1. Repeat | 1 | 2 | 3 | 4 | 5 |
| 2. Repeat | 3 | 5 | 1 | 2 | 4 |
| 3. Repeat | 5 | 4 | 2 | 3 | 1 |
| 4. Repeat | 4 | 3 | 5 | 1 | 2 |

Abr.: 1: Normal time, normal dosage, 2: normal time, double dosage, 3: overdue time, normal dosage, 4: overdue time, double dosage, 5: Control parcels.

Aims our experiment were investigation phytotoxic effect of hormonal products on winter wheat. In support of this action or refute the following measurements were performed:

- plant height
- ear length
- thousand seed weight
- seed number per ear

After the measurements were carried out a comparative analysis, which credibility factor we supported analysis of variance.

**Results and Discussion**

The weed infection was weak on the field (under 10 % before the threatment), the most common weeds were tipical grain weeds, including *Papaver rhoeas, Tripleurospermum inodorum*, but the weed with the highest coverage was *Ambrosia artemisiifolia* and the *Chenopodium* and *Amaranthus* species. After the treatment only the control parcel left infected, the other parcels were clear, with under 1 % weed coverage.

First we measured the plant height. The results we obtained shows, that the active ingredient 2,4-D does not affected the height of the plants in the single dose plots. In the double dose in normal and delayed-time plots the plant heights were decreased. This decreasing was statistically justified. It was also observed, that in the control plot plant height was significantly lower than the rest of the parcels. This significant decrease is caused by the continuous weed competition (Fig. 1).
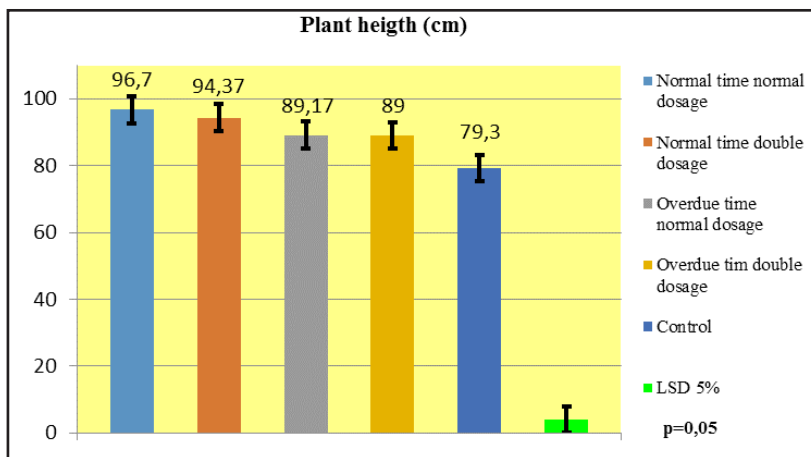


Figure 1.: Effect of treatments on the plant heights

When the harvest started, we measured the ear length, the seed number per ear and the thousand seed weight. The ear length between the normal treatments and the delayed treatments was different (approx. 1 cm). In the late-treated plots we found deformed and bent ears too. The reason for this is the phytotoxic effect of the applied herbicide. But, on the control parcels the measured values was significantly lower than the treated parcels. Our opinion is, that the reason of the weak results that on the control parcels were under strong competition of weeds (Fig. 2). The seed number per ear was also affected by the herbicide, but the weakest production was by the control plot, because of the competition between wheat and weeds. We found clear correlation between the number of ears per eye and ear length. The difference was also statistically justified. The herbicide causes yield loss, because the number of seeds within the ear decreased in any case. The last treatment, just before flowering causes fertility problems, and because of that the ear length decreased (Fig.3). We observed, that in those ears were shrivelled, shrunken seeds too.
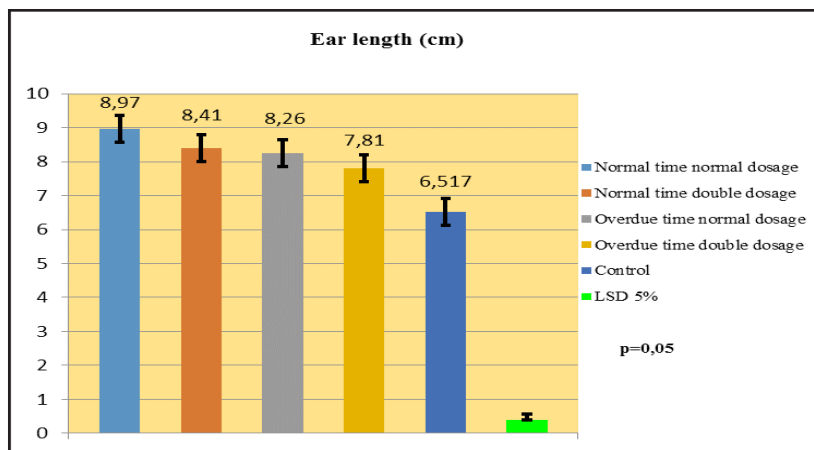
*Figure 2.: The length of the ears in differently treated parcels*
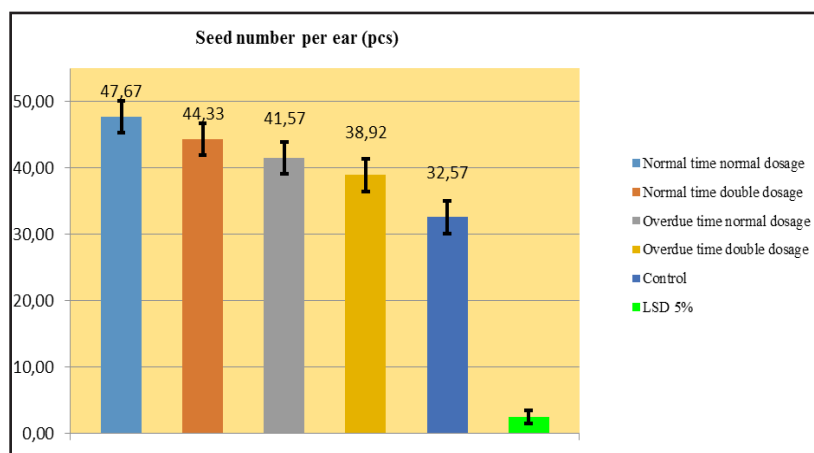


*Figure 3.:The number of the seeds per ears influenced by 2,4-D*

The seed number per ear was also affected by the herbicide, but the weakest production was by the control plot, because of the competition between wheat and weeds. We found clear correlation between the number of ears per eye and ear length. The difference was also statistically justified. The herbicide causes yield loss, because the number of seeds within the ear decreased in any case. The last treatment, just before flowering causes fertility problems, and because of that the ear length decreased (Fig.3). We observed, that in those ears were shrivelled, shrunken seeds too.

The last factor, that we observed, was the thousand seed weight. We observed, that in the plots, where we used the herbicide, produced less seeds than the control parcels. The control showed an improved value compared to the delayed treatments. Statistically verifiable results were obtained in the single and double-dosed treatments to the normal treatment time. The result of the delayed treatment was statistically not justified. This means that the harmful effects of 2,4-D has not been proved in this case (Fig. 4).
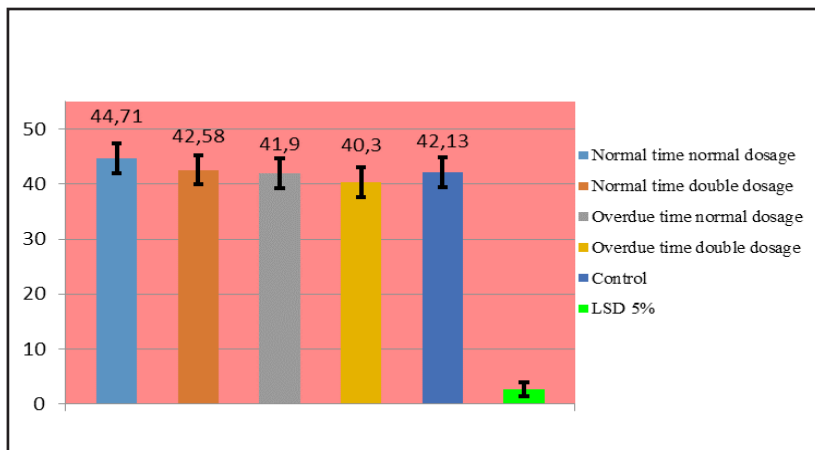
*Figure 4.: The thousand seed weights of wheat in the treatments*

## Conclusions

Our goal was to analyze the problem with the double dosage, if it occurs, when the spraying accuracy or in case of insufficient air movement causes overlapping. The conclusion is, that the treatments occurred phytotoxic symptoms. The ear length and the thousand seed number is demonstrably reduced in the treated area, and therefore the double dose showed yield reducing effect on wheat. On the environmental and economic point of view, in all cases, we need to use the rules set out in the spraying, the spraying machine maintenance should be carried out, and we need to use precise application. The delayed doses shows the problem, when because of the event of wet weather we cannot perform a normal treatment. In these plots we can clearly see the phytotoxic symptoms.

With the delayed, double dosage treatment we wanted to prove the problem with the overlapping. In this case, the plants showed an increased phytotoxic symptoms, including ear deformations, spike shortening and varying degrees of distortion. The results show, that if we need those delayed treatments, we must do it with utmost care.In the control plots , however, were all results weaker then the treated plots, for example the plant height was 18 percent lower than in the parcel threated with normal time and normal dosage. The seed number per ear was also affected, the weeds caused 32 percent of loss in the seeds in one ear. Those values were proven significant.Weeds cause an adverse effect to the wheat, it was falling behind in development. It follows, that the weeds can cause serious problems, especially in large groups from the $T_4$ weeds, and mixed with perennial weeds.

Overall, the evaluation of the experiment proved that we cannot grow wheat under extensive growing conditions. There is a need to intervene early weed control. On the control parcels were the biggest loss, and this loss was also proven signicifant. It was proved by the measurements, that the double-dose treatment causes the biggest rate of decline (for example the number of seeds per ear was affected by the double dosage and overdue time threatment, the loss was high - 20 % - , but the magnitude of declining is still not bigger, than in the control plot value (32 %). It is clearly established, that the treatment undergone by the non-optimal time is still better than the missed treatment. In any case, we need to use the applied regulations, so neither the environment nor the plant will be not damaged.

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# Effect of gluten formation on wheat quality

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Abstract: Wheat gluten contains two type of protein molecules namely gliadins and glutenins. Gliadin and glutenins play main role in determining viscoelastic properties of wheat dough and other technological quality parameters. Gluten proteins can cause intestinal disorders or celiac disease in some gluten intolerant individuals. This require the selection of cultivars having low gluten content. In this paper gluten content of 10 old wheat cultivars created in former Yugoslavia were analyzed during two year (2011-2013) with different climatic conditions (temperature and precipitation). In first year the dry gluten content varied between 24.21% (Lasta) and 32.16% (Macvanka 2), while in the second year all cultivars had higher gluten content and changed from 26.95% (Loznicanka) to 36.36% (Crvenkapa). The protein content and loaf volume were also higher in all cultivars in the second year in comparison to the values of the first experimental year. The presence of gliadin alleles at *Gli-A1* and *Gli-A2* loci, controlling α-, β-, γ- and ω-gliadins were also analyzed and probability of their origin were estimated. Five alleles (*a, b, f, h, k*) were present in *Gli-A1* locus in Yugoslav cultivars while 7 alleles (*b, e, g, j, k, o, p*) were present at *Gli-A2* locus in cultivars originating from other countries (Italy, Hungary, Romania, France, Great Britain, Mexico and the late Soviet Union), *Gli-A1h* allele was not present in any studied foreign cultivars. Lasta variety was identified with the lowest gluten content and being the most suitable for breeding and bread making for celiac patient.

Key words: gliadin alleles, gluten, quality, wheat.

## Introduction

Gluten is a complex protein which formed from proteins of flour, such as gliadins and glutenins, by mechanical mixing with water. Hydrated gliadins and glutenins begin to stick to each other and begin to interact through the formation of chemical bonds. Properties of dough, -viscoelasticity, -strength and -resistance depends on the structure and interaction of these proteins (Menkovska et al., 2002; Torbica et al., 2007). Gliadins and glutenins composed of many different molecular types, which differed in their molecular weight. Gliadin contain single polypeptide chains, globular confirmation, with intra disulfide bonds (Bietz, 1997; Shewry and Halford, 2002) and most of them have low molecular weight (16kDa to 50kDa), glutenins have intermolecular disulfide bonds between polypeptides. One group of glutenins consists of subunits with low molecular weight 20kDa to 50kDa (LMW GS) while the other group of glutenins have high molecular weight 50kDa to 200kDa (HMW GS). Gliadins are encoded by six *Gli*-loci, positioned at short arm of chromosomes 1. and 6. on A, B and D genomes of wheat. Each locus characterized

multiple alleles (Metakovsky et al., 1991). Gliadin proteins are similar, -considering their amino acid sequence and molecular weight and these proteins are rich in proline (~14%) and glutamine (~40%) Wrigley and Bietz (1988). Large number of gliadin and glutenin subunits were identified by electrophoretic separation (Bietz, 2002). In standard gel electrophoresis, the mobility of the proteins depend on the size and the charge (Waga and Zientarski, 2007).

Gliadins and glutenins are important for human nutrition. World Health Organization (WHO) recommends  bread eating several times per day (WHO, 2003). However, wheat products which consist of gliadins and glutenins can cause, celiac toxicity and allergenicity as well (Matsuo et. al. 2008). Three illnesses are associated with gluten intake: food allergy is present in 0.2-0.5% of the population; celiac disease (CD) appear in both children and adults in various frequencies (Kagnoff,, 2007) and gluten sensitivity with frequency of 6% in USA but the tendency is increasing all over the world (Kasarda, 2013).

The aim of this paper was to identify the alleles at the *Gli-A1* and *Gli-A2* loci, encoding gliadins and to study the variability of quality traits, such as: gluten content, protein content and loaf volume obtained from flour of wheat cultivars and grown under different environmental condition.

## Materials and methods

Grain samples of 10 wheat cultivars (Bacvanka 1, Balkan, Baranjka, Crvenkapa, Dukat, Gruza, Krajinka, Lasta, Loznicanka and Macvanka 2) were analyzed for gliadin composition, especially focusing on proteins encoded by alleles at the *Gli-A1* and *Gli-A2* loci. Electrophoresis was carried out by Metakovsky et al. (1991). Gliadins were extracted with 70% ethanol from 30 single kernels. Gliadin extract ($20\mu l$) were loaded on the gel by micropipette. Polyacryl-amide gel was polymerized by $10\mu l$ 3% hydrogen peroxid.  Gel electrophoresis was performed in 8.33% polyacrylamide for 2.5-3 hours, under constant voltage of 550 V and in $5m$M aluminum lactate buffer at pH=3.1 (Novoselskaya *et al.,* 1983). After running electrophoresis, gels were removed and immersed in $300ml$ of fixative for 15 minutes, and stained in 0.05% ethanol solution of Coomassie Briliant Blue R-250 by adding $250ml$ 10% trichloroacetic acid (TCA). Staining was carried out during night. Next day. Gels were washed in water and photographed. Photographs were used for determination of gliadin alleles (Metakovsky, 1991).

### *Weather condition during growing seasons*

In the years of experiment temperature and the quantity of precipitation were different. The values in comparison with 10 year's  average were also different (tab. 1). The average temperatures (8.13°C) of the first year was slightly lower than the 10 year's average (9.08°C), while the average temperature (9.73°C) in the second year of the experiment (2012/13) was higher than in first year and the 10 yearr's average. In the first year (2011/12) the average amount of precipitation (474.7mm) was significantly lower than in the second year of experiment (611.5mm), and lower than the 10 year's average (543.8mm).

The amounts of precipitation was high and had suitable distribution from sowing to ripening time in the second year of the experiment. In the first year, precipitation was extremely low at the period of germination, while from December to ripening time the

distribution of precipitation was enough at each stage of plant development. Precipitation in June of the first year (17.8mm) was enough for seed maturity at the end of grain filling stage, however this quantity of precipitation was much lower than the 67.6mm in second year, the 74.4mm average precipitation in the last ten year.

*Table 1. Monthly and mean temperatures and monthly and cumulative precipitation*

| Month | Temperature °C | | | Precipitation (mm) | | |
|---|---|---|---|---|---|---|
| | 2011/12 | 2012/13 | 2001-2010 | 2011/12 | 2012/13 | 2001-2010 |
| October | 10.4 | 13.7 | 12.2 | 30.4 | 56.7 | 64.3 |
| November | 3.2 | 9.1 | 7.0 | 1.7 | 11.1 | 57.4 |
| December | 3.3 | 0.4 | 2.0 | 63.7 | 97.6 | 48.5 |
| January | -0.1 | 2.9 | 0.9 | 107.1 | 62.4 | 42.8 |
| February | -4.2 | 4.0 | 2.4 | 54.9 | 84.3 | 44.7 |
| March | 8.8 | 6.4 | 7.6 | 24.5 | 102.0 | 52.5 |
| April | 12.7 | 13.3 | 12.0 | 69.1 | 41.2 | 66.6 |
| May | 16.0 | 18.0 | 17.2 | 105.5 | 70.8 | 74.9 |
| June | 23.1 | 19.8 | 20.4 | 17.8 | 85.4 | 92.2 |
| Average | **8.13** | **9.73** | **9.08** | 52.7 | 67.94 | 60.4 |
| Total | | | | **474.7** | **611.5** | **543.8** |

## Results and discussion

### *Variation in gliadin alleles*.

The gliadin allele composition at the *Gli-A1* and *Gli-A2* loci and the quality traits of wheat showed differences among the analyzed wheat cultivars. Five different alleles were identified (***a, b, f, h, k***) at the *Gli-A1* locus and 7 alleles (***b, e, g, j, k, o, p***) at the *Gli-A2* locus. The frequency of the identified alleles was different. The ***b*** allele (40.0%) was the most frequent at the *Gli-A1* locus, while frequency of alleles ***a, f*** were 20% and the lowest frequency had alleles ***h, k*** with 10%. At the ***Gli-A2*** locus the most frequent allele was the ***g*** allele (40.0%), while frequency of allele ***e*** was 20.0% and frequency for alleles ***b, k, o, p*** was 10%. Krajinka cultivar was heterozygous at the *Gli-A2* locus, where two different alleles *Gli-A2g, Gli-A2j* (table 2). The established polymorphisms of ***Gli-A1*** and ***Gli-A2*** was in agreement with previous finding (Menkovska et al., 2002; Djukić et al., 2011; Knezevic et al., 2016a). The variation of gliadin alleles identified in this analysis indicated that there is a lagre gene-pool which could be useed in breeding programmes to transfer superior genes to other varieties in order to improve the protein composition. The high frequency of the similar alleles is coming from the frequent use of parental genotypes carrying the sam protein alleles. Other reason could be the use of selection method based on phenotypic characters, that might also lead to high allele frequency of certain protein alleles (Knezevic et al.2016a).

The alleles identified at the *Gli-A1* locus were mainly γ-gliadins, but there were smaller number of β- and ω-gliadins as well. Alleles at the *Gli-2* locus encodes mainly α-gliadins components and smaller number of β-gliadins.

### *Grain protein content*.

The protein is the main compositional quality trait of the grain. In this study, the grain protein content varied from 10.40% (Lasta) to 13.50% (Crvenkapa) in the first year of investigation. In the second year of investigation grain protein content varied from 12.10% (Lasta) to 14.50% (Crvenkapa). Generally the protein content of all varieties were higher in the second year than in the first year of research (table 2).

The gentic control of the protein content is very complex, and it, also, depends on the environmental factors. The protein content is genetically determined. Althogh the environment/year can influence it, but the order of the variaties will be very similar in each year (Jolánkai et al, 2008; Johansson et al., 2008; Knezevic et al., 2016b). The pre- and post-anthesis N application, the temperature and the timings, the post-anthesis temperature, the regime of watering all have strong influence on the accumulation of the protein content (Martre et al., 2006; Godfrey et al., 2010).

Protein content and protein quality are highly associated with the baking quality and positively correlated with wet gluten content, farinogrph dough stability and bread loaf volume (Menkovska et al. 2002).

*Table 2. Gliadin allele composition and technological quality of winter wheat cultivars*

|  | Gli alleles | | Dry gluten % | | Grain protein content % | | Loaf volume (*ml*) | |
|---|---|---|---|---|---|---|---|---|
|  | *A1* | *A2* | 2011/12 | 2012/13 | 2011/12 | 2012/13 | 2011/12 | 2012/13 |
| Bacvanka 1 | a | b | 28.22 | 32.44 | 11.60 | 13.20 | 440 | 480 |
| Balkan | f | g | 30.46 | 34.42 | 13.40 | 14.60 | 450 | 500 |
| Baranjka | b | e | 26.89 | 32.28 | 12.80 | 13.40 | 420 | 450 |
| Crvenkapa | k | g | 31.12 | 36.36 | 13.50 | 14.50 | 520 | 540 |
| Dukat | f | o | 24.52 | 29.10 | 11.20 | 12.30 | 340 | 390 |
| Gruza | b | k | 29.23 | 32.21 | 12.30 | 12.80 | 400 | 420 |
| Krajinka | h | g+j | 25.34 | 31.89 | 12.00 | 13.25 | 360 | 400 |
| Lasta | b | p | 24.21 | 27.04 | 10.40 | 12.10 | 340 | 380 |
| Loznicanka | b | g | 29.57 | 26.95 | 11.60 | 12.85 | 420 | 420 |
| Macvanka 2 | a | e | 32.16 | 30.63 | 12.10 | 12.65 | 430 | 430 |

### *Gluten content.*

Gluten is like a rubbery mass, which remains after washing of starch granule and water-soluble components from wheat dough. The dry gluten content varied much depending on the wheat cultivars, wheater conditions and the year. In the first year of investigation, the dry gluten content varied between 24.21% (Lasta) and 32.16% (Macvanka 2). The gluten content in second year of experiment varied from 26.95% (Loznicanka) to 36.36% (Crvenkapa). Generally, each wheat cultivar had the higher gluten content in the second year than in  the first year (table 2).

The variation of the quality characters determined by the genotype and its interaction with the environmental factors (Naeem et al., 2012; Horváth et al., 2014). Temperature sums is the main factor in the polymersation of gluten proteins (Triboi et al., 2003). The high temperature contribute to shortening stage of development (grain filling, ripening). The high temperature at the end of the grain-filling period caused the greatest reduction in the mean gluten index (Vida et al., 2014). It will takes a long time to improve wheat quality characters by breeding (Zečević, et al., 2013).  Amount of gluten protein fraction will be higher when using fertilizer than without using. The high temperature increasing of gluten protein content per grain which is resulted by the inhibited synthesis of starch under high temperature (Hurkman et al., 2013). Gluten matrix have main role in determining baking quality of wheat flour by affecting capacity of water absorption, viscosity and elasticity of the dough, what is important for food industry and quality products in human nutrition. Gliadins determines the extensibility of the dough while glutenins determines the strength of the (Shewry et al., 2003). Their quantitative ratio is important determinant of gluten quality (Menkovska et al., 2002; Wrigley et al., 2006).

### Bread quality

Loaf volume is a parameter characterizing bread qualiy. In this study, 'Crvenkapa' variety had the highest loaf volume (520*ml*) in the first and second (540*ml*) year, while the lowest loaf volume was found for 'Dukat' and 'Lasta' (340*ml*) in the first year and 'Lasta' (380*ml*) in the second year of investigation (table 2). The quality characteristics are important for processing industries and bakeries. Uniform quality could be reached by blending of the flours originating form different trial locations, seasons and varieties. This can help for the industries to produce uniform quality wheat products. Further research would be needed to evaluate allergenic factors, and to use it for selection in breeding programs.

## Conclusions

On the basis of the identified gliadin alleles, polymorphisms were established at *Gli-A1* and *Gli-A2* loci i.e. 5 at *Gli-A1* and 7 at *Gli-A2* locus. The study of the ten wheat cultivars showed differences in their quality characteristics such as gluten content, protein content and loaf volume. The quality characteristics of wheat cultivars differed and varied among cultivars and years. The wheat cultivar 'Crvenkapa', had the highest dry gluten content (36.36%) in second year, grain protein content (13.50% and 14.50%) and loaf volume (520*ml* and 540*ml*) in both years of the investigation. 'Lasta' cultivar had low gluten content (24.21%) in the first year of investigation, and low protein content in both years. Flour from 'Lasta' and flour from other low protein varieties can be used to blend with flour with high protein content to getting uniforme quality. The decreasing of gluten content is important for prevention of "toxicity" and decreasing cause of intestinal disorders. Also, this cultivar is interesting for breeding wheat cultivars with low protein content, which flour and its products are more suitable for consumers untolerant to gluten.

## Acknowledgement

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# Subalpine Springs (The Krkonoše Mountains National Park): Species Diversity in Relation to Environmental Factors

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Abstract: The springs are unique ecotones that integrate ecological characteristics and human impacts associated with both underground and surface water as well as terrestrial ecosystems. A clear definition of the management of springs is a precursor to their effective protection and restoration. Species diversity of springs areas in the eastern part of the Krkonoše Mts. was investigated in eight various localities. By a cover-abundance scale species richness (plants and mosses) and habitat type (phytocenological survey) were evaluated.

Statistical analyses were used to compare springs by environs gradients with respect to environment conditions (Principal component analysis with environmental supplementary variables, Simpson's dominance index and Shannon's diversity index). A total of 59 vascular plants, 9 mosses and 4 communities were found, including 8 endangered taxa of them. The critically threatened taxa include *Cardamine amara* subsp. *opicii*; *Montia fontana* subsp. *fontana*. Those in the endangered list include *Delphinium elatum* and *Swertia perennis*. Vulnerable species include *Epilobium alsinifolium* and the lower risk (near threatened) include 3 taxa.

The springs of Modry brook and Renner brook had the highest species diversity and the largest number of vascular plants were observed on the headwater area of Upa River (so called "Deliquescent Rock"). The fewest endangered species were determined on the springs of Suchy brook and Javori brook. The resulting species richness is a function of the combination of altitude and exposure, where the highest species richness values were recorded in the highest located subalpine headwater areas.

Keywords: species richness, groundwater, phytocenological survey, Giant Mts.

## Introduction

Springs are ecosystems in which groundwater reaches the Earth's surface either at or near the land-atmosphere or land-water interface. At their sources (orifices, points of emergence), the physical geomorphic template allows some springs to support numerous microhabitats and large arrays of aquatic, wetland, and terrestrial plant and animal species. Yet, springs ecosystems are distinctly different from other aquatic, wetlands and riparian ecosystems (Stevens et al. 2005). They fundamentally contribute to the circulation of nutrients, soil formation, climate regulation, carbon accumulation, nutrient and water retention (Sorooshian and Whitaker 2003).

The Krkonoše was proclaimed as a government-protected area for water accumulation because of its significance as a headwater area. The collecting area of underground waters is shallow and has a relatively low springs discharge. The springs water maintains a year-around temperature between 4 - 6 °C, so the headwater areas do not freeze (Štursa et al. 2012). Thanks to various geologic and ecologic conditions, headwater areas include a wide range of locations (Danks and Williams 1991). Twelve springs types are recognized, not including paleo-springs (Springer and Stevens 2009). This differentiation of communities

is primarily conditioned by the ground water mode; water chemical composition, temperature, nutrition content, springs discharge, water flow rate and slope angle. The most significant species diversity is influenced by mineral richness and the reaction of springs water.

The class *Montio-Cardaminetea* comprises vegetation developing in springs with cold and well oxygenated water. Cold water with low nutrient content reduces vascular plant productivity, while leading to increasing bryophyte cover. Enhanced input of nutrients can therefore quickly change the structure of springs vegetation to the benefit of vascular plants (Hájková et al. 2006). With increasing distance from a springs, flow rates and oxygenation decrease and springs vegetation is replaced by other communities, typically mire and fen vegetation of the class *Scheuchzerio palustris-Caricetea nigrae*.

**Materials and methods**

A field survey was conducted at the The Krkonoše Mountains National Park. That survey was carried out from July to October during the 2015-2016 growing season, to quantify the vegetation structure. Eight springs were chosen: L1 – Zeleny brook 50°42′42.704′′N 15°40′26.351′′E | L2 – Modry brook 50°43′21.799″N 15°41′23.273″E | L3 – Javori brook 50°39′30.148′′N 15°44′27.238′′E | L4 – Weber brook 50°40′33.614′′N 15°44′19.803′′E | L5 – Renner brook 50°43′45.719′′N 15°49′29.460′′E | L6 – Max brook 50°39′26.506′′N 15°50′58.014′′E | L7 – Suchy brook 50°39′45.649′′N 15°51′26.606′′E | L8 – Upa River (so called ´Deliquescent Rock´) 50°40′1.685′′N 15°47′321.445′′E – for more characteristics, see Table 1.

*Table 1: Summary of different springs at different sites of the The Krkonoše Mountains National Park*

|    | Exposure  | Soil     | Altitude (m a.s.l.) | Slope (°) | Coverage E₁ (%) | Coverage E₀ (%) | Springs type |
|----|-----------|----------|---------|-------|---------------|---------------|-----------|
| L1 | East      | Podsol   | 1 375   | 12    | 70            | 40            | Limnocrene |
| L2 | South     | Podsol   | 1 350   | 39    | 70            | 50            | Helocrene |
| L3 | North     | Podsol   | 1 220   | 14    | 15            | 90            | Helocrene |
| L4 | Northwest | Podsol   | 1 110   | 9,5   | 60            | 50            | Helocrene |
| L5 | West      | Podsol   | 1 080   | 11,3  | 70            | 50            | Helocrene |
| L6 | West      | Cambisol | 965     | 54    | 90            | 15            | Rheocrene |
| L7 | West      | Cambisol | 940     | 10,5  | 60            | 20            | Helocrene |
| L8 | East      | Podsol   | 625     | 69    | 80            | 5             | Rheocrene |

Springs types follows Springer and Stevens (2009) | L1 – L8 see Materials and methods

The phyto-sociological part was worked out with the help of the Zürich Montpellier School of Phytosociology and included analysis, synthesis and identifying vegetation. Sixteen phyto-sociological relevés were taken (Rosenthal, 2003). The vegetation plot size was delimited in such a way as to represent full floristic composition of the phyto-coenosis. It varied from 3.75 to 12 m² depending on plant density and the homogeneity of vegetation cover.

For each vegetation plot, all vascular plants and cryptogams were recorded. These plant species were recorded according to the Braun-Blanquet cover-abundance scale. Geographic coordinates, elevations above sea level, soil types, aspect and slope inclinations (environs gradient) were noted for each relevé. The springs type was determined by analysing its groundwater environment. Simpson's dominance index and Shannon's diversity index was calculated for each individual location, using the PAST software (Hammer et al. 2001). A TWINSPAN analysis (Hill and Šmilauer 2005) was used to perform the

preliminary classification of communities (cluster analysis). Principal component analysis (PCA) of species composition (relevé diversity is expressed as Shannon-Wiener index) with environmental supplementary variables was analysed by CANOCO 5 software package (Šmilauer et Lepš 2014). Nomenclature by Danihelka et al. (2012), classification of threatened taxa by Grulich (2012).
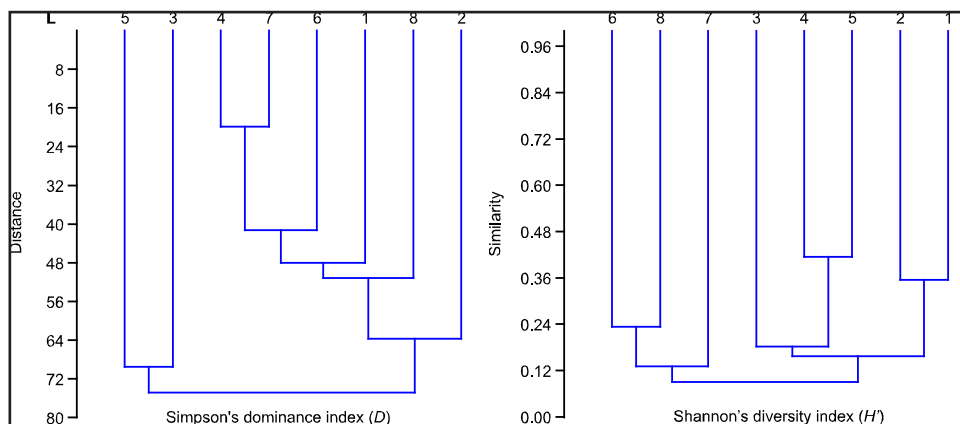


*Figure 1: Cluster analysis of species richness of different springs indicated by Simpson´s dominance index (D) and by Shannon's diversity index (H') - complete linkage (Euclidean distance) | L1 – L8 see Materials and methods*

### Results and discussion

On selected springs, a total of 59 vascular plants and 9 mosses were determined, including 8 endangered taxa among them (Table 2, Figure 3). Figure 1 (left side) shows highest species richness on L5 and L3 (with 19 vascular plants and 4 mosses, respectively 18 and 3). It is probably connected with this that the index is heavily dependent on the most numerous species and less sensitive to rare species. On the other hand, the lowest diversity was observed on L7 and L4 (5 vascular plants and 2 mosses) – which are closely similar. On right side of Figure 1 is a clear cluster with L1 and L2 (subalpine elevation) that also shows in other clusters, as well as springs.

*Table 2: Summary of threatened taxa determined at different springs*

| | Statutes by Red List (Grulich 2012) | Springs | Cover-abundance scale |
|---|---|---|---|
| *Cardamine amara* subsp. *opicii* | C1 b - Critically threatened taxa | L2 | 3 |
| *Delphinium elatum* | C2 r - Endangered taxa | L6 | 2 |
| *Epilobium alsinifolium* | C3 - Vulnerable taxa | L2, L4, L8 | 2, +, 1 |
| *Epilobium palustre* | C4 a - Lower risk – near threatened | L5 | 1 |
| *Chrysosplenium oppositifolium* | C4 a - Lower risk – near threatened | L8 | 1 |
| *Montia fontana* subsp. *fontana* | C1 b - Critically threatened taxa | L2 | 1 |
| *Swertia perennis* | C2 r - Endangered taxa | L2 | + |
| *Viola biflora* | C4 a - Lower risk – near threatened | L2, L3, L5 | +, r, r |

Cover-abundance 7-degree scale follows Braun-Blanquet | L1 – L8 see Materials and methods

The results of species diversity and phytocenological relevés show diverse types of communities in the springs. These are communities of the alliance *Swertio perennis-Dichodontion palustris* Hadač 1983 (L1-L4) and associations *Caricetum remotae* Kästner

1941 (L5, L6), *Cardamino-Chrysosplenietum alternifolii* Maas 1959 (L7) and *Pellio epiphyllae-Chrysosplenietum oppositifolii* Maas 1959 (L8).
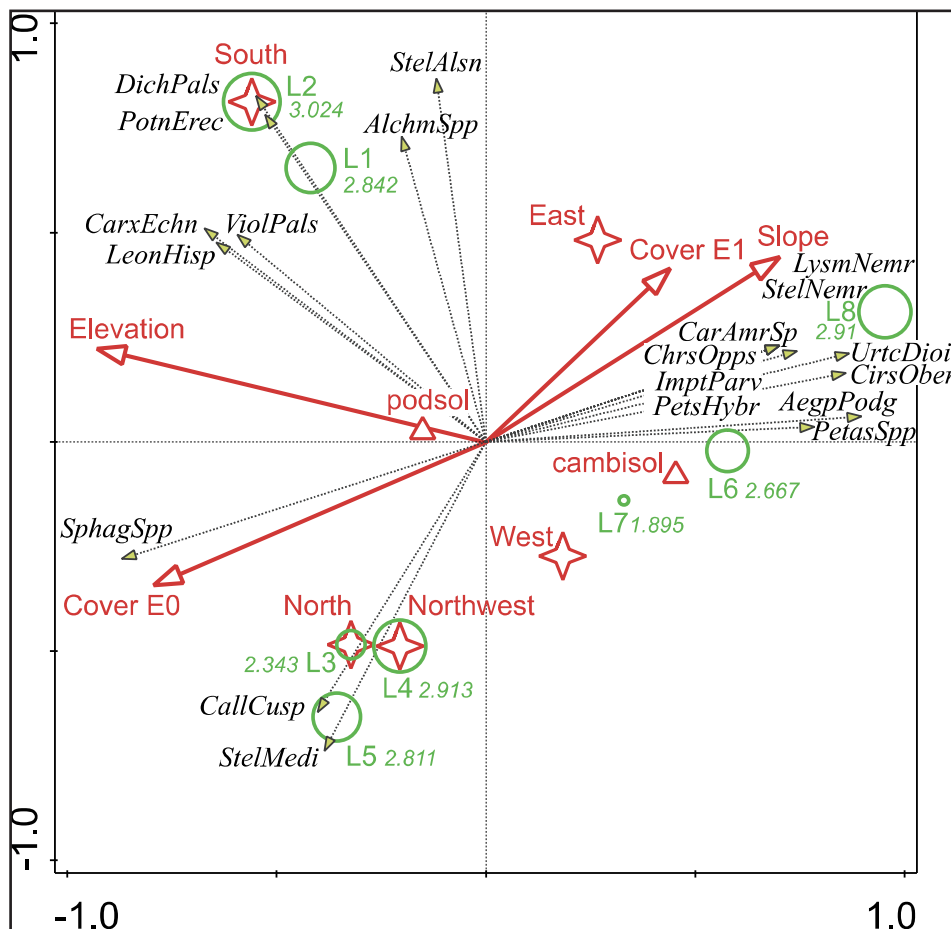


*Figure 2: An ordination diagram of PCA with environmental supplementary variables | red – supplementary variables; black - species; green – springs (L1 - L8) – point size and number correspond with Shannon-Wiener index | L1 – L8 see Materials and methods | AegpPodg - Aegopodium podagarium | AlchmSpp - Alchemilla spp. | CallCusp – Calliergonella cuspidata | CarAmrSp - Cardamine amara | CarxEchn - Carex echinata | ChrsOpps - Chrysosplenium oppositifolium | CirsOber - Cirsium oleraceum | DichPals - Dichodontium palustre | ImptParv - Impatiens parviflora | LeonHisp - Leontodon hispidus | PetasSpp - Petasites spp. | PetsHybr - Petasites hybridus | PotnErec - Potentila erecta | SphagSpp - Sphagnum spp. | StelAlsn - Stelaria alsine | StelMedi - Stelaria media | StelNemr - Stellaria nemorum | UrctDioi - Urtica dioica | ViolPals - Viola palustris | LysmNemr - Lysimachia nemorum*

The first and second axis in PCA are explained in 24.62 and 23.72, respectively. Figure 2 shows both species abundance and richness by various springs in relation to their environmental gradient. First axis represents the effects of elevation and slope while the second illustrates aspect. The resulting species richness is a function of the combination of altitude and exposure, where the highest species richness values were recorded in the highest located subalpine headwater areas with southern exposures.In terms of the overall

approach to management of habitats, springs are distinguished by two basic groups. The first involves the natural habitat (forest and subalpine springs as L1, L2 and L3; bogs and some types of transitional bogs), which should generally leave the spontaneous development with possible one-off interventions aimed primarily at restoring the natural water regime. This corresponds with Barquin and Scarsbrook (2008). The second group consists of semi-natural habitats – mainly springs L4 and L6. The existence of these semi-natural habitats is conditioned especially by human activities, including deforestation and subsequent traditional management in the past of non-forest springs, calcareous and non-calcareous moss springs and some types of transitional bogs. These habitats require more or less steady, albeit extensive management to replace the former traditional agricultural practices.

## Conclusions

The springs are unique ecotones that integrate ecological characteristics and human impacts associated with both underground and surface water and terrestrial ecosystems. Spring vegetation occurs across a broad altitudinal range from lowlands to the subalpine belt, both in open places and under forest canopies. A clear definition of the management of springs, with the help of vegetation and environmental factors surveys, is a precursor to their effective protection and restoration.
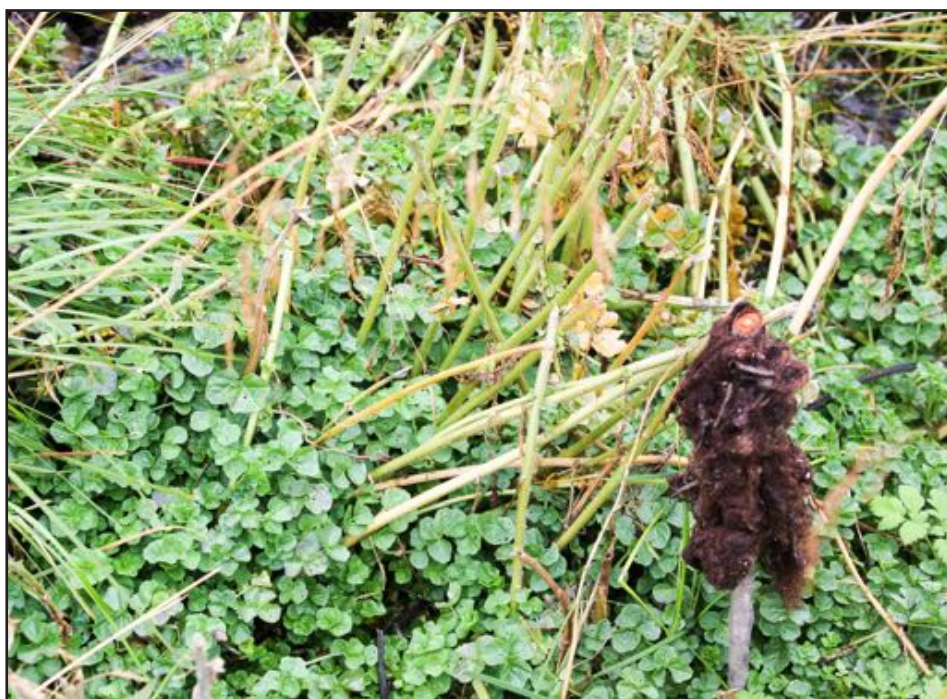


*Figure 3: The critically threatened plant Cardamine amara subsp. opicii on subalpine springs Modry brook (L2 | 18. 9. 2015) with protection against grazing forest animals.*

**Acknowledgement**

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# Environmental and production aspects of maize cultivation in relation with the different time-applied nitrogen

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Abstract: In order to determine the influence of nitrogen fertilization on status of mineral nitrogen in soil and yield of maize two stationary field experiments were set up in Croatian Pannonia agricultural region in vicinity of Vukovar city. Field trials include four fertilizer treatments in four replications. The treatments are:  (i) Fall (autumn) - 180 kg N/ha, (ii) Spring (pre-plant) - 180 kg N/ha, (iii) Fall and spring – 70 kg N/ha in fall and 110 kg N/ha in spring, (iv) Fall, spring and topdressing - 70 kg N/ha in fall, 70 kg N/ha in spring and 40 kg N/ha in topdressing application. Results of two-year studies at two location reviled that treatments did not significantly affected on maize grain yield and they were in the range of 13.4 t/ha to 14.0 t/ha in 2014 and 10.1 t/ha to 11.3 t/ha in 2015 at Vukovar location and 16.2 t/ha to 16.9 t/ha in 2014 and 8.3 t/ha to 8.8 t/ha in 2015 at Belje location. The relatively highest grain yields in both years of investigations and on both locations were achieved with the application of all nitrogen in the fall. Although the fall nitrogen applications is not recommended due to greater risk of N loss, results of nitrogen content up to 90 cm soil depth show that different nitrogen application time did not significantly influenced on nitrogen accumulation in soil. Fall (autumn) fertilizer application has relatively contributed an increscent of nitrate nitrogen accumulation in soil.

Keywords: fertilization, soil, mineral nitrogen, environment, yield

## Introduction

Over the past 40 years a broad range of environment legislation has been put in place. Among other things General Environment Action Programme of EU set the priority objective to protect, conserve and enhance the Union's natural capital which also includes soil fertility. All this imposes an obligation to scientists to provide the best advice how to preserve the environment. In terms of maize production in Croatia according to FAOSTAT (2017) data base in period from 1997 to 2014 average production was 1.98 M tonnes with average yield of 5.94 tonnes/ha and in average 338865 ha of land was harvested. That means that 6 % of Croatian terrestrial territory was fertilized in order to achieve appropriate maize yield. Due to the known fact that excessive fertilization has a negative impact on soil conditions and in order to protect the health of people, nature, environment and the interests of consumers an increasing number of producers implement the principles of integrated agriculture in their production. Integrated production means a balanced application of agro-technical measures for the production of environmentally and economically friendly products with minimal use of agrochemicals. In this mode of farming crop nutrient management plan is indispensable and highest average annual application of nitrogen (mineral and/or organic) is 170 kg/ha/year for arable crops except for silage maize when Regulation allows a maximum of 200 kg N/ha. Many authors worldwide investigated the influence of increasing doses of nitrogen fertilization on temporal and spatial distribution of mineral nitrogen in soils. The conclusions derived from these studies can be summarized as follows: nitrogen variability in soil is influenced primarily due to the amount and type of applied fertilizer, by the type and characteristics of the soil, by the type of crop, by the presence or absence of a particular culture, by schedule

and intensity of rainfall [Németh and Kádár (1999), Ikerra et al. 1999, Guo et al. (2001), Zebarth and Milburn (2003), Li et al. (2005), Fang et al. (2006), Mesić et al. (2007), Nance and Karlen (2007), Kristensen and Thorup Kristensen (2007),  Gami et al. (2009), Jurišić et al. (2014)]. In contrast to the above studies the goal of this research was to determine the influence of different application periods of the same quantity of mineral nitrogen (180 kg/ha, allowed amount of nutrient in integrated farming practise) on the yield of maize but also on nitrogen variability in soil up to 0.9 m of soil depth.

## Materials and methods

This study was carried out on two locations (Vukovar and Belje) in Croatian Pannonia agricultural region in vicinity of Vukovar city during two growing season (2014 and 2015) of maize (*Zea mays* L). The investigation area has a temperate continental climate. The annual mean temperatures slightly varied from 12.6 in 2015 to 13.4 °C in 2014. Total precipitation was in the range of 686.2 mm in 2015 to 823.0 mm in 2014. Soil types on locations and its chemical properties were listed in table 1. In order to determine the initial state of soil, samples were taken before the experiment was set up. Samples were air dried, milled, sieved and homogenized. The soil pH was determined in 1:2.5 (w/v) soil suspension in 1 M KCl. Plant available phosphorus and potassium were extracted by ammonium lactate (AL) solution (Egner et al., 1960) and detected by spectrophotometric and flame photometric, respectively. Total carbon and total nitrogen content for calculation of CN ratio were determined by dry combustion method (ISO 10694 and ISO 13878).

*Table 1: Soil type and chemical characteristics\* of soils*

| Location | Year | Soil type\*\* | $pH_{KCl}$ | mg $P_2O_5$ / kg | mg $K_2O$ / kg | C/N ratio |
|----------|------|-----------|------------|-----------|-----------|-----------|
| Belje | 2014 | Gleysoils, Mollic | 7.43 | 411.1 | 306.5 | 22.3 |
|  | 2015 | Gleysoils | 7.35 | 243.6 | 158.2 | 26.9 |
| Vukovar | 2014 | Eutric Cambisol | 6.79 | 188.3 | 266.4 | 12.8 |
|  | 2015 | Chernozem Haplic | 7.17 | 140.2 | 227.1 | 14.4 |

\*surface soil layer (0-30 cm); \*\* WRB (2006)

The experimental design was a randomized block with four treatments and four replications. The size of each plot is 25 x 25 m and in total experiments included 16 plots. Treatments vary by the applications of different nitrogen fertilizer (Urea – 46 % N and potassium ammonium nitrate (KAN) - 27% N) or by the different times of fertilizer application (Table 2). In total on each treatment 180 kg N/ha was applied but in different time. Depending on the location and year of investigation, fall (autumn) nitrogen application was conducted in late October or early November, early spring or pre plant nitrogen application was initiated in April, while the topdressing fertilization was done in late May or early June.

*Table 2: Treatments and applied amounts of nitrogen*

| Treatment | Nitrogen application (kg/ha) | | |
|-----------|------------------|------------------------|-------------|
|  | Fall (autumn) | Early spring (pre-plant) | Topdressing |
| Fall (F) | 180 kg N (Urea) | - | - |
| Spring (S) | - | 180 kg N (Urea) | - |
| Fall + spring (F+S) | 70 kg N (Urea) | 110 kg N (Urea) | - |
| Fall + spring + topdressing (F+S+T) | 70 kg N (Urea) | 70 kg N (Urea) | 40 kg N (KAN) |

Grain yield and moisture content (gravimetric method) in maize were determined after the harvest. During the vegetation of maize soil sampling was conducted six times. Composite samples per plot were taken from three depths (0-30 cm, 30-60 cm and 60-90 cm). During one vegetation season 288 soil samples were taken per one location. Totally, in 1152 soil samples nitrate-nitrogen content was determined by colorimetric method. Wet soil samples were extracted u 1M KCl solution. After the extraction, samples were centrifuged; filtrated and $NO_3$-N content was detected by spectrophotometric method (Cd reduction). Statistical analyses of differences in soil nitrate-nitrogen content according to fertilization treatments for soil depth and differences in grain yield according to fertilization treatments were computed by analysis of variance (ANOVA) (SAS 9.1, SAS Institute Inc., USA). The significance test was performed at probability level of $P < 0.05$. Differences among treatment means were separated using Fisher's least significant difference procedure.

## Results and discussion

Results of two years of investigations relives that different application time of 180 kg



*Figure 1: Effect of different time applied amount of nitrogen on grain yield of maize, Belje location; Pr>F: 0.6490 (2014) and 0.7400 (2015)*

nitrogen / ha did not significantly affected on yield of maize grain neither on the Belje location (P = 0.6490 in 2014 and P=0.7400 in 2015) neither on the Vukovar location (P = 0.3342 in 2014 and P=0.8109 in 2015). Yield of grain varied from 16.2 t/ha to 16.9 t/ha in 2014 and from 8.3 t/ha to 8.8 t/ha in 2015 at Belje location (Figure 1).

At Belje location average grain yield in 2015 (8.4 t/ha) was 49 % lower than average grain yield recorded in 2014 (16.5 t/ha). Results can by partly explained by the fact that in 2015 according to the Thornthwaite water balance, water deficit (247.9 mm) was recorded in June, July, August and September, in months which are very crucial for maize development. Also, soil (Gleysoils, Mollic) at Belje location in 2014 was more supplied with phosphorus (411.1 mg/kg) and potassium (306.5 mg/kg) (Table 1).

Yield of grain varied from 13.4 t/ha to 14.0 t/ha in 2014 and 10.1 t/ha to 11.3 t/ha in 2015 at Vukovar location (Figure 2). According to the results and although this is only a relative differences the highest grain yield at Vukovar location in both investigations years and at Belje location in 2014 were achieved at treatment when all nitrogen was applied in fall (autumn). According to these results the rate of nitrogen leaching, especially nitrogen applied
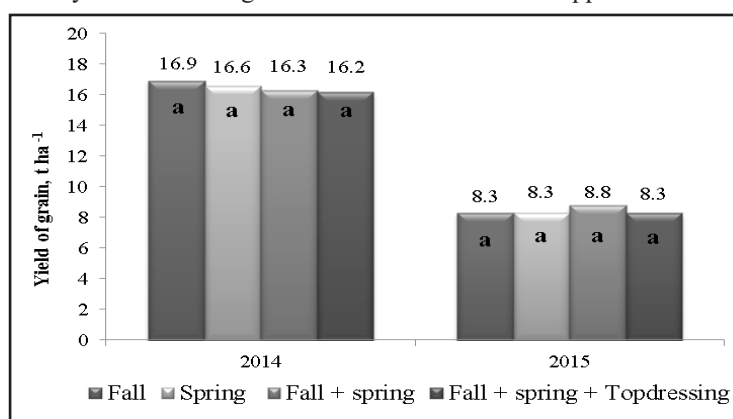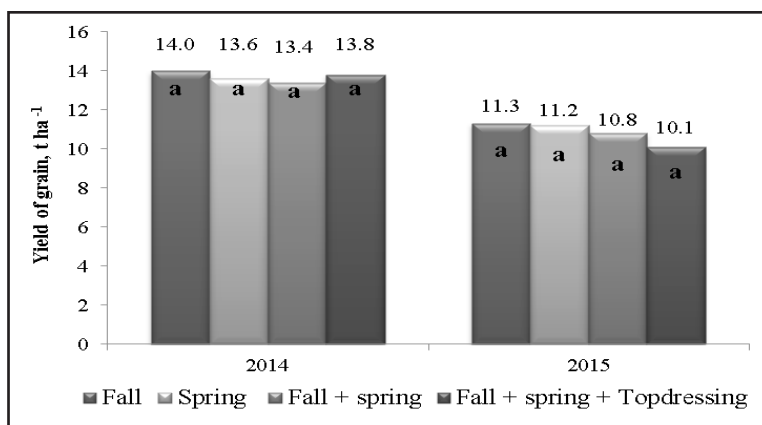
*Figure 2: Effect of different time applied amount of nitrogen on grain yield of maize, Vukovar location; Pr>F: 0.3342 (2014) and 0.8109 (2015)*

in the fall, is not in a large-scale that would have a negative impact on yield. The results shown in Tables 3 and 4 represent the average nitrogen content for six samplings times which were conducted during each growing season of maize. Variability of nitrate-nitrogen ($NO_3$-N) content in soil per treatments and soil depth at Belje location is presented in table 3.

*Table 3: Variability of nitrate-nitrogen content in soil per treatments and soil depth at Belje location*

| Belje location, 2014 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Soil depth** | **Treatments** | | | | **Statistics** | | | | |
| | Fall | Spring | Fall+Spring | F+S+T | LSD | Pr>F | Std | Cv | N |
| | mg $NO_3$-N/kg | | | | mg/kg | | mg/kg | % | |
| 0-30 cm | 35.6 | 33.9 | 34.6 | 32.6 | 11.8 | 0.9599 | 9.32 | 27.6 | 24 |
| 30-60 cm | 37.1 | 33.3 | 30.8 | 33.7 | 11.7 | 0.7311 | 9.32 | 27.7 | 24 |
| 60-90 cm | 31.8 | 30.1 | 30.3 | 32.1 | 9.9 | 0.9568 | 7.73 | 24.9 | 24 |
| Belje location, 2015 | | | | | | | | | |
| 0-30 cm | 37.5 | 36.7 | 38.9 | 31.3 | 15.7 | 0.7604 | 12.5 | 34.7 | 24 |
| 30-60 cm | 36.4 | 42.0 | 38.2 | 33.3 | 21.8 | 0.8690 | 17.2 | 45.8 | 24 |
| 60-90 cm | 46.5 | 37.1 | 41.3 | 37.6 | 21.2 | 0.7839 | 16.9 | 41.6 | 24 |

F+S+T – Fall+Spring+Topdressing; LSD – least significant difference; Std – standard deviation; Cv –coefficient of variation; N – number of observations

According to LSD values and results of analysis of variance different time of nitrogen application did not significantly influenced on nitrogen content in soil neither in 2014 neither in 2015 at Belje location.

With average coefficient of variation of 26.7 % nitrogen content at Belje location in 2014 varied in range from 30.3 mg/kg at last depth (60-90 cm) on treatment with spring nitrogen application to 37.1 mg/kg on treatment with fall nitrogen application in subsurface soil layer (30-60 cm). In 2015 up to 90 cm of soli depth and different time of nitrogen application, $NO_3$-N content in average varied 40.7%.

Although the nitrogen applied in fall can be lost before the crop uptake and although topdressing applications is much recommended in terms of environmental protection, results reviles no significant differences between nitrogen content in fall N application and topdressing application treatments.

        

Relativity higher amount accumulated $NO_3$-N in soil at fall treatment (Table 3) compared to other treatments was recorder at first depth in 2014 (35.6 mg/kg), second depth in 2014 (37.1 mg/kg) and at the last depth in 2015 (46.5 mg/kg). Similar results were established at Vukovar location (Table 4).

*Table 4: Variability of nitrate-nitrogen content in soil per treatments and soil depth at Vukovar*

| Soil depth | Treatments | | | | Statistics | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fall | Spring | Fall+Spring | F+S+T | LSD | Pr>F | Std | Cv | N |
| **Vukovar location, 2014** | | | | | | | | | |
| | mg $NO_3$-N/kg | | | | mg/kg | | mg/kg | % | |
| 0-30 cm | 46.2 | 39.1 | 39.8 | 40.5 | 17.8 | 0.8289 | 14.1 | 34.1 | 24 |
| 30-60 cm | 44.9 | 43.5 | 42.6 | 41.2 | 17.6 | 0.9750 | 13.7 | 31.8 | 24 |
| 60-90 cm | 38.3 | 40.3 | 41.1 | 37.9 | 16.8 | 0.9728 | 13.1 | 33.2 | 24 |
| **Vukovar location, 2015** | | | | | | | | | |
| 0-30 cm | 45.5 | 34.7 | 31.4 | 34.7 | 14.9 | 0.1556 | 11.4 | 31.2 | 24 |
| 30-60 cm | 39.7 | 39.8 | 38.7 | 36.9 | 11.8 | 0.9499 | 9.22 | 23.7 | 24 |
| 60-90 cm | 50.8 | 43.8 | 36.2 | 41.5 | 15.7 | 0.1485 | 11.2 | 26.0 | 24 |

F+S+T – Fall+Spring+Topdressing; LSD – least significant difference; Std – standard deviation; Cv –coefficient of variation; N – number of observations

## Conclusions

In terms of environmental and production aspects of maize cultivation in relation to the different time-applied nitrogen, two years investigation at two location lead to the conclusion that the application rate of 180 kg nitrogen/ha in fall did not significantly increased content of accumulated nitrogen up to 90 cm soil depth. Nitrogen applied only in fall, or only in spring (pre-plant), or in fall and spring and even applied and distributed in fall, spring and during vegetation (topdressing) equally affected the spatial distribution of nitrate nitrogen in the soil. It also indicates that yield of grain was not under the influence of different time of nitrogen application. The extension of this research to the monitoring of groundwater quality would give much more detailed insight and answers about the risks associated with application of nitrogen in fall. The dynamics of nitrogen depends on precipitation and temperatures, so this investigation should be conducted for several more years in order to confirm the conclusions presented in this paper.

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# Elaboration of the methodology of dew measurement by means of weighing lysimeters

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Abstract: Records of the frequency of occurrence of or the total amounts of dew are scarce. The lack of such records can undoubtedly be attributed to the difficulty of making quantitative measurements of dew. Within some limitations large precision weighing lysimeters can be suitable for the accurate determination of dew amounts. The lysimeter data completed with meteorological data provide the possibility to identify such periods with positive water balance when no rainfall occurred hence dew formation is probable. Nevertheless some "data noises" (oscillations) are characteristic to weighing lysimeter data gained with a high frequency for a longer period. These oscillations can be easily mixed up with the weight increase due to dew formation, therefore filtering or smoothing functions must be used to separate them. Our research aimed to elaborate a method that is suitable to separate true dewfall periods from oscillations and to quantify the amount of dew occurred on the surface of 2 weighing lysimeters of Karcag Research Institute of RISF UD for a longer period. The method we applied for filtering was the application of weight data measured with 1 hour frequency instead of 10 minutes frequency. The investigation period lasted from 1/4/2015 to 30/9/2016. Two high precision weighing lysimeters were involved, one with bare soil surface and one with grass cover. 43.11 and 49.35 mm dew was calculated for the grass covered and the bare surface lysimeters for the total 18-month-long investigation period with the application of our method.

Keywords: dew, data filtering, weighing lysimeter

## Introduction

Dew is the moisture which condenses from the atmosphere on plants, soil, or other surfaces near the ground. Due to its dependence on radiation balance, dew amounts can reach a theoretical maximum of about 0.8 mm per night; measured values, however, rarely exceed 0.5 mm. In most climates of the world, the annual average is too small to compete with rain. In regions with considerable dry seasons, adapted plants benefit from dew (Agam and Berliner, 2006). Records of the frequency of occurrence of or the total amounts of dew are scarce. The lack of such records can undoubtedly be attributed to the difficulty of making quantitative measurements of dew.

Dew can be considered from quite different points of view. For meteorologists, dew is, like rain, a natural phenomenon of condensation that proceeds from the atmosphere and starts on a substrate, submicron particles for rain, a larger surface for dew. For hydrologists, dew is a potential source of water. However, the volume of vapour that can condense at the surface of the ground is obviously much smaller than for rain, where the substrate particles are immersed in a large atmospheric volume from which they collect water (Beysens, 1995).

The amount of dew formed on plants is not well known. It would appear that during dew nights the amounts vary from very small quantities to about 0.51 mm estimated that the maximum possible amount is about 0.76 mm for a 10-hour night, but such amounts would occur only under exceptional circumstances. Total annual dew precipitation may lie between about 12.7 mm in cold climates and in nearly arid warm climates, to about 76.2 mm in semihumid warm climates (Hofmann, 1955). The actual amount of dew in a specific place is strongly dependent on surface properties. For its measurement, plants, leaves, or whole soil columns are placed on a balance with their surface at the same height

and in the same surroundings as would occur naturally (Agam and Berliner, 2006; Uclés et al., 2014). Recent advances in lysimetry allow measuring water balance components with high accuracy and high temporal resolution. Hence, precipitation as well as dewfall as a part of it can be determined directly from lysimeter data. Within some limitations large precision weighing lysimeters can thus be suitable for the accurate determination of dew amounts (Meissner at al., 2007). Furthermore, an adopted method of data interpretation is supposed to be necessary to separate dewfall from other measured water balance components (Nolz et al., 2013).

On the base of the scientific and practical experiences gained during the long-term operation of the weighing lysimeters at Karcag, Zsembeli (2005) figured out that the facilities provide very accurate data of moisture content changes of the lysimeter units. These changes caused by the different water regime of the different soil surface formations and plant covers can be compared and expressed numerically, since all the components of the water balance can be measured or calculated even for short terms.

## Materials and methods

The determination of the dewfall periods was done on the base of the weight data recorded by means of a weighing lysimeter system of Karcag Research Institute RIEF University of Debrecen (KRI). There are six weighing lysimeters (the only ones in Hungary) with surface area of 1.7 m$^2$ and depth of 1 m. The sensitivity of the scales of the weighing system is 0.1 kg (0.06 mm). The basis for calculation was a water balance equation with measured quantities on the left-hand side and the (yet unknown) boundary fluxes between soil and atmosphere on the right-hand side (Eq. 1).

$$\Delta W + SW = P + I - ET \tag{1}$$

($\Delta W$ = change of profile water content, SW = seepage water at lysimeter outlet, P = precipitation on the lysimeter, I = irrigation on the lysimeter, ET = evapotranspiration from the lysimeter; all dimensions are lengths)

The fundamental dataset contained 10-min-data of lysimeter mass (changes equaling changes of water content) and seepage water collected at the bottom outlet of the lysimeters, from which a nominal time series (W+SW) was calculated. The determination of the dewfall periods was done on the base of the weight data recorded by means of the weighing lysimeter system. Those periods are considered dewfall periods when positive weight changes are recorded by the lysimeters and at the same time neither natural precipitation nor irrigation occurred. The amount and duration of the natural precipitation data were determined on the base of the records with 10 minutes frequency of the meteorological station (belonging the official national network operated by the National Meteorological Service of Hungary) located in the territory of KRI at approximately 250 m distance from the lysimeter station. During the investigation period (1st April 2015 – 30th September 2016) the measurement frequency of the weight data of the lysimeters was also 10 minutes in order to harmonize them with the meteorological data. The weight changes of two weighing lysimeters – a grass covered and another with bare soil surface – were determined for each day of the investigation period and put an Excel data base.

**Results and discussion**

*Determination of the dewfall periods*: Analysing all the weighing data of our data base we signed those periods when positive weight changes were characteristic regardless their extent. All these periods were compared to the precipitation data gained by means of the meteorological station and we determined such periods when positive weight changes are recorded by the lysimeters and at the same time neither natural precipitation nor irrigation occurred. For those periods we calculated the daily water balances of the soil columns of the lysimeters taking all the relevant inputs and outputs into consideration according to Eq. 1.

Due to oscillation of the weighing system (which depends on environmental factors such as wind velocity), simple averaging methods are not sufficient to obtain an appropriate accuracy. These 'data noises' can easily be detected by the sensitive (0.1 kg) scales of the lysimeters as temporary increase of the weight. It is obvious that the higher is the measurement frequency set in the lysimeter system, the higher is the chance of the detection and store of false values. In order to distinguish the true dewfall events from the oscillations filtering of the data is necessary. There are several options to filter the data, in this paper one of them is introduced that was developed and found the easiest by our research team.

In this paper one typical day (9th September 2015) is presented when dewfall on the surface of the lysimeters was evident (visible). *Fig. 1* shows the daily weight changes (relative values, no correspondence with the real weight of the soil columns) recorded with the frequency of 10 minutes for the grass covered (left) and for the bare soil (right) lysimeters. Since no natural precipitation was detected on that day by the meteorological station, and we did not apply irrigation, theoretically the positive changes mean dewfall events, while the negative changes indicate evaporation (E) or evapotranspiration (ET). Nevertheless several oscillations also occurred during that day that cannot be explained with dewfall or evaporation/evapotranspiration as these processes are not likely to take place after each other in such a short time.
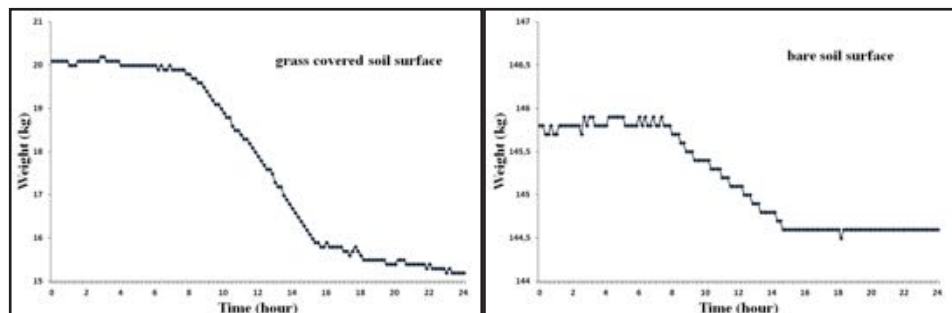


*Figure 1: Daily weight changes in the lysimeters on the base of the data measured at 10 minutes frequency*

As the oscillations cannot be distinguished from the true dewfall events, it was necessary to elaborate a method how the oscillations can be excluded. Our filtering method is based on the principle that dew typically forms during the evening, night and early in the morning when a surface cools through loss of infrared radiation down to a temperature which is colder than the dewpoint of the air next to that surface, hence in such periods when the chance of evaporation is practically zero. In such a case if the moisture gain of

the soil surface by dew formation does not disappear by evaporation soon, the sudden and short-term up and downwards changes in the weight of the soil column of a lysimeter are probably due to oscillations, while such increases in the weight that last longer (like an hour) are most likely due to dew formation. Therefore we changed the weight measurement frequency from 10 minutes to 1 hour (taking only every 6th data into account) in order to exclude such positive weight changes that disappear soon assuming that the longer lasting positive changes are caused by dew falling.

In *Fig. 2* the daily weight changes in the lysimeters on the base of the data measured at 1 hour frequency are illustrated for the grass covered (left) and for the bare soil (right) surfaces. In the graphs shown as examples, we indicated the dewfall periods according to the principle of our estimation with black circles. In the case of the grass covered lysimeter 2x0.058 mm (0.116 mm) dew precipitated on the base of the water balance calculated from the weight changes between 2 and 3 a.m. on 9th September 2015. The first dewfall period was the same as in the case of the grass covered lysimeter with similar weight decrease after it, but then there was another weight increase lasting from 4 to 6 a.m. to resulting in 4x0.058 mm (0.232 mm) dew. The difference is primarily considered to the difference in the soil surfaces, as while in the case of the bare soil lysimeter radiational cooling of the ground surface could cause moisture to condense within the pore spaces of the surface layer of soil and that moisture condenses out of the air which is in the soil pores, in the case of the grass covered lysimeter this process is not likely to take place. Nevertheless it also must be admitted that the possible measurement error is also close to these values.
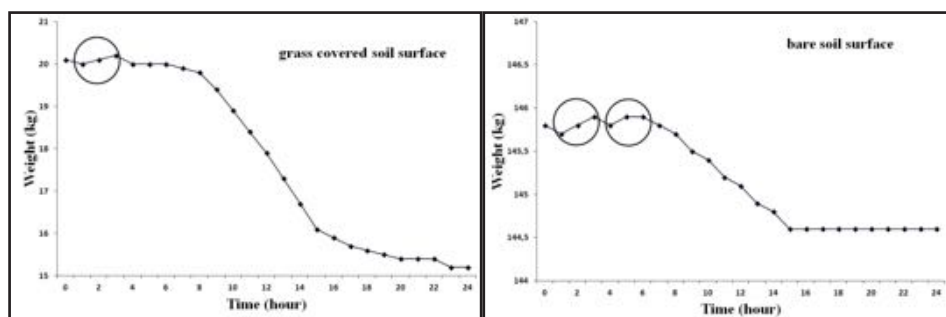


Figure 2: Daily weight changes in the lysimeters on the base of the data measured at 1 hour frequency

In harmony with the literature data our results describing that typical day with dewfall events also prove that dew is most likely to form at night, as temperatures drop and the surfaces (objects) cool. However, dew can form whenever a dew point is reached.

*Determination of the amounts of dew for a longer term*: By means of this dewfall identification method based on the filtering we determined the daily amounts of dew fallen on the grass covered and bare soil lysimeters during all the 549 days of the whole investigation period and calculated the total values. Altogether 43.11 mm dew was detected on the grass covered surface calculated on the base of the water balance data during the 18 months of the investigation period. In order to judge this amount being high or low, we can compare it to the literature data, but unfortunately we have no data for a complete year from January to December yet. As the annual distribution of dew is not even, we cannot compare our values directly to the literature data. Nevertheless splitting the investigation period to broken years (2x9 months) we get 20.82 mm dew between 1st of April and

31st of December 2015, while 22.29 mm between 1st of January and 30th of September 2016, respectively in the case of the grass covered soil surface. Taking these values three quarters of the total amount, the annual amount of dew would be approximately 26-28 mm, which is quite close to the value published by Szász (1992) who calculated 28.67 mm of atmospheric dew. These values are also within the range according to Hofmann (1955) who estimated the total annual dew precipitation between 12.7 mm and 76.2 mm ranging our climate closer to nearly arid warm (12.7 mm) than to the semi-humid warm climates (76.2 mm). In the case of the bare soil surface altogether 49.35 mm dew was detected during the 18 months of the investigation period, which is somewhat higher compared to the grass covered surface. Splitting the investigation period to broken years we detected 20.48 mm dew precipitated on the bare soil surface between 1st of April and 31st of December 2015, while 28.87 mm between 1st of January and 30th of September 2016, respectively. If we estimate the total annual amount taking these values three quarters, we get approximately 26-36 mm, which can be considered realistic taking the investigated conditions into consideration and are in harmony with the literature data.

*Seasonality of dew*: According to the relevant literature the largest chance of dew formation is during the summer months as the conditions of the condensation of water vapours. The dew formation is more when the sky is clear and less when it is cloudy. In a typical summer day, when the sky is clear and the ground and plant surfaces are cooler at nights, there is more evaporation of water and hence more dew formation. When it is cloudy, the ground and plant surfaces do not get cool in the night and hence there is less dew formation. Contrary to these, the amount of dew measured on the grass covered soil surface in the 6 summer months (June, July, August of 2015 and 2016) was only 6.58 mm giving 15% of the total 18 months (*Fig. 3*).



*Figure 3: Monthly dew amounts measured on the lysimeters*

The dewiest period was detected on the grass covered surface in the three-month-long period between November 2015 and January 2016 with its 13.77 mm (40% of the total amount). Obviously most of the dew precipitated during this period was frost. In the case of the bare soil surface a bit more even time distribution of dew formation was characteristic. The amount of dew measured on bare soil surface in the 6 summer months (June, July, August of 2015 and 2016) was 14.11 mm giving 28% of the total 18 months. The dewiest period was a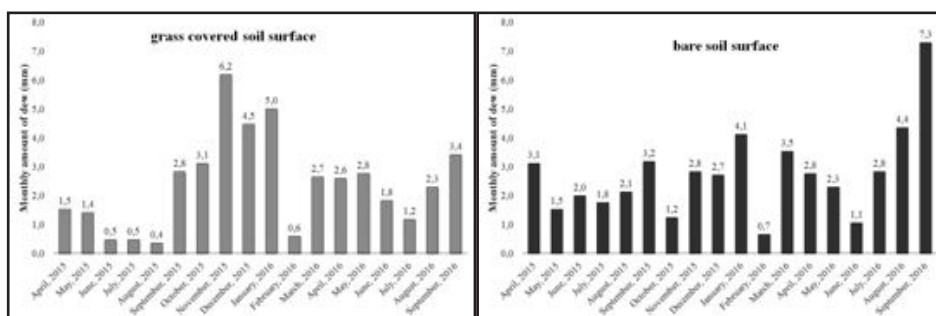lso during the period from November 2015 to January 2016, but with somewhat lower amount (9.65 mm) and percentage (20% of the total amount). Extreme amount of due was detected in September 2016 with its 7.29 mm.

## Conclusions

We established that the application of the weight data measured with the frequency of 1 hour seems to be more suitable to filter and exclude the oscillations and get only the weight increases that describe dewfall than the application of the weight data measured with the frequency of 10 minutes. Continuing our measurements we intend to determine the amount of dew for different soil surfaces for several years in order to have the basis for further comparisons.

## Acknowledgement

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# Complex effect of secondary salinization and composting on soil respiration

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Abstract: Salt-affection has been and still is a critical issue facing croplands in a large portion of the Great Hungarian Plain. Compost is known to have a positive effect on salt-affected soil, but little is known about the correlation between soil organic matter and bacterial activity. Microbial biomass and activity is found to be relative to levels of soil organic matter (SOM). Bacterial activity is affected by both salts and organic matter, and this research aims to determine the breakpoint and relationship between those two parameters. A pot experiment with 27 pots was set in Karcag Research Institute of RISF UD where soil respiration was measured under controlled conditions over a 16-week-long period. During the initial phase of the experiment, compost was added to non-salt-affected soil in differing doses (0, 25, 50 t/ha), and each sample was irrigated with a solution of different salt concentrations (0, 600, 1800 mg/l) with three test replications. At the tenth week, the compost was added to each at second time. At the thirteenth week, soil conditioner, rich in N, S and Ca, was added equally to each soil sample. Following the experimental phase, nutrients and bacterial colony measurements were conducted. During the phase of the experiment with compost application alone, the results demonstrated that salt concentration of irrigation water and compost ratio had negligible influence on soil respiration. However, following the addition of soil conditioner, a dramatic increase in respiration was recorded with initial spike of chemical reaction and second spike with biological reaction. Nutrient components were highly affected by salt and SOM, therefore, soil microbial activity fluctuates with variable reasons including combination of nutrients under salt stress environment.

Keywords: $CO_2$ emission, composting, soil organic matter, secondary salinization

## Introduction

Salt-affected soils are often found in arid or semi-arid regions. 230 million hectares of land are available for irrigation, 45 million hectare (19.5%) are salt affected currently. Furthermore, increased salinization of arable lands may result in a 30% loss of land, with some estimates as high as 50% by 2050 (Wang et al., 2003). The problem is caused by excessive salts inhibiting plant mineral nutrients uptake which, in most cases, leads to prematurity and low crop yield. However, salt resistance in plants is a complex interaction of characteristics determined by a number of genes and gene combinations, and there have been limited successes in developing salt-resistant species (Ahmad and Rasool, 2014; Roy et al., 2014). In order to utilize the salt-affected soils, many studies have been conducted to improve the quality of soil physically, chemically and biologically through the implementation of organic wastes as fertilizers and amendments (Diacono and Montemurro, 2015; Tejada et al., 2006; Yadav and Agarwal, 1961).

However, it is still unknown whether plant and soil quality is affected negatively due to the direct salt pressure, or to the indirect effect of a decline in soil organic matter which has a positive effect on soil structure (Wong et al., 2010). The relationship between salinity and soil organic matter on a microbial community needs to be studied further in order to develop the fundamental understanding and resulting applications. The aim of our research is to investigate the effect of compost on salt-affected clay soil. Microbial biomass and

activity is found to be relative to levels of soil organic matter (SOM). It is little known whether low microbial biomass is affected directly by salt accumulation or by reduced levels of SOM due to poor plant growth in salt-affected soils. Bacterial activity is affected by both salts and organic matter, and this research aims to determine the breakpoint and relationship between those two parameters.

**Materials and methods**

A pot experiment was conducted at the Karcag Research Institute RIEF University of Debrecen (KRI) in Karcag, Hungary where the wide range of soil is affected by salinization and sodification due to shallow and saline underground water with high clay contents. It is a representative place where secondary salinization has been observed, so this place was chosen for experiments with high applicability.

The experiment was conducted about 120 days. Parts of experiment design including duration, parameters of measurements, and compost rates were referred to Wong et al. (2009). This experiment investigated the correlation between compost and salt concentration on microbial activity. All treatments were replicated three times.

Soil samples were taken from the grounds of the Karcag Research Institute where underground water did not affect soils with soluble salts. Soils were carefully sieved through 5 cm mesh, and mixed well to provide uniform samples. Soil properties including soluble cations, pH, and humus contents were analyzed prior to field experiment. In this study "Terrasol," which is a mature compost mainly composed of sheep manure products released from KRI, was used. The doses of application were followed by the producer's recommendation.

In the region of Karcag, well water is rich in soluble salts, which leads to salinization and sodification. In this experiment, well water was used to simulate conditions similar to the real situation happening in this region. Well water contains approximately 1800 mg/L of soluble salts and is approximately pH 7.4 year-round with little fluctuations from the past experiments in the institute. Well water was used in this experiment because secondary salinization has been observed around this area with well-water irrigation (Zsembeli et al., 2011).

Each sample was irrigated after the soil became dry (roughly once in one to two weeks) to maintain humidity. Samples were irrigated and monitored for the microbial response to salt accumulation over time. Obviously accumulation of salts increased as time proceeded.

6kg of soil was placed in each 10 L bucket, and incorporated with different amounts of compost during the 1st week of the experiment. All the samples were irrigated after soil dried. Table 1 indicates the experimental design. During the tenth week, compost was added to each sample a second time, effectively doubling the compost/soil ratio. During the final stage, beginning in the thirteenth week, 4.1 g of soil conditioner as recommended by the producer was added equally to each sample (since the sample results were not significantly different at this point). The soil conditioner was a product called "*Solactive*" from Timac Agro. It is rich in nutrients; N 14%, $SO_3$ 28%, CaO 22%, and MgO 2%. Hand

tillage was performed after both compost and soil conditioner application to create a more uniform mixture.

The experiment was conducted in a room with moderate temperatures to avoid cold outside weather, and moved outside with overhead cover once the weather became warmer during June. Samples were irrigated 28 times in total amount of 2.6 litres.

*Table 1. Design of the pot experiment*

| Organic Matter / Salt Concentration | Control (0 t/ha) | Compost (25 t/ha) | Compost (50 t/ha) |
|---|---|---|---|
| 0 mg/L (control) | 0-0 | 0-25 | 0-50 |
| 600 mg/L | 600-0 | 600-25 | 600-50 |
| 1800 mg/L | 1800-0 | 1800-25 | 1800-50 |

To measure soil respiration, $CO_2$ measurement was often used to monitor the microbial activity. $CO_2$ measurement was conducted on the day after irrigation to have same conditions of wetness since the water content of soils has correlation with microbial activities. $CO_2$ was measured with a Gasalert Micro 5 infrared gas analyser. The same methodology was used in KRI by Zsembeli (2006) previously.

**Results and discussion**

Our hypothesis was that microbial activity is influenced positively by compost and negatively by salt stress. In order to have some ideas about the correlation between those two parameters we determined the carbon dioxide emission (soil respiration referring to microbial activity) from the treated soils of the pot experiment.

Fig. 1-3 show the results of soils respiration of the soil samples with the same salt/water concentration levels for each compost/soil ratio over the four-month-long duration of the experiment. The results show that application of compost twice did not show significant differences in soil respiration. However, after the application of soil conditioner which is rich in N, S, and Ca, there were significant differences between different irrigation salt concentrations at 98th day ($P < 3.3e{-}17$). The samples irrigated with 1800 mg/L water had the highest soil respiration, and the lowest respiration level for deionised water. However, after the initial spike, salt concentration was found to have no significant effect on soil respiration when measured at 113th day ($P = 0.59$).

Overall, our data indicate that irrigation did not affect soil respiration with compost application alone. However, high compost ratios slightly improved soil respiration at all levels of salt concentration. Application of soil conditioner after compost triggered the two spikes in soil respiration, and the interaction between salt and compost can be seen ( 98th day: $P < 0.026$, 113th day: $P < 0.030$).

First spike was occurred with different salt levels. To amend saline soils, $CaCO_3$ (limestone) is usually applied to neutralize exchangeable Al or Na, and supplies Ca in acid soils. Ca precipitates as calcite ($CaCO_3$) instead of reaching with infiltration.
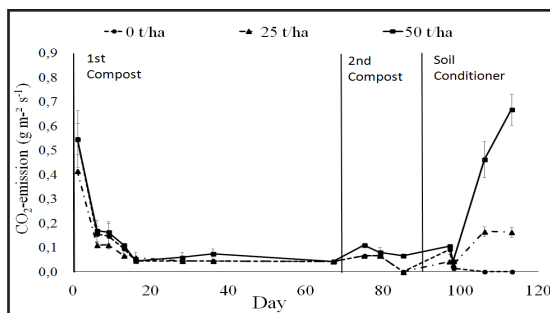
Figure 1. $CO_2$ emissions under 0 mg / L irrigation with different compost doses
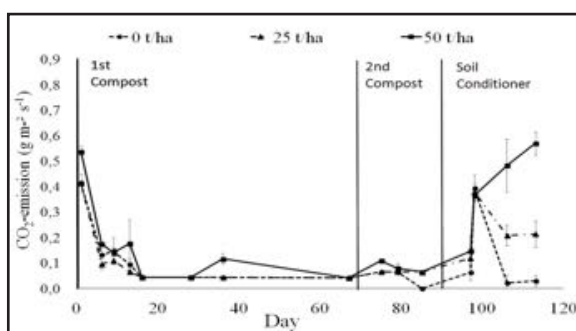


Figure 2. $CO_2$ emissions under 600 mg / L irrigation with different compost doses
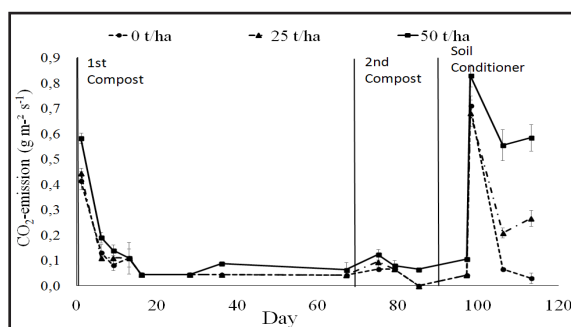


Figure 3. $CO_2$ emissions under 1800 mg / L irrigation with different compost doses

However, in sodic soils, lime is not suitable for reclamation. Limestone can be only used when acidifying amendment is applied prior to lime application. A common amendment procedure is application of gypsum ($CaSO_4 \cdot H_2O$). Common acidifying procedure for the reclamation of sodic soils is sulphuric acid and elemental sulphur. It is critical to oxidize sulphuric acid by soil microorganisms, and a lag time is several weeks to months before leaching begins. In general, those reactions take place in soils, which could lead emission of $CO_2$ from soil by chemical reaction (Abrol et al, 1988; Bohn et al., 2001).

Thus, it can predict that sulphur in soil conditioner reacted with water and elements in well water such as sodium. Brinck and Frost (2009) also indicate that Ca can be mobilized through the addition of $H_2SO_4$. Gypsum reduces deterioration of clay-rich soil structure and improves infiltration rates. Under high Na and $HCO_3^-$ concentrations, elemental S application converts some of $HCO_3^-$ to $CO_2$ and reduces the precipitation of $CaCO_3$ and suppresses SAR.

The second spike on 116 days was led by the compost level. Although there were enough compost applied before, soil conditioner was the inducing reason on second spike. It is predicted that composition of compost or total elements were modified after addition of soil conditioner. It could happen because of two possible reasons, either leaching of excess salts or addition of nitrogen causing lower C/N ratio.

In some cases, glucose addition promotes microbial growth, and nitrogen or phosphorus increased at even higher ratio, while carbon addition does not affect respiration (Aldén et al., 2001). Liming has the potential to decrease C mineralization of SOM and plant residues, but mineral N has a positive effect on increasing $CO_2$ derived from organic matter. However, N effect was cancelled out if lime and N were applied simultaneously (Wachendorf, 2015).

In terms of salt concentration, salinity did not necessary correlate with $CO_2$ flux in this experiment. Yan and Marschner (2013) also found a similar result that soil respiration rate was highest in the low-salinity treatments and lowest in the mid salinity treatments, while the soil microbial biomass was highest in the high salinity treatments and lowest in the low-salinity treatments. This was attributed to increased substrate availability with high salt concentrations through either increased dispersion of soil aggregates or dissolution or hydrolysis of soil organic matter, which cancels the stresses on the microbial population from high salt concentrations.

Other studies focus respiration from the point of carbon-use efficiency (CUE). CUE is defined as a ratio of growth over C uptake. Riggs and Hobbie (2016) conducted seven-year of N addition, decomposition rate and respiration of SOM were both measured with decline trends. Additional nitrogen addition decreases CUE due to decreased oxidative enzyme activity. However, the mechanism remains uncertain. According to Manzoni et al. (2012), CUE of soil microbial communities increased as the C/N ratio of the soil or decomposing substrate decreased.

**Conclusions**

There were no significance of compost and salt on the microbiological activity of the soil till addition of a soil conditioner rich N, S, and Ca. After this addition, there was a chemical reaction, and followed bacterial reaction. Thus, there was more impact of SOM than salt in this experiment. Our results indicate that compost has played a role to suppress salt stress by increasing C/N and essential nutrients. Even though the addition of salt may not have been enough load to be stressed or not enough sufficient time compared to under practical situations where salinity develops to higher degrees, this experiment indicates the situation of starting point of salt-affected soils.

**Acknowledgement**

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# Land cover changes in the Visegrád Group between 1990 and 2012

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Abstract: The countries of the Visegrad group share many common traits in their historical development. During the socialist era the countries followed a largely similar development path, and the subsequent social and economic transformation also involved similar processes, which had a great impact on the land cover. Extensive suburbanisation and greenfield investments became the driving force of a rapid urban sprawl, the disintegration of Comecon and loss of protected markets led to agricultural abandonment and fallow lands, while the EU integration and access to CAP support again altered the circumstances for agricultural activity.

The aim of my paper is to analyse the land cover change processes in the Visegrad Group following the political and economic transition. To identify the similarities and differences between the countries, the analysis was carried out for the whole group and also for each country.

The analysis was based on Corine Land Cover raster datasets with 100-meter resolution for 1990 and 2012. The high number of the original categories (34 for the whole Visegrad Group) was reduced by merging them into the following categories: artificial surfaces, arable land, vineyards and fruit cultivations, grasslands, complex cultivation patterns, forests, wetlands and other natural areas and water bodies. For the analysis, the Terrset Land Change Modeller and ArcGIS software were used.

The results indicate that even in the case of the most general land cover change trends, which are common in country-level (increase in the share of artificial surfaces and forests, loss of arable land), there are big differences in the spatial patterns of the change. The expanse of arable land of the Polish eastern periphery and the decrease in the Czech periphery clearly illustrates the effect of region- and country-specific factors. The limitations of the Corine Land Cover database are also observable through the false increase of artificial surfaces in Poland.

Keywords: Visegrád Group, Corine Land Cover, political and economic transition, artificial surfaces, heterogeneous agricultural areas

## Introduction

The historical development of the Visegrád countries shows many similarities. It is especially true for the second half of the twentieth century, when the region was incorporated into the Eastern Bloc. The economies of the state socialist countries often considered very similar to each other. While there is a truth in it for example in case of their industrial policy, in fact, the three Visegrád countries implemented markedly different agricultural models during the decades of communism.

Czechoslovakia adapted the soviet sovkhoz-kolkhoz model without inclinations (Doucha and Divila 2008). Hungary also adapted the main elements of this model (albeit the transformation only finished after the revolution), but in a less constrained way. In some territories (like the Danube-Tisza Interfluve) the farmers could retain their land ownership, and agricultural activity in small household plots was also encouraged (Csatári and Farkas 2008). Opposed to this, small-scale family-farming system persisted in Poland during the socialist era, with a significant share of state farms only in the so called "regained territories". But in spite of these differences, some of the main land cover change processes were similar in the three countries (decrease in arable land, increase in artificial surfaces and forests) (Bezák and Mitchley 2014; Farkas and Lennert 2015; Gubka et al. 2013). After the political and economic transition, significant changes in the land cover patterns could be anticipated. From the five types of driving forces of land use change (political, economic,

cultural, technological and natural), four underwent drastic changes during and after the transition (Hersperger and Bürgi 2009). Just like in the preceding historical periods, many of the changes were common for each country during these two decades: the disintegration of Comecon and the loss of protected markets, loosely controlled urban sprawl in the wake of socioeconomic transformation, accession to the EU and the introduction of the CAP subsidiaries and regulations. All these changes had an impact on the land use, but the question is: combined with the unique national and regional characteristics, have they had the same effect?

The aim of my paper is to analyse the main land cover patters and their changes after the political and economic transition, and to identify the common and country-specific land cover change trends. Since land cover change is a key element of the post-productivist rural restructuring, my focus will be on the rural areas.

**Materials and methods**

The Corine Land Cover database was used for analysing the land cover changes of the Visegrád Group. This data collection was coordinated by the European Environmental Agency for more than thirty years, and uses the same methodology for all the participating countries. For the Visegrád group, datasets are available for the reference years of 1990, 2000, 2006 and 2012. To ensure compatibility with the Terrset Land Change Modeller software, I used raster datasets with the resolution of 100*100 meters.

From the original 44 land cover categories, 32 appear in the Visegrád countries. At this number, it is hard to highlight the general land cover change trends, so the number of land cover categories has to be reduced for the analysis. The eight aggregated categories are the following: artificial surfaces, arable land, vineyards and fruit cultivations, grasslands, heterogeneous agricultural area, forests, wetlands and other natural areas and water bodies.

The changes between the aggregated categories were analysed in country and local administrative unit (LAU-2) level (in case of Poland, the urban and rural part of the urban-rural gminas were considered as independent analytical units). I also introduced two spatial categories – rural and remote rural. I consider all local units rural, which have less than 5000 inhabitants (regardless of administrative status) or do not possess city rights. Those rural areas, from where it takes at least 45 minutes to reach the nearest city with at least 50000 inhabitants are considered remote rural (while the remaining rural settlements are considered commutable rural). For the data processing and the analysis, the ArcGIS and Terrset Land Change Modeller software were used.

**Results and discussion**

The Table 1. shows the distribution of the eight land cover categories in the Visegrád group. In three countries (Czechia, Hungary and Poland) arable land is the largest category, while due to its generally elevated landscape, forests cover the most area in Slovakia. Natural physical conditions play an important role in case of other categories too. Because of its semi-arid puszta-type habitats, Hungary has the highest share of grasslands from the four countries. Some categories cover only a small part of the Visegrád group (vineyards and fruit cultivations, wetlands and other natural areas), but play an important role in the formation of its landscape or as habitats with rich biodiversity.

*Table 1: The share of the aggregated land cover categories in the countries of the Visegrád Group*

|  | **Poland** | **Czechia** | **Slovakia** | **Hungary** |
|---|---|---|---|---|
| **Artificial surfaces** | 3.3% | 6.0% | 5.7% | 5.6% |
| **Arable land** | 44.9% | 45.1% | 34.4% | 53.5% |
| **Vineyards and fruit cultivations** | 0.3% | 0.6% | 0.8% | 2.3% |
| **Grasslands** | 9.0% | 3.7% | 7.1% | 9.7% |
| **Heterogeneous agricultural area** | 10.5% | 9.1% | 8.7% | 5.2% |
| **Forests** | 30.2% | 34.7% | 42.1% | 20.7% |
| **Wetlands and other natural areas** | 0.5% | 0.2% | 0.6% | 1.1% |
| **Water bodies** | 1.4% | 0.7% | 0.4% | 1.8% |

*Source: Own elaboration based on Corine Land Cover database*

Due to the urbanisation characteristics of the earlier periods, the artificial surfaces display an especially uneven spatial pattern. Besides the agglomeration of the capital cities, the Upper Silesian conurbation and the Ore Mountains Mining Region are the most notable concentrations. While the share of artificial surfaces is quite similar in Czechia, Hungary and Slovakia, Poland shows significantly lower proportion of artificial area (Table 1).
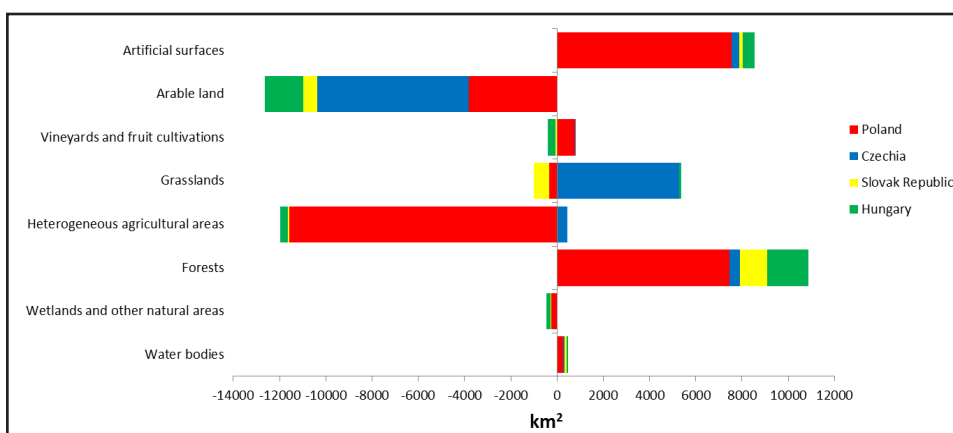


*Figure 1: Change in land cover by each category between 1990-2012. Source: Own elaboration based on Corine Land Cover database*

The heterogeneous agricultural area is a very diverse category. It contains lands principally occupied by agriculture with significant areas of natural vegetation, and the original Corine category called complex cultivation patterns. It was a diverse category in itself, connected to intensive agricultural activity like horticulture. Complex cultivation patterns include scattered farm areas and garden zones, which are typical accessory elements of the market towns of the Hungarian Plain. This also reveals that not all inhabited areas belong to the category of artificial surfaces.

As we can observe in Figure 1., the political and economic transition did not disrupt some of the most important land cover change trends. The increase of artificial surfaces and forests and the decrease of arable land continued in the period of 1990-2012. These are common trends for each country. In the case of other land cover categories (e.g. vineyards and fruit cultivations, grasslands), the direction of changes is not so unambiguous.

*Figure 2: Relative change in land cover by each category, 1990-2012 (in percentage of the land cover in 1990)*
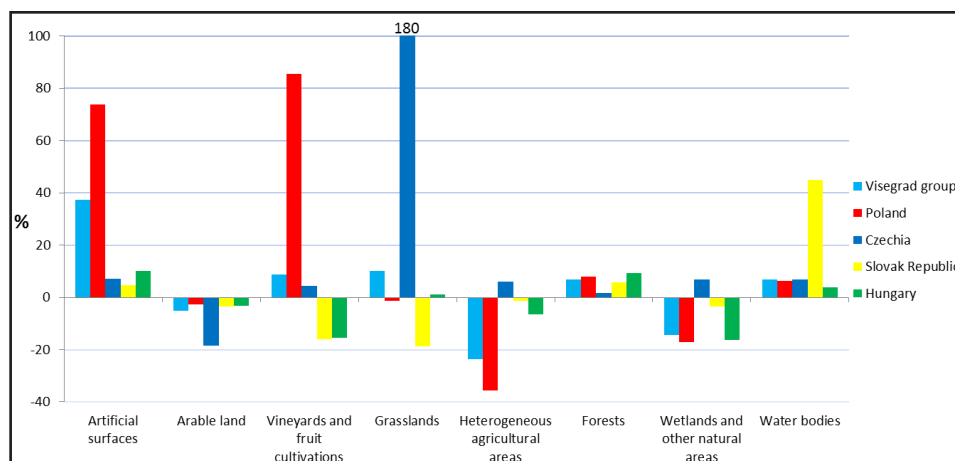*Source: Own elaboration based on Corine Land Cover database*

In Figure 2., we can observe that there are vast differences in the rate of changes even in the case of common trends. For example, the share of artificial surfaces increased everywhere, but in Poland, it is almost doubled just in two decades. There are also big differences in the loss of arable land - in Czechia, this category decreased with almost one-fifth of its original extent. We can also observe some radical changes in the case of categories where common trends are not apparent, like the drastic increase in the area of vineyards and fruit cultivations in Poland or in the area of grasslands in Czechia. We can suspect that country- or region-specific circumstances are behind these unique processes. The case of Slovakia supports this claim: the significant increase in water bodies can be connected to a single event: the opening of the Gabčíkovo water reservoir.

The analysis in the local administrative (LAU-2) level uncovered some of these unique factors. In case of artificial areas, the significant differences between Poland and the rest of the countries reappear in the change patterns. In Czechia, Hungary and Slovakia, increase is limited to the local units in the urban areas of capitals and regional centres. This process can be adequately explained with intensive suburbanisation and greenfield investments following the political and economic restructuring (Stanilov and Sýkora 2014). However, in Poland, a more general increase can be observed. After analysing the changes with the introduced urban/commutable rural/remote rural categories, this land cover change trend seems more and more peculiar. The increase was moderate (a little more than 20%) in the urban local units, in the commutable and even in the remote rural areas the area of artificial surfaces more than doubled just in two decades. No spatial process (e. g. counterurbanisation) can explain such an immense expanse in the periphery. Further investigation revealed that the main source of this transition was the heterogeneous agricultural area land cover category. It was already mentioned that besides agricultural areas, this category can also include inhabited areas. It is also true to Poland, where the houses of the villages are often spread out along the roads in a scattered pattern, mixed with agricultural lands. A large part of these settlements was categorised as heterogeneous agricultural area in 1990, but this methodological decision was revised in the following datasets, and these areas later appeared as artificial areas. It indicates that in many cases real transformation to artificial surfaces did not occur, and gives an answer to why was Poland's artificial land cover so low in 1990.

      

The influence of region- or country-specific factors can be found in other transformations too. While the decrease of arable land was general, there are large areas of Poland where the area of arable land actually increased. These areas can be mostly found in the eastern part of the country. In these areas small-scale family farming persisted during the socialist era (Bański 2011). The relatively low unemployment of these regions indicates that many former industrial workers returned to subsistence farming. This safety net function explains why market-controlled land abandonment avoided the region (Zgliński 2008).

The Czech peripheries serve as an opposite example, where a significant transformation (more than 20%) from arable land to grassland occurred. Behind this we can also suspect country-specific reasons – these areas underwent population exchange after the Second World War, and the new residents could not get attached to their land before its nationalisation. After the restitutions, this lack of emotional ties led to land abandonment in the changing market environment. Also, some of the LFA subsidiaries in the mountainous parts of Czechia also favour grasslands (Štolbová 2007).

Incidentally, the increase in grasslands can also be observed in urban and commutable rural areas. For example in Hungary, the increase in the aforementioned areas exceeds the increase in the remote rural areas. Arable land abandoned in hopes of later investments often turned into grassland for the time being – thus the more intensive expanse.

Amongst the categories with modest overall share, the land cover change of vineyards and fruit cultivations is also heavily dependent on local conditions, which indicates that the special characteristics of the producing regions have growing importance in the market economy.

**Conclusions**

Land use change is one of the key processes of rural restructuring in the developed world. Using the Corine Land Cover database, the land cover changes in the Visegrád Group after the political and economic transition were analysed using eight aggregated land cover categories. The results indicate that the political and economic transition did not disrupt the most important land cover change trends (decrease in arable land, increase in artificial surfaces). The most general land cover change trends are common for each county (increase in the share of artificial surfaces and forests, loss of arable land), but apart from that, the effect of regional and national factors is clearly observable. Typical examples for that are the increase in arable land in the Polish eastern periphery, and the significant decrease of the same category in the peripheral Czech regions. Moreover, the reasons behind the exceptional increase of artificial surfaces in Poland also reveal the limitations of the Corine Land Cover database.

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# The determination of the areas at risk of soil degradation by water erosion

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Abstract: Water erosion (except from intra-soil and raindrop erosion) occurs only upon the formation of rainfall surface runoff and a puddle creation on the soil surface. Both of these phenomena are observed when the intensity of rainfall, resp. melting snow is greater than the intensity of infiltration. Moreover, the formation of puddle erosion is dependent on the zero soil surface slope gradient. Surface runoff from the investigated slope (catchment) begins when the depth of the design rainfall exceeds the initial retention of the soil surface on slope, resp. in the catchment. By the application of Soil Conservation Service curve number method the depth of initial potential retention of the study area can be calculated. This calculation uses the representative value for the study area (or its part), which is, in the case of the design rainfall, a function of the soil hydrological properties, the quality of soil cover and land-use. The aim of the contribution is to present a simple approach of initial determination of potential surface retention at the catchment scale (Tajná Stream catchment, West Slovakia). The analysis shows that whole agricultural as well as forest land in the study area is potentially prone to water erosion. The advantage of the proposed approach is that inputs for this method are generally available for the whole Slovakia (and probably for the other countries too) as well as the fact that it can be successfully implemented into the GIS interface.

Keywords: water erosion, surface retention, intensity of design rainfall, surface runoff, CN value

## Introduction

Soil erosion is recognized as one of the most common physical soil degradation processes in Slovakia. Although being one of the processes naturally occurring in the environment, when the soil erosion intensity evidently exceeds the intensity of soil formation, various social, economic, environmental and agricultural problems emerge. Particularly, water erosion that threatens more than 55% of agricultural soils in Slovakia needs to be taken into consideration when managing and planning human activities by application of erosion control principles (e.g. land consolidation, soil fund organization, landscape planning, integrated catchment management) (Kondrlová, Antal, 2015). For the purposes of information processing, analysis and decision making geographical information systems are widely used nowadays (Kondrlová, 2009). Water erosion (except from intra-soil and raindrop erosion) occurs only upon the formation of rainfall surface runoff and a puddle creation on the soil surface. Both of these phenomena are observed when the intensity of rainfall, resp. melting snow – $i_R$ is greater than the intensity of infiltration – $v_i$ (at the saturation related to saturated hydraulic conductivity of the near-surface soil). Moreover, the formation of puddle erosion is dependent on the zero soil surface slope gradient. To determine the depth of the surface runoff ($H_{S,R}$), water balance method can be used as the simplest option at the slope as well as catchment scale (Antal, Igaz, 2008):

$$H_{S,R} = H_P - H_I - H_A - V_i \qquad (1)$$

where:  $H_{S,R}$ - depth of surface runoff (mm)
  $H_P$ - depth of precipitation (mm)
  $H_I$ - depth of rainfall interception by the soil surface vegetation cover (mm)
  $H_A$ - depth of rainfall accumulated on the soil surface (mm)
  $V_i$ - depth of water that will infiltrate into the soil during rainfall duration (mm)

Water balance model is often used also for modelling purposes (e.g. van Dijk, 2010). Since it is difficult to estimate the soil infiltration rate for different landuse types at varying quality of land cover, we recommend to apply the worldwide known Soil Conservation Service curve number (CN) method (Chow, 1964) that is used e.g. in catchment scale calculations of sediment delivery ratio and the amount of direct surface runoff (Šinka, Kaletová, 2013; Šinka, 2009). This approach estimates the potential (maximum) retention (2) and the depth of direct runoff while it takes into account specific soil hydrological properties, the quality of soil cover and landuse. This procedure finds its application also at width design of vegetation filter strips (Antal, 1985; Soulis, Valiantzas, 2012; Ishtiyaq, Vivek, Mukesh Kumar, 2015):

$$A = 25.4 \: [(1000/CN)-10] \tag{2}$$

where:    A - potential surface retention (mm)
           CN - representative CN value for specific landuse category

$$D \geq L_n * H_{O,L} / (0.2 * A - H_p) \tag{3}$$

where:    D - width of the vegetation filter strip (m)
           $L_p$ - the length of the adjacent slope (m)
           $H_{O,L}$ - amount of surface runoff from the upslope area (mm)
           A - potential surface retention of the filter strip (mm)
           $H_p$ - the amount of design rainfall (mm)

To gain a mathematical solution, the expression $(0.2 * A - H_p)$ has to be greater than 0. After employing the formula (2) the initial potential surface retention of the study area can be calculated as follows (Antal, 1985):

$$H_{R,i} = (5080/CN_i - 50.8) \tag{4}$$

where:    $H_{R,i}$ - depth of potential surface retention (mm)
           $CN_i$ - representative CN value for specific landuse category

The aim of the contribution is to present a simple approach of initial determination of potential surface retention at the catchment scale using the GIS interface.

## Materials and methods

The Tajná Stream catchment (647 ha) is located in the northern part of the Nitra region in the Danubian Upland, in western Slovakia. Tajná Stream (4.7 km) flows into Širočina River in the cadastral area of the Tajná municipality (48°15'42.6" lat., 18°21'42.58" long.). The climate varies from warm to temperate with either Atlantic or continental influence. The average annual air temperature is 10° C and average annual rainfall is 590 mm (Kondrlová, 2009). The catchment is used predominately for forestry (51 %) and agricultural production (Table 1) on Cambisols and Luvisols, respectively.

*Table 1: Representation of the landuse categories in the Tajná Stream catchment*

| Category | Area, ha | % | Category | Area, ha | % |
|---|---|---|---|---|---|
| forest | 332.30 | 50.81 | shrubs | 10.14 | 1.55 |
| arable land | 271.19 | 41.46 | built-up area | 5.93 | 0.91 |
| orchard | 10.12 | 1.55 | garden | 4.90 | 0.75 |
| pasture | 9.19 | 1.40 | roads | 3.50 | 0.53 |
| meadow | 5.03 | 0.77 | water | 0.34 | 0.05 |
| vineyard | 1.13 | 0.17 | quarry | 0.29 | 0.04 |

All the digital analyses and map outputs were processed in ArcGIS 10.2.2 (© ESRI) using various processing tools, mainly the Spatial Analyst and Conversion Tools. Landuse map was created after digitizing the topographic maps (at scale 1:25000). The information on soil types was obtained from seven-digit code of soil-ecological units (© Soil and Soil Protection Research Institute, Bratislava), that were processed as a result of Complex Soil Survey in 1961 – 1970 in Slovakia. The information on forest soils was obtained from the National Forest Centre in Zvolen.

## Results and discussion

To convert the soil map into hydrological soil groups (HSG) tables available in Slovak literature were used (Antal, Igaz, 2008; Šinka, Muchová, Konc, 2013). The soils of the hydrological soil group B, C, D were identified around the catchment. The same literature sources were used for assigning CN values (adjusted to Slovak conditions) on individual forest land (forests, shrubs) and agricultural land (arable land, meadow, pasture, orchard, and vineyard) categories occurring on represented HSGs (Table 2). In case that such a source missing, the original methodology of Chow (1964) can be used. CN values closer to 100 interpret a low potential of surface retention and a high potential of surface runoff or puddle formation. The risk of puddle formation was limited to areas with the slope gradient less than 1°. According to our analysis the puddles are more likely to form on the ridges as well as valleys - where very flat areas naturally occur. The potential surface retention in the study area was estimated according to the formula (4). Forests showed to be the most stable landscape feature regarding the water erosion control, with the potential surface retention reaching up to 42 mm of water depth (Figure 1). Due to combination of soils with low infiltration potential (group D) and weak vegetation cover (predominantly patchy shrubby areas) the lowest retention potential was identified on pastures and shrubby areas (down to 5.6 mm).

Table 2: Recommended CN values according to hydrological soil group (HSG), landuse management and the quality of vegetation cover (Hrádek, 1989* ; Šinka, Muchová, Konc, 2013[+])

| HSG | Landuse categories | | | | | | |
|-----|-------------|----------|---------|---------|---------|---------|----------|
|     | Arable land* | Pasture* | Meadow* | Shrubs* | Forest* | Orchard[+] | Vineyard[+] |
|     | CN values | | | | | | |
| B | 75 | 80 | 60 | 80 | 55 | 73 | n.a. |
| C | 80 | 85 | n.a. | 90 | 65 | 82 | 82 |
| D | 85 | 90 | 80 | 90 | n.a. | 86 | n.a. |

n.a. - no applicable for current study area

Taking into account the rainfall depth at specific return period (10-years, periodicity = 0.1 and 50-years, periodicity=0.02) obtained from the closest meteorological station in Vráble (Antal, Igaz, 2008) it was possible to estimate the depth of the water layer that would be created on the soil surface at the specific surface retention capacity (Figure 2 and Figure 3).

Localities with the risk of surface runoff or puddle formation were identified on areas where the depth of the design rainfall – $H_{D,R}$ exceeded the initial retention of the soil surface on slope, resp. in the catchment – $H_{R,i}$. In general whole study area is threatened by water erosion at some extent - in the forest land water layer of 12 mm of depth (at $H_{D,R}$ = 53.8 mm), respectively 24 mm (at $H_{D,R}$ =65.9 mm) would be potentially formed.
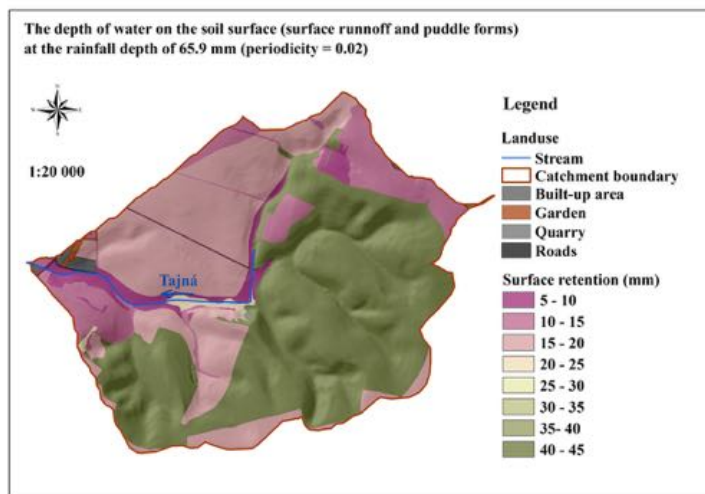
*Figure 1. Potential surface retention (mm) of the study area estimated according to CN method*

The depth of surface runoff for other landuse categories can possibly rise up to 48 mm (at $H_{D,R}$ = 53.8 mm), resp. up to 60 mm (at $H_{D,R}$ = 65.9 mm). According to the proposed methodology, the specific zones where $H_{D,R}$ exceeded $H_{R,i}$ were determined as areas with risk of soil degradation formation by water erosion.

These results can be used for the purposes of preliminary assessment in various areas of landscape planning and watershed management (Ishtiyaq, Vivek, Mukesh Kumar, 2015). The importance of the results increases with the depth of the estimated surface runoff and its location according to specific landuse types (build up areas, water dams, etc.).

The calculation procedure can be extended taking into account various further characteristics of the catchment, such as flow accumulation (Šinka, 2009) to calculate only the depth of the surface runoff, but also its volume, sediment delivery ratio and other characteristics of erosion-deposition processes (Šinka, Muchová, Konc, 2015; Kondrlová, 2009). Besides with more detailed approach also the requirements on the obligatory input data and GIS skills will increase.

It is obvious, that the performance of this approach is highly dependent on the precise CN value estimation. Besides the landuse, soil cover quality and soil type characteristics, also the antecedent moisture conditions of soil prior to specific rainfall event need to be taken into account considering the fact that more moistened soil will have lower retention capacity than the dry soil.

Chow (1964) presents CN value conversion table for individual antecedent moisture condition groups (AMCs). Calculation of the CN values for AMC-I and AMC-III is possible also by using the calculations present by Ishtiyaq, Vivek, Mukesh Kumar (2015).
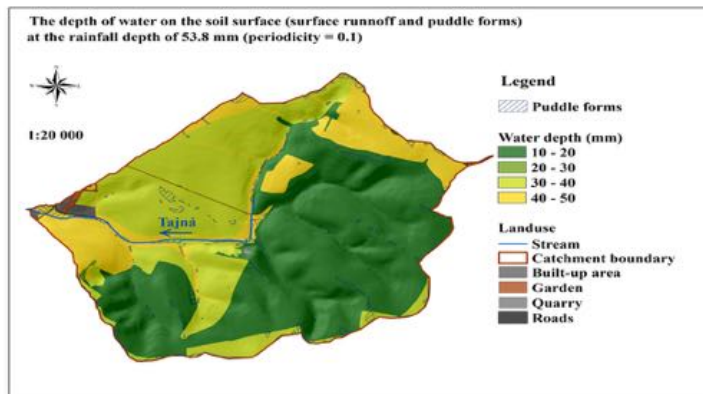
*Figure 2. The depth of water on the soil surface (surface runoff and puddle forms) at the rainfall depth of 53.8 mm (periodicity = 0.1)*
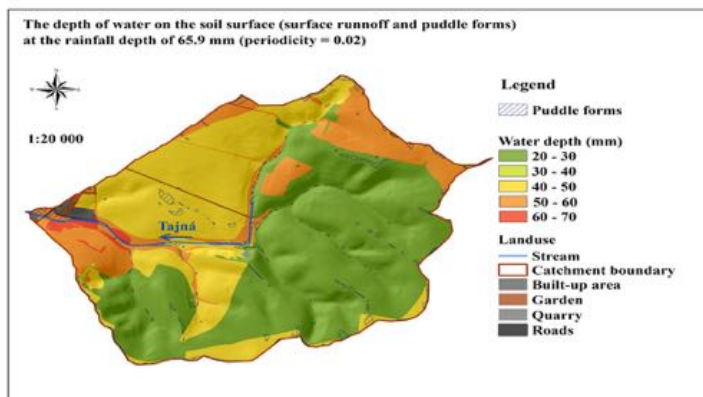


*Figure 3. The depth of water on the soil surface (surface runoff and puddle forms) at the rainfall depth of 65.9 mm (periodicity = 0.02)*

**Conclusions**

A methodical approach presented in this study enables to localize the specific zones where $H_{D,R}$ exceeds $H_{R,i}$ and thus areas that are at the risk of soil degradation formation by water erosion by using simple but worldwide-used curve number method. The advantage of the proposed approach is that inputs for this method are generally available for the whole Slovakia (and probably for the other countries too) as well as the fact that this approach can be successfully implemented into the GIS interface. Only basic up to moderate GIS skills are required to perform the analysis what potentially makes this procedure suitable and attractive for planners and environmental managers for the purposes of the preliminary assessment of water erosion risk at the catchment scale.

## Acknowledgement

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# Risk of soil salinization/sodification in the Danube Lowland after the realization of underground sealing walls between Komárno and Štúrovo, Slovakia

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Abstract: The paper deals with the evaluation of the characteristics of groundwater (GW) important for the formation and spreading of saline soils and their trends in the alluvium in the left bank-side of the Danube River between Komárno and Štúrovo, Slovakia after underground sealing walls realization for the period from1997 to 2012. The risk that this GW results in salinization/sodification of soils was determined from selected characteristics of GW mineralization ($HCO_3^-$, $SO_4^{2-}$, $Cl^-$, $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $K^+$, pH, EC), SAR (sodium adsorption ratio) and from the groundwater level (GWL) regime within the territory.

Keywords: alluvium of Danube River, GW regime, soil salinization/sodification, underground sealing walls

## Introduction

In areas with evaporative soil water regime, such as the Danube Lowland in south-western Slovakia or the Eastern-Slovakian Lowland, the main source of salts within soils is the mineralized groundwater (GW) located close to the ground surface. Dissolved salts are transported from the GW table to the soil profile via vertical upward capillary flow of water. Under evaporative soil moisture regime the GW rises up to the root zone of soil profile from where it is deflated by evapotranspiration, and the soluble salts may precipitate and accumulate within upper soil horizons to the same extent as by using of irrigation water with similar chemical composition (Kováčová, 2015; Fulajtár, et al., 1998; Burger and Čelková, 2003). The formation of saline soils is affected especially by total mineralization of GW, their chemical composition and by the groundwater level (GWL) regime. Moreover, utilization of this GW for irrigation leads to further accumulation of salts within the soil profile.

The processes of soil salinization/sodification in the left bank-side of the Danube River between Komárno and Štúrovo can be affected by the system of protective underground sealing walls which were built during the construction of Gabčíkovo – Nagymaros waterworks in Slovakia and finished in 1996. They were constructed to prevent the gravitation outflow of internal waters from the territory of Slovak Republic into the Komárno - Ipeľ river confluence section of Danube and thus may set conditions for increasing of mineralized GW table and subsequent soil salinization/sodification (Burger and Čelková, 2009; Burger, 2012). The aim of this paper is to characterise the chemical status and regime of groundwaters, which are relevant for formation and spreading of saline soils within the left bank alluvium of the Danube River and to determine whether the risk of salinization/sodification of local soils has been increased after the realization of underground sealing walls.

**Materials and methods**

The GW can be evaluated according to the criteria for the quality of irrigation water. There are various classification schemes used for the assessment of the irrigation water (Allison, et al., 1954; Ayers and Westcot, 1985; Rhoades, et al., 1992). Most of them are based on the assessment categories according to physiological impact on plants or on the quality of soils. We recognize: salinity – the total dissolved solids (expressed as specific electrical conductivity, EC); sodicity – the relative ratio of sodium to the calcium and magnesium (expressed as sodium adsorption ratio, SAR); alkalinity – the content of carbonates and bicarbonates; pH – acid or alkaline medium, and the proportion of specific ions: chlorides, sulphates, nitrates and boron.

As regards of EC, according the American Laboratory Classification System the irrigation water is divided into four classes: low mineralized water (EC < 25 mS m$^{-1}$); medium mineralized water (25 mS m$^{-1}$ < EC < 75 mS m$^{-1}$); highly mineralized water (75 mS m$^{-1}$ < EC < 225 mS m$^{-1}$) and very highly mineralized water (EC > 225 mS m$^{-1}$). As regards of SAR there are four classes: low sodium water (SAR=0 – 10); medium sodium water (SAR=10 – 18); highly sodium water (SAR=18 – 26) and very highly sodium water (SAR > 26). On the basis of the values of EC and SAR we can determine the risk of soil salinizatin/sodification under the influence of irrigation water or shallow GW.

The chemical composition of water influences the character of saline soils formation. According to concentration of particular ions (Cl$^-$, SO$_4^{2-}$, HCO$_3^-$, CO$_3^{2-}$, Na$^+$, Ca$^{2+}$, Mg$^{2+}$) the character of soil salinization is determined (the chloride salinization – the limit value of 300 mg l$^{-1}$ Cl$^-$, the sulphate salinization – the limit value of 250 mg l$^{-1}$ SO$_4^2$, the soil alkalinity – the concentration of bicarbonates and carbonates anions exceed that of sulphates and chlorides and the ions of Na$^+$ dominate in the cationic composition (Allison, et al., 1954; Fulajtár, et al., 1998).

The GWL and its fluctuation is another factor affecting the formation and spreading of saline soils. The degree of mineralization of GW in terms of soil salinization/sodification is important only under conditions of capillary rise of mineralized GW to the root zone of soil profile or to the soil surface (Várallyay and Rajkai, 1989; Várallyay, 2011). The "critical" depths of GWL in the areas of medium and high mineralized GW differ for various soil textural classes and range between 1-2.5 m below the soil surface within the studied region (Jambor, et al., 1988).

The values of selected characteristics of GW mineralization (HCO$_3^-$, SO$_4^{2-}$, Cl$^-$, Ca$^{2+}$, Mg$^{2+}$, Na$^+$, K$^+$, pH, EC) and monitoring of GW table regime were provided by the Slovak Hydrometeorological Institute in Bratislava. Samples were taken twice a year during the spring and autumn times. Chemical analyses (ion chromatography, AES-ICP, electrometry, gravimetry) were performed by the State Geological Institute of Dionysus Štúr. The SAR values were calculated by the equation: $SAR = Na^+ / \sqrt{Ca^{2+} + Mg^{2+}/2}$ , where Na$^+$, Ca$^{2+}$ and Mg$^{2+}$ are the ions concentrations in mmol.l$^{-1}$ (Allison, et al., 1954).

***Definition of the area of interest***

The area of interest is located in the south-eastern part of the Danube Lowland in the

alluvium between Komárno and Štúrovo. The location of the monitoring objects of GW quality is shown in Figure 1. The hydrological regime of GW flow and its quality affects a system of protective measures in the left bank side of Danube River between Komárno and Štúrovo. Protective measures include the reconstruction of dams with the construction of underground sealing walls, construction of seepage and drainage channels and pumping stations. The construction of underground walls was realized in the year 1982 – 1996. There are three so called "windows" omitted in the non – permeable underground walls near the village Iža, Kravany nad Dunajom and Mužla Kendeleš (Figure 1).



*Figure 1. The map of GW quality objects (Komárno 260290, Iža 602390; Iža Bokroš 52990; Moča 602490; Kravany nad Dunajom 602690; Mužla Kendeleš 251490)*

### Results and discussion

In the GW in the alluvium in the left bank side of Danube River between Komárno and Štúrovo after underground sealing walls realization for the 1997 to 2012 the concentration of bicarbonates ions ($HCO_3^-$) exceeded that of sulphates ($SO_4^{2-}$) and chlorides ($Cl^-$), while $Ca^{2+}$ and $Mg^{2+}$ dominated in the cationic concentrations. $HCO_3^-$ concentrations ranged from 280.6 mg.l$^{-1}$ – 1052.6 mg.l$^{-1}$ and the concentrations of $SO_4^{2-}$ ranged from 37.3 mg l$^{-1}$ – 891 mg l$^{-1}$. Concentrations of $Cl^-$ in GW were low and ranged between 18.2 mg l$^{-1}$ – 139.0 mg l$^{-1}$ except of Iža where the maximum of $Cl^-$ concentration reached 255 mg l$^{-1}$. In terms of hydrochemical classification of GW they are the type of calcium-magnesium-bicarbonate and the type of calcium-magnesium-sulphate, with high bicarbonates and sulphates content. Concentrations of $Na^+$ were low in most monitored objects except of Iža and Iža Bokroš and ranged between 14 mg l$^{-1}$ – 210.3 mg l$^{-1}$. The pH values ranged from 6.84 – 7.69. The time courses of electrical conductivity values (EC) and sodium adsorption ratio values (SAR) in the monitored GW and their trend lines for 1997 – 2012 are documented in Figure 2.

The EC values ranged from 84.4 mS m$^{-1}$ to 278.0 mS m$^{-1}$, and showed increasing tendency from 1997 to 2012 in most of the monitored waters. The highest values of EC > 225 mS m$^{-1}$ were measured in Iža. The SAR < 4 values (except of Iža Bokroš where the SAR values

ranged from 2.0 to 6.8), showed an increasing tendency within the objects Iža Bokroš, Moča and Mužla Kendeleš and decreasing tendency in Komárno, Iža and Kravany nad Dunajom (Figure 2).
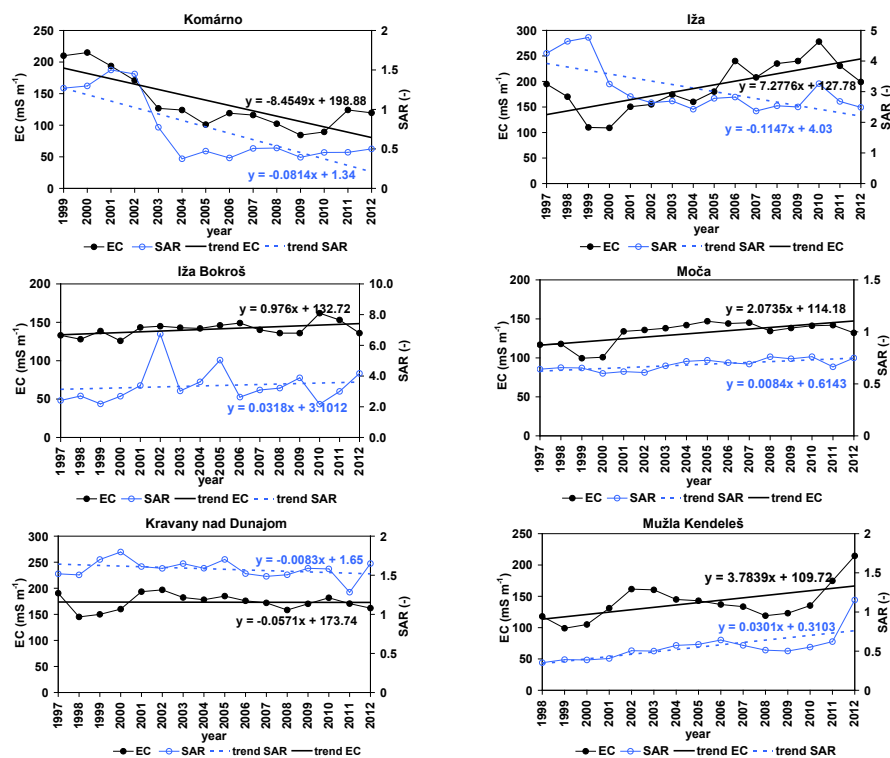


*Figure 2. The time courses of the EC and SAR values in GW in the localities of Komárno, Iža, Iža Bokroš, Moča, Kravany nad Dunajom, Mužla Kendeleš and their trend lines from 1997 to 2012*

Most of the monitored GW are the highly mineralized waters with a high salinity hazard and low sodium hazard for influenced soils according to criteria of USSL Staff. The GW of Iža and Iža Bokroš can be classified as the water with high to very high salinity hazard and low to medium sodium hazard for soils under influence of GW.

The time courses of the average annual GWL for the 1997 to 2012 period are documented in Figure 3. The depth of GWL under the surface ranged from 2.75 m – 6.80 m, except Iža and Iža Bokroš where it was situated in a depth of 0.52 m – 2.03 m.

Because of existing openings ("windows") in underground sealing walls the trends in average annual GWL for 1997 to 2012 decreased in most monitored wells, except for Iža Bokroš and Mužla Kendeleš where the trend of GWL was increasing.

Thus, in spite of high GW mineralization and its upward trends generally the risk of secondary soil salinization/sodification within the alluvium on the left bank-side of Danube River between Komárno and Štúrovo has not increased. Only within the objects

of Mužla and Iža Bokroš the realization of underground sealing walls crops really caused the rise of GWL above the critical depths of 0.52 – 2.03 m.
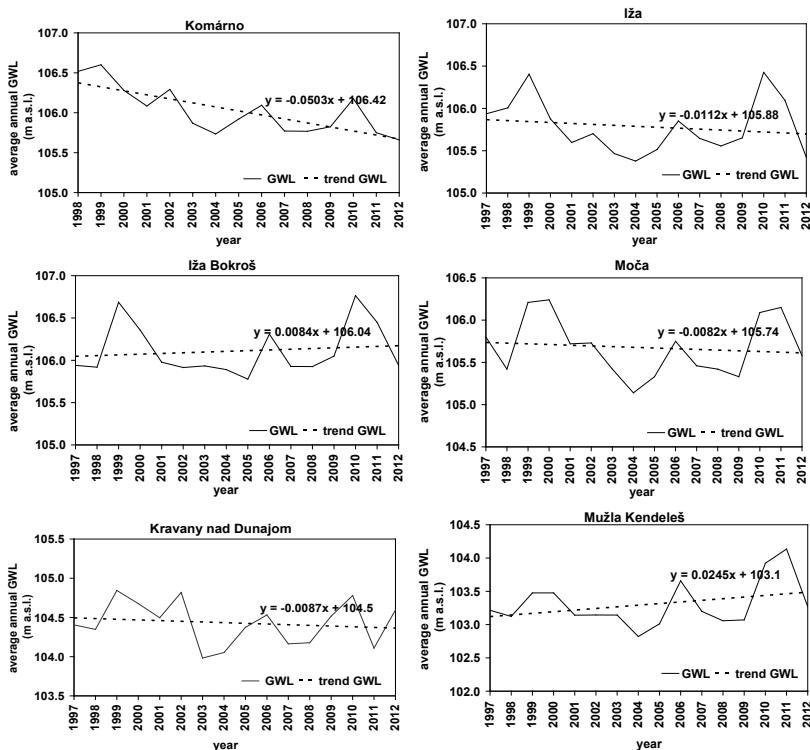


*Figure 3. The time course of average annual levels of GW tables in Komárno; Patince; Iža; Iža Bokroš; Moča; Kravany nad Dunajom and Mužla Kendeleš and their trend lines from 1997 to 2012*

The risk of secondary soil salinization/sodification under the influence of GW in the area of interest is high with rising trend only in the drainless depressions, then during the floods on Danube through the "windows" in the underground sealing walls and also by using of highly mineralized GW for irrigation (Hraško, 1971; Burger and Čelková 2009).

## Conclusions

Finally, we can conclude that after the completion of underground sealing walls construction despite the high mineralization of GW and its upward trends, there was no risk of secondary soil salinization/sodification by means of vertical flow from a GW level to the root zone of soil profile, or on the surface of the soil in most observed localities in the alluvium in the left bank side of Danube River between Komárno and Štúrovo, due to that the GW levels were lower than critical GWL except Iža Bokroš and Iža, where the highly mineralized GW was situated near the surface, and due to decreasing trends in the annual average levels of GWL for 1997 – 2012. Priority attention should be paid to the quality of water for irrigation, which is necessary for optimizing of water and salt regime of soils due to the fact that in studied area the saline soils were already occured. The highly

mineralized GW of the monitored objects is not suitable for irrigation and the use of this water represents a high risk of secondary soil salinization and a low to medium risk of soil sodification.

## Acknowledgement

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# Economic Structures of Tobacco Farms: The Case of Denizli Province in Turkey

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Abstract: Tobacco is important for Turkey in terms of the economic, employment and consumption gains it provides. In addition, "Oriental Tobacco", also known as "Turkish Tobacco" abroad, has also provided great awareness. This study was carried out in the Denizli province which constitutes 20% of the tobacco produced in Turkey. Data were obtained by face-to-face survey with 79 farmers. The number of farms to be surveyed was calculated by using the Stratified Sampling Method. Purpose of working; to reveal the economic structures of tobacco growing farms in Denizli and to determine the effects of privatization of TEKEL on tobacco producers. In Turkey, imports of cigarettes have been started since the 1980s. In 1986 foreign companies were allowed to produce cigarettes. In 2002, TEKEL was privatized with the law no. 4733 and tobacco production was started to be contracted and government support was also abolished. After this ban, there has been a great decline in tobacco production. Therefore, sampling of Denizli province in tobacco farmers and the reasons of this change were aimed. With the privatization of TEKEL, the number of farmers producing tobacco and numbers decreased and the share of imported tobacco increased. Farmers who gave up production have migrated from the village to the city. The farmers were not satisfied tobacco farming after the privatization of TEKEL, and also there were problems in the contracted system. The farmers interviewed said that the number of producers decreased due to falling profits in tobacco production and labour demand and thus the production of tobacco has been decreasing in the investigated area.

Keywords: Tobacco, Farmers, Privatization, Policy, Turkey

## Introduction

Tobacco cultivation areas in Turkey are distributed to the Aegean, Marmara, Black Sea, East and South East regions depending on climate and soil characteristics. Approximately 96% of the produced tobacco is Turkish (Oriental) tobacco. The remaining tobacco is Virginia, Burley, Puro, Tömbeki and Hasankeyf tobacco (Yücer et al., 2006). Changes in imports of cigarettes were made with the policies implemented after 1980. By law no. 1177, cigarettes were started to be imported. With this law and another law coming out in 1986, cigarette manufacturing was allowed in the country. In the past years, tobacco stocks and uncompleted inventory costs, which were caused by the fact that the production amounts exceeded the export and domestic consumption requirements, revealed the fact that the tobacco policies were re-sighted and thus the government-supported production policies were abandoned (Orman, 2011). With the law numbered 4733 issued in 2002 in Turkey, the tobacco market has been left with a special sector. With this law; Contract manufacturing, auction system, leaving all the market administrations to the Tobacco and Alcohol Market Regulatory Authority (TAPDK) on behalf of the state, and opening the privatization of TEKEL (Alıcı, 2010). Along with the change in policies towards tobacco, the biggest impact is the producer number between 2000 and 2013. The number of farm households, which was 586.311 in 2000, decreased by about 86% and reached 81,840 farms by the year 2013. Parallel to the decline in the number of tobacco producers, there has been a significant decline in the amount of production. The share of regulations made after 2002 was enormous in this decrease in the number of tobacco growers. In some regions, alternative crops have been passed and in some regions, families were withdrawn

completely from the agricultural sector (Alıcı, 2010). Table 1 listed the tobacco production and cultivated areas between 1993 and 2013 in Denizli province of Turkey. The amount of tobacco production both in Turkey and Denizli has been decreasing until 2011, and an increase has been taking place since 2012. Parallel to this, there was a decrease in the cultivated area until 2011, an increase after 2012.

*Table 1. Number of tobacco producers by region in Turkey*

| Years | Production (tons) | | Cultivated area (decares) | |
|---|---|---|---|---|
| | Turkey | Denizli | Turkey | Denizli |
| 1993 | 338.796 | 25.732 | 3.398.560 | 295.150 |
| 1995 | 204.440 | 20.557 | 2.099.190 | 244.220 |
| 2000 | 200.280 | 23.199 | 2.365.690 | 325.840 |
| 2003 | 112.158 | 15.942 | 1.830.430 | 287.540 |
| 2005 | 135.247 | 18.954 | 1.853.420 | 278.050 |
| 2010 | 53.018 | 10.839 | 813.335 | 192.288 |
| 2011 | 45.435 | 11.558 | 766.575 | 191.297 |
| 2012 | 73.285 | 19.084 | 1.076.984 | 255.117 |
| 2013 | 93.158 | 22.120 | 1.330.733 | 308.985 |

Source: TÜİK, 2016

The highest tobacco production was in Denizli province. The products grown in Denizli in 2014 constituted 20% of the total tobacco production. Therefore, it was aimed to determine the causes of this change with reference to Denizli province of tobacco producers. The tobacco legislation has caused a significant reduction in production.

## Materials and methods

The main material of this research was obtained from the tobacco producers in Denizli province. The sample size was found to be 79 producers according to the Stratified Sampling Method, with a permissible error amount (average deviation of 5%) and a 90% confidence limit from the average of the population. These farmers were distributed in 3 groups by their tobacco area. The farmers with 1 to 15 decares tobacco area were defined as the first group (24 farmers), while farmers with 15.1 to 30 decares tobacco area were defined as the group II. group (24 farmers), farmers with 30.1 and over decares tobacco area were III. group (31 farmers) (Table 2). During the visit to the farmers, prepared questionnaires were directed to the producers. In analyses for farmers, both the tobacco area was calculated separately for size groups and for the average of farmers. The effects of privatizations in the tobacco market were taken on questionnaires to the producers and likert scale (5).

*Table 2. Sample size*

| Farmer groups | Population | Standard deviation | Variance | Mean | Sample volume |
|---|---|---|---|---|---|
| I. group (1 to 15 decare) | 7697 | 3.2 | 10.5 | 10.9 | 24 |
| II. group (15.1 to 30 decare) | 5637 | 4.5 | 20.1 | 23.1 | 24 |
| III. group(30.1 and over decare) | 2050 | 16.1 | 258.2 | 46.3 | 31 |
| Total | 15384 | 13.6 | 184.0 | 20.1 | 79 |

1 decare equal 0.1 hectare

## Results and discussion

It is determined that farmers have education at primary school level (5.5 years) There was no statistically significant difference (P> 0.00; P = 1.017) between tobacco field groups and the level of education of farmers, although the level of education with tobacco field

groups increased. The average age of the farmers interviewed was calculated as 46.4 years. Farmer ages, 46.2 years in the first group, 46.3 years in II. group, and it was 46.6 years in II. group (Table 3). There was no significant difference between the groups and farmer's age criterion. The duration of experience of tobacco farmer was 29.4 years. According to tobacco land size groups; this value was 26.2 years in group I, 23.7 years in group II, 36.4 years in group III (Table 3). As the area of tobacco grows, the experience of the farmer was also increasing. On the other hand, there was no statistically significant relationship between tobacco field width groups and the farmer's experience in tobacco production (P> 0.00; P = 0.995). The average household size was 4.1 persons. The lowest number of household members was with 3.7 persons in the group II, and the highest number of household members 4.6 in the group III. There was a statistical relationship between the size of the household and the size of the enterprise. The tobacco cultivation area ranged from 0.8 to 3.7 hectares in the groups, with an average of 2.2 hectares. In the farmer groups, between 44.2% and 73.1% of the total crop production areas constituted tobacco cultivation areas. This ratio was 62.5% in the farms average (Table 3). The operational land area was 3.49 hectares and 45.1% of the land was owned land and 54.9% was rented land. Farmers were producing tobacco generally by renting land. The share of non-irrigated land was 93%. The share of non-irrigated land varies between 82.0% and 95.1% in the groups (Table 3). So the rate of irrigation in the production of tobacco growers was also very low.

Tobacco has the highest share in gross production value at 55.0%. This ratio was followed by 33.8% of livestock activity and 11.2% of other products. The share of tobacco gross value in the III. group was 58.4% and was the highest value. The most important production value item in group I farmers was livestock. The share of tobacco cultivation in farmer groups also varies from 44.2% to 73.1%. Farmers' satisfaction with agricultural activities, tendency to continue tobacco growing, level of satisfaction with tobacco growing, level of knowledge with tobacco growing were also asked and the results were given in Table 3. These answers were taken on a 5-point Likert scale. Farmers' levels of satisfaction with agricultural activities and tobacco growing were found to be low (2.3 and 2.3). Farmers tend to continue to grow tobacco (3.7) and knowledge level in tobacco growing was moderate (3.4). There was no statistical difference between these criteria and the groups of tobacco cultivated area.

The attitude of producers interviewed in the field of research to various applications in tobacco farming was evaluated according to the 5 Likert scale. When the growers' attitudes to various practices in tobacco farming were examined, it was determined that the farmers participated in the following statements: "difficult to supply tobacco seed"; "Input prices are high"; "There is a problem in the supply of labour in tobacco growing"; "Tobacco production declined after privatization of TEKEL"; "With TEKEL privatization, inputs in tobacco production were more expensive"; "The privatization of TEKEL increased the farmers' migration from the village to the city"; "With the privatization of TEKEL, the number of unemployed in the village has increased"; "Increased costs of switching to contracted tobacco production"; "Most tobacco manufacturers have left tobacco production after privatization"; "Tobacco growing is an important source of livelihood in the region"; "The marketing situation of tobacco products affects production"; "Climate change affects tobacco growing"; "Tobacco growing is a profitable activity"; "Privatization negatively affected tobacco production"; "Tobacco is no longer as important as it used to be"; "Privatization of TEKEL facilitated tobacco marketing" (Table 4).

*Table 3. Some social-economic indicator in tobacco production*

| Indicators | Groups of farms | | | Average |
|---|---|---|---|---|
| | I | II | III | |
| Farmers age (year) | 46.2 | 46.3 | 46.6 | 46.4 |
| Farmers education level (year) | 5.1 | 5.6 | 5.7 | 5.5 |
| Household size (head) | 3.8 | 3.7 | 4.6 | 4.1 |
| Farmers experience on tobacco production (year) | 26.2 | 23.7 | 36.4 | 29.4 |
| Owned land (%) | 60.0 | 62.4 | 32.2 | 45.1 |
| Rented land (%) | 40.0 | 37.6 | 67.8 | 54.9 |
| Irrigated land (%) | 18.0 | 5.6 | 4.9 | 7.0 |
| Non-irrigated land (%) | 82.0 | 94.4 | 95.1 | 93.0 |
| Irrigated land for total tobacco cultivated area (%) | 2.8 | 0.0 | 5.7 | 4.0 |
| Tobacco land (%)* | 44.2 | 51.4 | 73.1 | 62.5 |
| The share of tobacco production value in total gross production value (%) | 37.6 | 55.1 | 58.4 | 55.0 |
| The share of other product production values in total gross production value (%) | 14.5 | 7.7 | 12.8 | 11.2 |
| The share of livestock production value in total gross production value (%) | 47.9 | 37.2 | 28.8 | 33.8 |
| Share of total agricultural income in total income (%) | 72.1 | 92.2 | 93.8 | 90.3 |
| Share of non-agricultural income in total income (%) | 27.9 | 7.8 | 6.2 | 9.7 |
| Parcel numbers of tobacco cultivated area (per) | 2.2 | 2.4 | 3.7 | 2.8 |
| Tobacco cultivated area (hectares) | 0.8 | 1.7 | 3.7 | 2.2 |
| Satisfaction level with agricultural activity* | 2.3 | 2.2 | 2.3 | 2.3 |
| Satisfaction level of tobacco growing* | 2.5 | 2.2 | 2.1 | 2.3 |
| Level of knowledge on tobacco growing* | 3.3 | 3.4 | 3.4 | 3.4 |
| A tendency to continue tobacco growing** | 3.7 | 3.7 | 3.7 | 3.7 |

*1 Very low 2 Low 3 Medium 4 High 5 Very high; **1 Definitely no 2 Not thinking 3 Undecided 4 Thinking 5 Definitely yes*

*Table 4. The participation of farmers in various statements about tobacco growing*

| Statements | Groups of farms | | | Avg* |
|---|---|---|---|---|
| | I | II | III | |
| Supply of tobacco seed was difficult | 4.8 | 5.0 | 5.0 | 4.9 |
| Input prices were high | 5.0 | 5.0 | 4.9 | 4.9 |
| There was a problem in the supply of labour in tobacco growing | 4.7 | 4.7 | 4.4 | 4.6 |
| Tobacco production decreased after privatization of TEKEL | 4.4 | 4.3 | 4.6 | 4.4 |
| With the privatization of TEKEL, inputs in t. produc. become more expensive | 4.7 | 4.5 | 4.1 | 4.4 |
| The priv. of TEKEL increased farmers' migration from the village to the city | 4.5 | 4.1 | 4.4 | 4.3 |
| With the priv. of TEKEL, the number of unemployed in the village has increased | 4.3 | 4.2 | 4.3 | 4.3 |
| Increased costs of contracted tobacco production | 4.2 | 3.8 | 4.5 | 4.2 |
| After privatization, tobacco producers have stopped tobacco production | 4.4 | 4.1 | 4.0 | 4.2 |
| Tobacco growing was an important source of livelihood in the region | 4.0 | 3.8 | 4.1 | 4.0 |
| The marketing situation of tobacco products affects production | 3.3 | 3.8 | 4.5 | 3.9 |
| Climate change affects tobacco growing | 3.5 | 3.5 | 4.2 | 3.8 |
| Tobacco growing was a profitable activity | 3.8 | 3.8 | 3.8 | 3.8 |
| Customization adversely affected tobacco production | 3.8 | 4.0 | 3.5 | 3.8 |
| Tobacco was no longer as important as it used to be | 3.9 | 3.7 | 3.2 | 3.6 |
| The privatization of TEKEL has facilitated tobacco marketing | 3.5 | 3.4 | 3.8 | 3.6 |
| Knowledge of business management for t. farm (profit, account, etc.) was sufficient | 3.2 | 3.5 | 3.3 | 3.3 |
| Tobacco growing infrastructure was sufficient (farmer) | 2.7 | 3.1 | 3.7 | 3.2 |
| Marketing infrastructure of tobacco products was sufficient (farmer) | 2.7 | 3.0 | 3.8 | 3.2 |
| Tobacco growing was more advantageous than other crop growing | 3.0 | 2.8 | 3.1 | 2.9 |
| Tobacco farming cannot be done without government support | 3.3 | 2.9 | 2.7 | 2.9 |
| Organization in tobacco growing was important | 2.6 | 2.1 | 3.5 | 2.8 |
| There was a lack of technical knowledge in tobacco growing | 2.5 | 2.9 | 2.7 | 2.7 |
| Organizing was important in marketing tobacco products | 2.3 | 3.3 | 1.7 | 2.4 |
| Agricultural organizations (union, cooperative) provide adequate info. support | 2.6 | 2.5 | 1.6 | 2.2 |
| After privatization the income from the tobacco has increased | 2.4 | 2.3 | 2.0 | 2.2 |
| Organization in tobacco growing was sufficient | 1.8 | 1.5 | 2.1 | 1.8 |
| Tobacco growing was an easy activity | 1.7 | 2.0 | 1.8 | 1.8 |
| Satisfaction with contractual crossing | 1.8 | 2.0 | 1.7 | 1.8 |
| I am pleased with the privatization of TEKEL | 1.9 | 1.8 | 1.4 | 1.7 |
| With the privatization of TEKEL, more farmers have begun to produce tobacco | 1.7 | 2.0 | 1.1 | 1.6 |
| The privatization of TEKEL did not change the number of tobacco producers | 1.3 | 1.4 | 1.1 | 1.3 |
| Tobacco growing was also a hobby | 1.3 | 1.2 | 1.0 | 1.2 |
| With the privatization of TEKEL, the net profit of t. production has increased | 1.2 | 1.2 | 1.1 | 1.1 |

*:1 = I definitely do not participate 2 = I do not participate 3 = Partially 4 = I participate 5 = I fully agree*

On the contrary, it was determined that they were not agreed with the following statements: "Agricultural organizations (union, cooperative) provide sufficient information support"; "After privatization the income from the tobacco has increased"; "Organization in tobacco growing is sufficient"; "Tobacco breeding is an easy task"; "Satisfied with the passage of contractual agriculture"; "Satisfied with the privatization of TEKEL"; "With the privatization of TEKEL, more farmers started to produce tobacco"; "The privatization of TEKEL did not change the number of tobacco producers"; "With the privatization of TEKEL, the net profit in tobacco production has increased further" (Table 4).

Ceylan (1995) pointed out that due to the changing consumer tastes and anti-smoking campaigns in the world, tobacco, known all over the world in the name of Turkish tobacco at the beginning of the century, is facing the danger of extinction today. Bas implementation of Law No. 4733. He reported that 98% of the tobacco farmers did not want to privatize the TEKEL. Gül et al. (2009) stated that most of the few producers who gave up tobacco production and benefited from the project support, were directed to cereal farming. They pointed out that profit of wheat, aspirants and canola products that may be an alternative to tobacco. According to the authors, canola was the best alternative product. Gümüş (2009) reported that producers were not satisfied with applications after the new tobacco legislation. The author stated that several multinational cigarette companies, which the contracted system makes dependent on the producers and which are handled by the auctioned sales system, play a decisive role in the tobacco market. He found that the tobacco real price of the producer has declined by half in ten years. Orman (2011) examined the changes in the economic structure of tobacco producers in Adıyaman tobacco production with the policies applied after 2000. According to the author, the changes made in the privatization and tobacco law have greatly reduced TEKEL's market share. The author claimed that the withdrawal of producers from tobacco production did not create an alternative production model, so a wave of migration to cities from rural areas began and an idle labour force emerged. Gültekin Karakaş (2014) reported that the tobacco sector in Turkey had undergone a market-oriented transformation with neoliberal policies implemented after the 1980s, during which the state control over the production and distribution conditions of tobacco production and cigarettes as an input of cigarette production was gradually phased out. The tobacco market has been left to the determination of transnational tobacco companies. The author pointed to the need to establish public control over tobacco products, production and trade, in the perspective of protecting public health. In contrast to the above results, Alıcı et al. (2011) found that farmers were producing for the private sector and were satisfied with this situation.

With the law numbered 4733 which came into effect in 2002, privatization of TEKEL has been made and then a significant decrease in tobacco production has occurred. With the privatization of TEKEL, the number of farmers producing tobacco and numbers decreased and the share of imported tobacco increased. Farmers who gave up production have migrated from the village to the city.

## Conclusions

Farmers produced tobacco on a small scale. More than half of their income was from tobacco production. Farmers had more than thirty years of experience in tobacco production. It was determined that the producers were not satisfied with the privatization of TEKEL,

and there were problems in the contracted system. The tobacco sector is important in terms of evaluating the family workforce for farmers and also in terms of income in the region. Farmers' production scale was small. Farmers were also less satisfied with tobacco production. But their tendency to continue production was still high. This is caused by the fact that the share of tobacco in income is important. It can be said that if the farmers organized, it could be possible to be a market actor.

## Acknowledgement

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# Socio-Economic Structure of Buckwheat Farms in Turkey

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Abstract: Buckwheat has gained importance as a result of the people's demand for healthy foods. Buckwheat, which is grown in many countries as an annual plant, is a pseudo cereal. Its importance and commercial value are increasing so far. In Turkey, the cultivation of buckwheat has evolved in recent years. Statistics related to the product are inadequate. It intended to identify the trend of continual improvement by determining the technical and economic situation of buckwheat production at the producer level. In this context, this study was presented socio-economic and technical structures of buckwheat farmers and proposals were developed for the improvement identified problems in Konya, Karaman and Çankırı province. The main material of this study was obtained through a survey from the buckwheat farms in Turkey. Data were obtained from 47 farmers by survey method and belonged to the 2016 production season. A single budget method was used for the calculation of the production cost. The relative profits in the production of buckwheat by the interviewed farmers were calculated as 1.71. The profitability of the farmers in continuing to produce buckwheat was significant and profited on the present conditions. But buckwheat farming has just begun to develop in the region. For continuity in buckwheat farming, farmers need to gain experience in growing buckwheat, and the government and non-governmental organizations need to increase knowledge of the farmers in buckwheat farming.

Keywords: Buckwheat, Farmers, Absolute profit, Relative profit, Turkey

## Introduction

Buckwheat belongs to *Polygonaceae* family *Fagopyrum esculentum* Moench. Buckwheat is a very old plant, rooted in Central Asia. In general, it began to be cultivated in China and Japan, then spread to Russia and Europe (Oplinger et al., 1989; Izydorczyk et al., 2005; Tomar et al., 2008).

Having a good chemical composition distinguishes buckwheat grains, which are particularly rich in fiber and protein content. The fact that it is a plant used especially by celiac disease also gives different importance to buckwheat (Dizlek et al., 2009). It increases the functional features and nutritive values of the product that the buckwheat added with its phenolic materials, antioxidant and fibre components (Hayıt and Gül, 2015).

World buckwheat production was about 3.8 million tons in 2000, but declined by 38% in 2013 to about 2.35 million tons (FAO, 2016). The most important cause of this tension in world buckwheat production is the decline in production in China and Ukraine. As a matter of fact, Chinese buckwheat production decreased by 62%, Ukrainian buckwheat production by 63% and Russian production by 16%. In the world buckwheat production, Russia ranks first with 35.5% share in 2013. In 2000, this share was 26.4%. In 2000, China achieved more than half of world buckwheat production. Today, the share of China in buckwheat production has decreased to 31.2%. Ukraine is the third largest producer of buckwheat with 7.6% share. The share of Ukraine in world buckwheat production was 12.7% in 2000. Therefore, the Ukrainian buckwheat production has also experienced some recession. On the other hand, there was an increase in the production of buckwheat in France, Lithuania, Poland, USA, Nepal, Latvia, Tanzania, Japan, Brazil, and Kazakhstan.

The world buckwheat cultivation area was 3.46 million hectares in 2000, while in 2013 it decreased by 34% to 2.27 million hectares. The most important factor in the reduction of this sowing area is the reduction in the sowing area of 68% of Ukraine, 39% of China and 37% of Russia in the mentioned period. Russia is in the first place in world buckwheat production as well as in the sowing area (share 39.8%). This is followed by 31.0% in China, 7.4% in Ukraine, 3.6% in Kazakhstan, 3.4% in the USA and 3.1% in Poland. There is no official information on buckwheat production in Turkey and FAO. As a result of interviews with researchers working on the subject, it was stated that there is growing of buckwheat in Turkey and that they have turned to production of buckwheat in recent years.

In Turkey, buckwheat has shown significant improvement over the last four years. There are some researches on usage of buckwheat flour, whole flour or its bran in the production of breads, pasta, cakes, noodles, biscuits, breakfast cereals, ice cream cones, tarhana and gluten-free foods (Bilgiçli, 2009; Atalay et al., 2013; Hayıt and Gül, 2016a; Hayıt and Gül, 2016b). As a result of the literature search, no economic studies have been found about the subject. At this point, the study aims were expressed as follows: (i) determining the socio-economic structure of the farmers in the field of buckwheat production; (ii) determining the technical structure of the buckwheat production activity; (iii) determining the economic structure of the buckwheat production activity; and (iv) identifying problems and developing solutions.

**Materials and methods**

The main material of this study was obtained by questionnaire method in Konya, Karaman, Çankırı provinces among the farmers who cultivated buckwheat. Secondary data related to the study were research findings on the subject at the national and international level. The data used in the research belong to the production period of 2016.

It is essential to have the right information and make the right decision in scientific researches. Therefore, it is necessary to reach the right information and to generalize the obtained information (Arıkan, 1994). Data were obtained from 47 farmers in Konya, Karaman, Çankırı provinces. The data required for the analysis were obtained through questionnaires from farmers operating in the field of buckwheat cultivation. In the questionnaire, the following information was collected from the farmers. This information were farmers' household size and family labour force status, labour force status, farmer's land property and land saving style, crop production status of farmer and usage of crops, evaluation of the buckwheat production, buckwheat marketing structure of the farmer, labour force and periods in buckwheat cultivation, input use and periods in buckwheat cultivation, cost structure and profitability of buckwheat farming, interest of farmer with technology, loan amount used by farmer, the problems faced by farmers in buckwheat cultivation, the expectation of farmers about the future of buckwheat farming, the judgments and attitudes of the farmers regarding the cultivation of buckwheat.

Single-product budget analysis was used in cost analysis of farmers. Gross production value includes agricultural products of the farmer with sales value and productive value increases (Erkuş et al., 1995). The statement of the owner of the farm was taken as basis for determining the debt of the farmer. The daily wages paid to the salaried workforce were taken as a precedent in order to calculate the daily working allowances of farm

owners and family members in the farm. General administrative costs were calculated as 3% of the total variable costs (Kıral et al., 1999). Gross profit was obtained by subtracting variable costs from gross production value and net (absolute) profit was calculated by subtracting production costs (Aras, 1988, Oktay, 1989).

Absolute Profit: Profit is the difference between income and expense. Gross Profit = Gross Production Value - Variable Costs formula was used (Açıl and Demirci, 1984; Kıral et al., 1999). Absolute profit = Gross production value (GPV) - Production cost (Kıral et al., 1999). Relative profit: Relative profit is the ratio of gross production value to production cost. Relative profit measures the productivity of production activities better (Kıral et al., 1999). Relative Profit = Gross Production Value / Production Cost (Kıral et al., 1999).

Regression Analysis: According to Gujarati (2006) and Greene (2008) the primary objective of regression analysis is to determine the various factors which cause variations of the dependent variable. The multiple regression analysis was used to identify the factors that affect buckwheat production's relative profit in the study area. The data were presented as linear functions. The regression model in its implicit form is given as: $Y = F(x_1, x_2, x_3, x_4, u)$. Where $Y$ = Relative profit of buckwheat production; $X_1$ = Yield of buckwheat in kilogram per decares; $X_2$ = Farmers age in years; $X_3$ = GPV of buckwheat production in Turkish Liras; $U$ = Error term. The functional forms are Linear Regression Model. $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + U$

**Results and discussion**

The results showed that the age of the respondents range between 23 and 66 years. The mean age was 45.57. One can infer from this result that the buckwheat farmers in the study area were at their economic active age (Table 1). The mean of household size was about 5 persons per house which indicate that the study area was an extended family dominated (Table 1). Küçükçongar et al. (2014) in their study in Konya also revealed that the average household size was 5 members. This implies that the respondents have a relatively large household sizes which they utilize as a source of family labour. Educational level of the respondents in the study area was 8.38 years. The education levels of the farmers interviewed were higher than the findings of Küçükçongar et al. (2014) and Çelik et al. (2016). The farmers experience on buckwheat production in the study area was very low (1.70 years). That the farmers who were interviewed had just begun cultivation of buckwheat, so that there might be shortcomings in the technical issues related to the breeding. The buckwheat cultivation area of farmers interviewed was 27 decares (2.7 hectares) and the number of parcels was 2. The share of buckwheat cultivation area in total arable land was 5.9%. The irrigation rate of buckwheat cultivation area was 86.1%. The share of agricultural income in farmers' income was 92.5% and the share of the gross production value obtained from buckwheat in the total GPV was 17.2% (Table 1).

Information source of the respondents on the production of buckwheat in the study area was generally from Ministry of Food, Agriculture and Livestock, agricultural research institutes. The interviewed farmers were influenced by the Provincial Directorates of Agriculture and the Agricultural Research Institute in the starting of buckwheat farming. Kara et al. (2016) carried out with aim to determination the efficient of nitrogen forms on nitrogen use efficient for buckwheat in Isparta and found that the highest grain yield (1456

and 1325 kg ha$^{-1}$) were obtained from ammonium sulphate. They identified that ammonium sulphate was positive effect to yield and some quality parameters of buckwheat. Kara and Telli (2016) also obtained highest grain yield (132.3 kg da$^{-1}$) from 8 kg da-1 phosphorus dose. Okudan and Kara (2015) found that highest grain yield (1254 kg ha$^{-1}$) of buckwheat were obtained from 75 kg ha$^{-1}$ N doses. In various regions, research such as fertilizer use and planting distance in buckwheat production is required. This kind findings need to be transferred to farmers.

*Table 1. Some social-economic indicator in buckwheat production*

| Some indicators | Average |
| --- | --- |
| Farmers age (year) | 45.57 |
| Farmers education level (year) | 8.38 |
| Household size (head) | 4.49 |
| Farmers experience on buckwheat production (year) | 1.70 |
| Irrigated land (%) | 34.90 |
| Non-irrigated land (%) | 65.10 |
| Irrigated land for total buckwheat cultivated area (%) | 86.10 |
| Buckwheat land (%)* | 5.90 |
| Gross production value of buckwheat production (%) | 17.20 |
| Agricultural income (%) | 92.50 |
| Parcel numbers of buckwheat cultivated area (per) | 2.00 |
| Buckwheat cultivated area (decares) | 27.00 |

A specific amount of inputs and services are required in order to produce a good or service. Cost can be defined as the amount and value of the altruistic behaviours in order to produce goods and services. Production costs can be defined as the monetary value of the inputs required for the buckwheat production. Accordingly, some economic indicator for buckwheat was given in Table 2. The production cost were examined under variable and fixed cost of which variable cost had the highest share of total production cost with 69.69% whiles fixed cost was amounted to 30.31%.

The interviewed farmers market the buckwheat they produce with different distribution channels such as merchants, brokers. However, some producers market their products directly or through electronic commerce in the study area. The production cost per kg, gross margin, absolute profit and relative profit were given in Table 2 below. The production cost per kg in the study area was TRL3.89. The mean average gross margin value was amounted to TRL508.56. Production cost varies in the farms with TRL519.11 being the average mean of the total production cost. The absolute profit mean average was calculated to be TRL353.89. The relative profit also varies from 0.64 to 3.93 with 1.71 as the average mean of the relative profit. In the study area, lack of technical know-how was the most pressing problem. The problems with planting materials, diseases and pests were the most pressing problem for the buckwheat farmers. Insufficiencies support policies by the government and marketing were the secondary problems.

*Table 2. Some economic indicators in the buckwheat production*

| | Average |
| --- | --- |
| Relative profit | 1.71 |
| Production cost per kilogram (TRL) | 3.89 |
| Gross profit per decares (TRL) | 508.56 |
| Absolute per decares (TRL) | 353.89 |
| Production cost per decares (TRL) | 519.11 |
| Variable cost (%) | 69.69 |
| Fixed cost (%) | 30.31 |

Regression analysis was carried out to determine the factors that affect relative profit of buckwheat production in the study area. The model specified relative profit of buckwheat production Y as a function of buckwheat yield $(X_1)$, farmer's age $(X_2)$ and GPV of buckwheat $(X_3)$. The summary of the linear form of production function result was given in Table 3 below. The result of the estimated parameter can be written thus: $Y = -0.1648445 + 0.0051250X_1 + 0.0203014 X_2 + 0.0000069X_3$ . The value of co-efficient of determination $R^2$ of 0.52526 (52.526%) indicates that about 53 percent of variation in relative profit of buckwheat could be explained by the explanatory variables in the stated regression model. The F-test was statistically significant at the 1% level, meaning that the production function existed $(F_{Calculated} > F_{Scale\ value}, 15.888 > 4.01)$; that is, all the explanatory variables jointly explained the variations in the output. Buckwheat yield, farmer's age and GPV of buckwheat production were identified as the significant factors affecting the relative profit score of buckwheat production in the study area. The positive coefficient of the variable indicates increase in these parameters to their buckwheat farm increases the relative profit of buckwheat.

*Table 3. Showing the regression analysis of buckwheat output*

|  | Coefficient | Standard error | t-values | P values |
|---|---|---|---|---|
| Constant | -0.1648445 | 0.3864798 | -0.4265 | 0.6719 |
| Yield (kg per decares) | 0.0051250 | 0.0009818 | 5.2200 | 0.0000 |
| Farmer's age | 0.0203014 | 0.0072875 | 2.7858 | 0.0079 |
| Gross production value of buckwheat (TL) | 0.0000069 | 0.0000029 | 2.3989 | 0.0209 |
| *F test* | *15.888* |  |  | *0.000* |
| *Adjusted R Square* | *0.49214* |  |  |  |
| *R Square* | *0.52526* |  |  |  |

## Conclusions

The farmers who were interviewed should make profit to continue producing buckwheat. In the present case this is possible. Relative profit must be greater than one (1) and relative profit was 1.71 which indicates that buckwheat production in the study area is profitable. However, buckwheat cultivation began only recently known, for continuity in buckwheat farming, farmers must gain experience in growing buckwheat. The government and non-governmental organizations need to increase the knowledge of producers about buckwheat agriculture. The use of buckwheat in gluten-free products should be widespread. Therefore, the farmer can increase the production amount.

## Acknowledgement

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# Nitrogen fertilization analysis on small plot winter wheat (*Triticum aestivum* L.) experiments

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Abstract: Our research at the Institute of Crop Production of the Faculty of Agricultural and Environmental Sciences at Szent István University was comparative tests of nitrogen fertilization analysis on small plot winter wheat experiments in which production was examined with a view to the different volume of used N fertilisation in the period of four years (2008, 2009, 2010, 2011). The comparative small plot winter wheat we have arranged is a split-plot experiment. In this case, the cultivar represented the main parcel factor and the various fertiliser treatments represented the sub-parcel factors. In our experiment the effects of 6 nitrogen doses (one control and 5 treatments as top-dressing) were examined for 5 cultivars (Mv Csárdás, Mv Magdaléna, Mv Suba, Mv Toborzó, Alföld 90) in 4 repetitions. Three years (2008, 2009 and 2011) of the experiment showed significant differences among the yields as a result of the applied different level of N. According to the comparative study of the vintages, there were statistically proved differences between the yields every year which proves that the weather has a huge impact on the harvest.

Keywords: winter wheat, N-fertilization, top-dressing, small-plot experiment, yield

## Introduction

Biological agents such as variety and it's nutrient reactions in winter wheat fertilization are commanding affects. High differences appear in variety's nitrogen demand and usage which are affected by agro-ecological (vintage soil) and agro technical (forecrop plant protection etc.) factors (*Harmati*, 1975; *Pepó*, 1995). Adverse weather effects can be moderated by appropriate nutrient replenishment (*Fowle*r, 2003).

Various authors – especially from the international literature – are suggesting different amount of fertilizer as well as different fertilizing time depending on the soil and ecological situation of the field they are working on. Most authors agree that the quality development caused by nitrogen application on winter wheat is very high (Ragasits, 1998), however there is a limit on applied amount of fertilizers (*Neményi*, 2008).

Nitrogen (N) fertilization is one of the most important agronomic management practices, yet the amount and timing of N remains a management challenge. Crops growing with N deficiency lose greenness, they are usually smaller with less biomass, and have reduced photosynthetic capacity resulting in poor yield and low protein content (*Basso et al.*, 2013).

## Materials and methods

*Location:* The experimental agricultural plot of the Institute of Crop Production is approx. 5 ha in size. The location of the small plot experiment is in the outer area of the municipality of Hatvan-Nagygombos.

*Weather in the examined years:* The annual average temperature of the area is 10.3 °C and annual precipitation varies between 560-580 mm. In the first year of the experiment (2008), in the period from August to July, the region's overall precipitation was 695.7 mm which is 120-140 mm more than the long term average. Overall precipitation in the

2nd year of the experiment (480.3 mm, in 2009) was 200 mm less in comparison to the previous year. 2010 was an extremely rainy year in Hungary with respect to the long term average, as the total precipitation (799.2 mm) was well above it. The large quantities of rainfall resulted in inland water over a large part of the experimental area, having a depressive effect on its flora. In 2011, total precipitation was 665.8 mm, which is above the average, just as it was during the preceding year.

*Soil:* The experimental area's soil is chernozem-brown forest soil, its most important average soil test result are shown below (5 August 2007): organic matter content: 2.65%; $CaCO_3$:1.86%; pH (KCl):7.30; KA: 45; $P_2O_5$ (mg kg$^{-1}$):463 (AL-soluble); $K_2O$ (mg kg$^{-1}$):293 (AL-soluble); N (mg kg$^{-1}$):0.9 (total mineral cont.)

From the measurement results of the soil samples, that the area has a satisfactory supply of phosphorus and potassium, therefore basic fertilisation only plays a sustaining role. As for feeding the flora, nitrogen is of exceptional significance, as this is the only macro element in limited supply for the plants.

### *Setting and sustaining the experimental conditions and the applied treatments*

*Layout:* The comparative small plot winter wheat we have arranged is a split-plot experiment. In this case, the cultivar represented the main parcel factor and the various fertiliser treatments represented the sub-parcel factors. In out experiment the effects of 6 nitrogen doses (one control and 5 treatments) were examined for 5 cultivars in 4 repetitions.

*Cultivars:* The varieties set in the experiment were selected with the aim of picking those with high yields as well as high protein and gluten contents as these react easier to the doses of active ingredients delivered. Four of the five selected cultivars were Martonvásár wheat (Mv Csárdás, Mv Magdaléna, Mv Suba, Mv Toborzó) and an older, but well-performing cultivar which had received state recognition back in 1987, the Alföld-90 (later known as Alföld).

*Plant nutrition:* As a basic fertiliser, one unit (300 kg ha$^{-1}$ total amount of fertilizer) of complex (N:P:K=15:15:15) fertiliser was delivered using a splash plate broadcast spreader. The various single N doses (0, 40, 80, 120 kg ha$^{-1}$) were delivered in spring as top-dressing, at the time of tillering.  In the case of double and triple N doses (split top-dressing: 80+40, 80+40+30 kg ha$^{-1}$), the first application was delivered at the time of tillering, the second at stem elongation, and the third nitrogen dose was delivered upon the appearance of the flag leaves. Top-dressing was performed manually in each case, given the small size of the plots, after doses had been precisely measured.

*Tillage:* The area managed according to the sequencing requirements of crop rotation was selected so that the preceding crop should be the same in each year examined and in our case it was dried beans.

*Sowing:* Sowing was performed every year with the institute's 8 row, sliding coulter Wintersteiger Plotman plot seeder (Wintersteiger GmbH., Ried, Austria).

*Cultivation routes:* The cultivation routes across the tillering winter wheat field sowed using the split-plot system were created during the spring with the help of a rotary tiller.

*Plant protection:* The plant protection treatments (weed control, application of fungicides,

etc.) was carried out in each case with a knapsack-type portable sprayer, therefore no wider access routes than created for sowing were necessary.

_Harvesting:_ Harvesting was performed using the Wintersteiger Nurserymaster (Wintersteiger GmbH., Ried, Austria) plot harvester owned by the Institute of Crop Production.

### Statistical data analysis

Statistical analysis (two variable analysis of variance /ANOVA/) of yield was carried out with the help of the GenStat programme. Variance analysis only shows whether significant difference exists among the results evaluated, but it doesn't indicate the groups among which the difference emerged. In order to identify this data, a Duncan multiple range post hoc test was carried out. The reason was the creation of homogeneous groups.

### Results and discussion

_Table 1 Results of the variance analysis of the four years (2008-2011)_

| Source of Variance | Degree of freedom | F-values | | | |
|---|---|---|---|---|---|
| | | 2008 | 2009 | 2010 | 2011 |
| Repetition | 3 | | | | |
| Varieties (A) | 4 | 11.11*** | 112.3*** | 60.1*** | 78.03*** |
| Fault (A) | 12 | | | | |
| Nutrition (B) | 5 | 84.6*** | 150.55*** | 63.93*** | 130.77*** |
| A x B | 20 | 1.48$^{ns}$ | 2.12* | 1.16$^{ns}$ | 1.19$^{ns}$ |
| Fault (B) | 75 | | | | |
| All | 119 | | | | |

**Table 1** shows the results of two-factor analysis of variance that was performed in each year of the study. From the results obtained it is obvious that each year there was a significant difference between the cultivars already on 0.1% significance level. The effect of - nutrient treatment (0.1%) was also significant in all years of our study. In case of the cultivar x nutrient interactions there was a significant difference only in 2009 on 5% level.

_2008:_ After having done the Duncan-test (**Figure 1**) significant differences were observed in three types (Alföld, Csárdás, Magdaléna) however the yields of Suba and Toborzó varieties didn't differ significantly either from each other or from the yields of Csárdás and Magdaléna. The comparison of the different N data showed a significant difference in all level of N. The only exceptions were seen in the case of the single 120 kg N application and in the case of the divided N doses (80+40 kg) where there was no significant difference in yields.
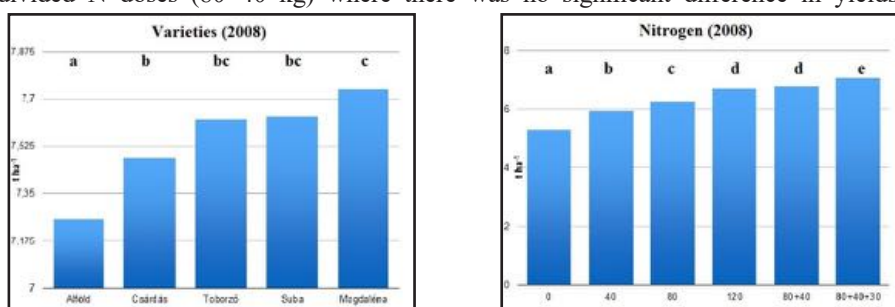


_Figure 1 Comparision of varieties and applied N doses, Duncan-test (2008)_

*2009:* According to the Duncan-test (**Figure 2**) there was a significant difference in yields between the Csárdás and Magdaléna wheats and in the other 3 varieties (Alföld, Toborzó, Suba). The Duncan-test didn't show significant difference among the yields of Alföld, Toborzó and Suba types. Differences in the doses of N were compared by the Duncan test as well. The result of the test falls in with the previous year's result as significant differences were measured in the applied doses of fertilizer. In 2009 the exception was again the divided and the single applications of the 120 kg N as there was no significant difference between the yields.
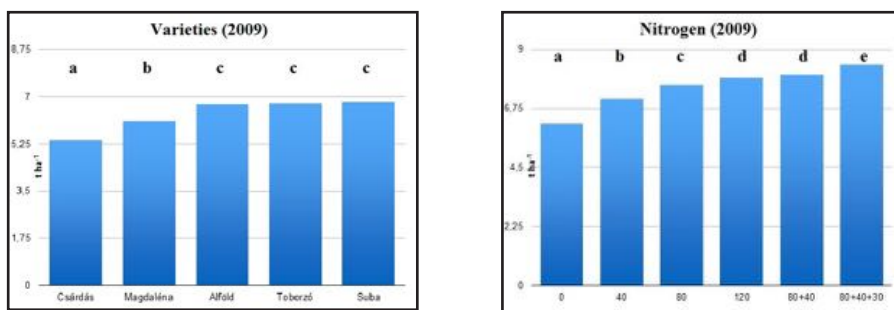


*Figure 2 Comparision of varieties and applied N doses, Duncan-test (2009)*

*2010:* Duncan-test (**Figure 3**) showed that there were significant differences among all types of wheats. Significant difference was shown among the 0, 40, 80, 120kg N, the 80+40kg N doses and the 80+40+30kg N doses. However, there were no significant differences among the 80 and 120 kg, and 80+40 kg and 80+40+30 kg N-doses.
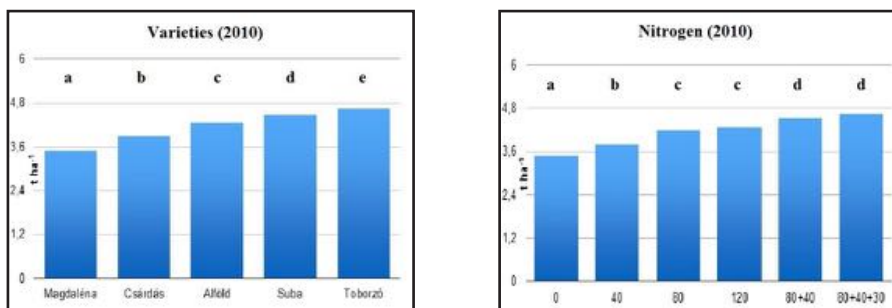


*Figure 3 Comparision of varieties and applied N doses, Duncan-test (2010)*

*2011:* Duncan-test (**Figure 4**), aimed to compare the different types, identified significant differences among the Csárdás, Magdaléna and Alföld types and between the mentioned types and the pair of Suba and Toborzó varieties. Significant difference was not shown between Suba and Toborzó types. The Duncan-test for N treatment showed the same result as it was in the first two years of experiment (2008-2009). The test didn't show significant difference between the use of single and divided use of the 120kg N however, apart from this result there was significant difference in all other nutrition levels.
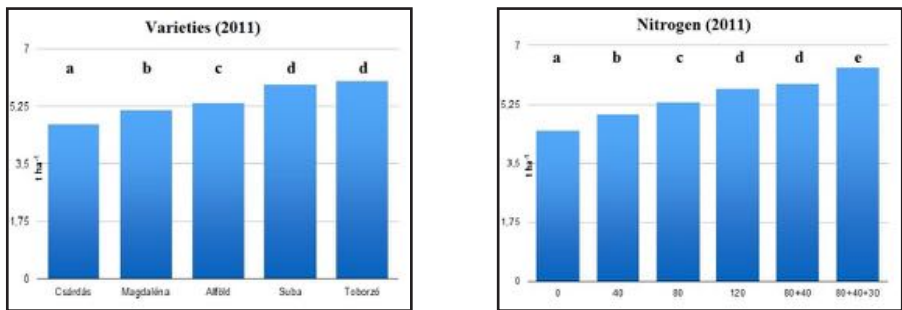
*Figure 4  Comparision of varieties and applied N doses, Duncan-test (2011)*

<u>2008-2011:</u>The follow-up of the the avarage yield per year was carried out by the Duncan-test as well. In all four years of the test period the yearly yield differed significantly (***Table 2***) from the other three years' results. As a result of the experiment we came to the conclusion that vintage has the greatest impact on the winter wheat yield that is followed by N fertilisation and the variety affect. The impact of the N fertilisation was two times as important as the variety impact however the interaction of the vintage and N fertilisation and vintage and variety were also significant.

*Table 2 Results of combined variance analysis*

| Source of Varrience | Repe-tition | Variety (A) | Fault (A) | Nutri-tion (B) | A x B | Fault (B) | Year (C) | A x C | B x C | A x B x C | Fault | All |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Degreeof freedom | | 4 | 12 | 5 | 20 | 75 | 3 | 12 | 15 | 60 | 270 | 479 |
| Calculated | | 125.26 | | 357.18 | 1.58 | | 2998.85 | 33.56 | 7.5 | 1.43 | | |
| F-volume | | *** | | *** | ns | | *** | *** | *** | * | | |

## Conclusions

Based on comparison of the examined four years of small plot winter wheat varieties and the N fertilisation trial we came to the conclusion that increased use of N doses resulted in yield increase in the case of all varieties. Three years (2008, 2009 and 2011) of the experiment show significant differences among the yields as a result of the applied different level of N. The yield of the tested winter wheat varieties increased as a result of the increased doses of N fertilisation. Exceptions are the utilization of the single and divided uses of the 120kg N, as there was no significant difference between the two N levels (method of application) in the mentioned three years. It means that the division of the top dressing's dose increased the yields but it can't be verified statistically. According to the comparative study of the vintages, there were statistically proved differences between the yields every year which proves that the weather has a huge impact on the harvest. Based on the combined analysis of variance test, done in the mentioned four years it is proved that greatest impact on winter wheat yield was the vintages, followed by the effects of N fertilization and the variety. The effect of the N fertilization was double of the varieties' effect though there were significant interactions between the vintages and N fertilisation and between the vintage and the variety.

**Acknowledgement**

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# Impacts of nutrition and age on the angiological state

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Abstract: Nutritional impacts on the angiological state of 54 random patients of an ambulance case study have been studied. Body Mass Indices (BMI) and age (Year) has been correlated with the General Angiological State (GAS) of the patients. The results obtained support a conclusion, that human nutrition – especially BMI increment - represents a characteristic stress factor in relation with vascular diseases. Within the patients, gender impacts have been evaluated. Correlations were found between overweight state and the severity of vascular diseases in case of patients. It can be stated, that over-weight and obesity, especially in male population is a major stressor that may have an influence on vascular diseases. Age proved to be a strong factor in vascular disease performance and could be observed in both genders. The strongest correlation with age was found in the state of the female lot.

Keywords: Angiological state, nutrition, Body Mass Index

## Introduction

Nutrition is a major source of most human health problems (Banczerowski et al, 2008; Szentpétery et al, 2002). Dietary patterns of the population often results in overweight or obesity (Lugasi and Marton 2010; Marton et al. 2012). Obesity is a serious medical condition that can cause complications such as metabolic syndrome, high blood pressure, atherosclerosis, heart disease, diabetes, high blood cholesterol, cancers and sleep disorders. Several authors have reported, that vascular diseases are often related to nutrition as well as to the age of patients (Romeo et al, 2008; Yuemang et al, 2006). Microvascular dysfunction in human hypertension has been reported by Poirier et al. (2006), and Kádár (2008). Angiological state of the population is being observed by various methods (USP, 2004, Michel 2008). In most countries objective measurement methods are combined with empirical observations.

The risks of improper human over nutrition on the circulatory system are as follows: Raised BMI increases the risk of hypertension (high blood pressure), which is itself a risk factor for coronary heart disease and stroke and can contribute to other conditions such as renal failure. The risk of coronary heart disease (including heart attacks and heart failure) and stroke may be both substantially increased. Risks of deep vein thrombosis and pulmonary embolism are also increased (Poirier et al. 2006).

The present study focuses on the presence of vascular diseases in relation with nutritional patterns and age of patients.

## Materials and methods

Nutritional impacts have been studied in a random population within a case study run at the Railway Health Care Company Budapest in 2016 following the methodology of an earlier study of Ross et al. (2009). 54 patients have been studied within an age range of 33 to 92 years. Age, Body Mass Index (BMI) and General Angiological State (GAS) were evaluated in general and in relation with gender of patients. Statistical analyses have been applied in accordance with statistical evaluation package of Microsoft Office 2003.

General angiological state (GAS) has been determined by a 1-7 ranking as follows:

- Three angiological diseases + diabetes          7
- Three angiological diseases          6
- Two angiological diseases + diabetes          5
- Two angiological diseases          4
- One angiological disease + diabetes          3
- One angiological disease          2
- Normal state          1

The three vessels' diseases observed were as follows: carotic artheric stenosis, coronaria stenosis, and peripherial arterial disease PAD. BMI and GAS, as well as age and GAS relations have been evaluated by regression analysis.
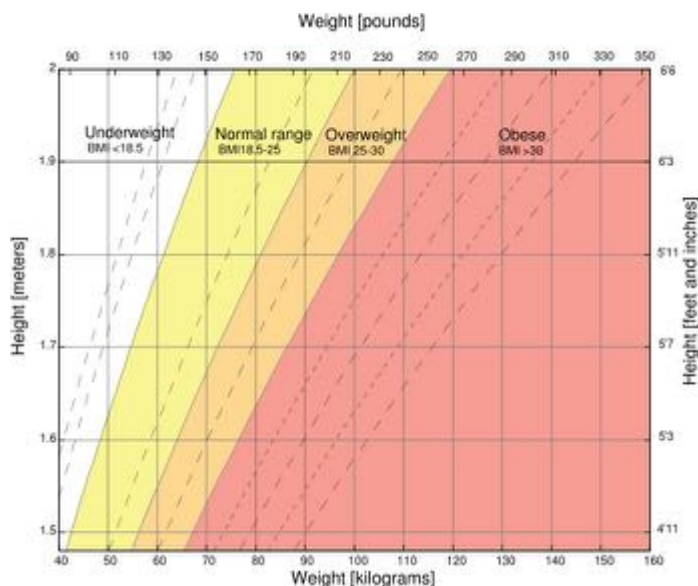


*Figure 1. Body Mass Index (BMI). source WHO 2012*

**Results and discussion**

The baseline data of patients, gender, age, BMI and GAS are presented in *Table 1*. The performance of patients was within a wide range in relation with these characteristics.

General Angiological State (GAS) of patients was gradual regarding the number and severity of vascular diseases and their combination with diabetes. BMI, GAS and age were evaluated both in general, and in relation with gender of patients. Gender ratio of the observed patients was 26 females and 28 males. Age range of the population was within 33 and 92 years. BMI range was 16.8 to 40.1 from which the following distribution was observed. Under-weight (<18.5): 3.7 %; normal (18.5-25.0): 42,6 %; over-weight (25.5-30.0): 37.0 %; obese (>30) 16.7 % as presented in *Figure 4*. The severity of angiological

state is as follows: normal and/or a single disease (GAS 1-2) is 20.3 %, medium (GAS 3-5) is 57.4 %, serious state (GAS 6-7) is 22.3 %.

*Table 1. GAS ratings of studied cases in relation with BMI, gender and age*

| No | Gender (male/ female) | a g e (year) | BMI (body mass index) | GAS rating (general angiological state) 1-7 |
|---|---|---|---|---|
| 1 | Male | 72 | 20.7 | 4 |
| 2 | Male | 62 | 40.1 | 7 |
| 3 | Female | 63 | 20.5 | 4 |
| 4 | Female | 67 | 24.9 | 4 |
| 5 | Female | 88 | 22.8 | 6 |
| 6 | Female | 83 | 23.2 | 6 |
| 7 | Female | 62 | 33,8 | 2 |
| 8 | Male | 66 | 25,7 | 5 |
| 9 | Male | 73 | 32.2 | 7 |
| 10 | Male | 80 | 25.5 | 5 |
| 11 | Male | 70 | 28.0 | 2 |
| 12 | Male | 64 | 30.7 | 4 |
| 13 | Male | 74 | 20.2 | 3 |
| 14 | Female | 74 | 29.8 | 4 |
| 15 | Female | 77 | 30.1 | 5 |
| 16 | Female | 70 | 26.8 | 7 |
| 17 | Male | 56 | 29.7 | 4 |
| 18 | Male | 83 | 25.2 | 5 |
| 19 | Female | 77 | 23.0 | 6 |
| 20 | Male | 83 | 25.2 | 5 |
| 21 | Male | 68 | 28.0 | 1 |
| 22 | Male | 66 | 26,9 | 5 |
| 23 | Male | 77 | 35.4 | 4 |
| 24 | Female | 86 | 22.2 | 4 |
| 25 | Female | 73 | 26.2 | 4 |
| 26 | Female | 75 | 16.8 | 6 |
| 27 | Male | 72 | 25.5 | 5 |
| 28 | Male | 64 | 24.3 | 4 |
| 29 | Female | 70 | 27.3 | 6 |
| 30 | Female | 70 | 30.1 | 5 |
| 31 | Female | 80 | 28.3 | 6 |
| 32 | Male | 82 | 24.7 | 5 |
| 33 | Male | 72 | 28.6 | 7 |
| 34 | Male | 66 | 27.7 | 6 |
| 35 | Female | 82 | 24.8 | 5 |
| 36 | Male | 80 | 29.4 | 5 |
| 37 | Male | 72 | 30.1 | 4 |
| 38 | Male | 63 | 27.1 | 2 |
| 39 | Female | 82 | 23.5 | 4 |
| 40 | Female | 92 | 22.9 | 4 |
| 41 | Female | 75 | 23.8 | 4 |
| 42 | Male | 78 | 29.1 | 4 |
| 43 | Male | 78 | 24.2 | 2 |
| 44 | Female | 71 | 19.2 | 5 |
| 45 | Female | 64 | 18.0 | 4 |
| 46 | Female | 73 | 25.1 | 2 |
| 47 | Male | 76 | 23.9 | 4 |
| 48 | Female | 65 | 18.7 | 2 |
| 49 | Male | 87 | 24.2 | 3 |
| 50 | Male | 74 | 23.5 | 2 |
| 51 | Female | 33 | 21.6 | 1 |
| 52 | Male | 36 | 24.2 | 1 |
| 53 | Female | 67 | 36.2 | 4 |
| 54 | Female | 67 | 24.2 | 1 |
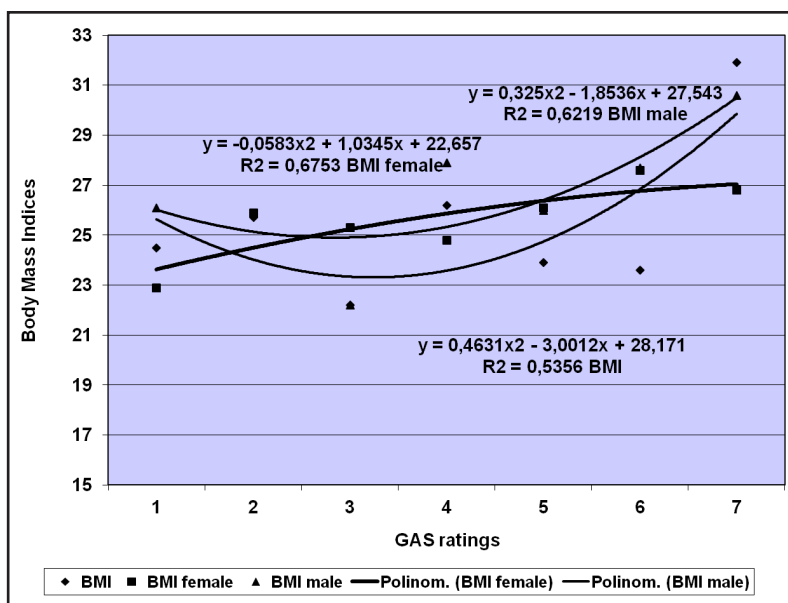
*Figure 2. BMI and gender impacts on Generel Angiological State GAS performance*

A statistical analysis is presented in *Figure 2* and *Figure 3* evaluating the performance of GAS in relation with nutrition patterns resulting in various levels of body mass like under-weight, normal state, over-weight and obesity and age of patients regarding both genders. The results obtained support the evidence that human nutrition – especially BMI increment - represents a peculiar stress factor in relation with vascular diseases. Correlations were found between overweight and the severity of vascular diseases in general, and in case of both sexes. Male patients had a specific pattern of this correlation, since the trend line of the BMI increment was almost linear in their case. Age of patients was in accordance with the general angiological state in both genders however vascular problems were increased significantly in the female lot with the strongest correlation.

## Conclusions

Nutritional patterns of the human population may have an impact on health conditions. In the present study the General Angiological State (GAS) was evaluated within a random population regarding gender, age and obesity. Three vascular diseases - carotic artheric stenosis, coronaria stenosis, peripherial arterial disease PAD - and their combination with diabetes have been studied in correlation with BMI. It can be stated, that over-weight and obesity, especially in male population is a major stressor that may have an influence on vascular diseases as it was reported by Poirier et al. (2006). Age proved to be a strong factor in vascular disease performance and could be observed in both genders. The strongest correlation with age was found in the state of the female lot. The results obtained have similarities with that of the survey of Ross et al (2009). There was only one detectable difference between the recent study and that of the 2008 year. The range of BMI proved to be broader and the number of over-weight and obese patients was increased.

Further studies are needed to explore nutritional interactions in relation with causes of obesity. Also, broader sampling of future case studies is needed to precise the results obtained.
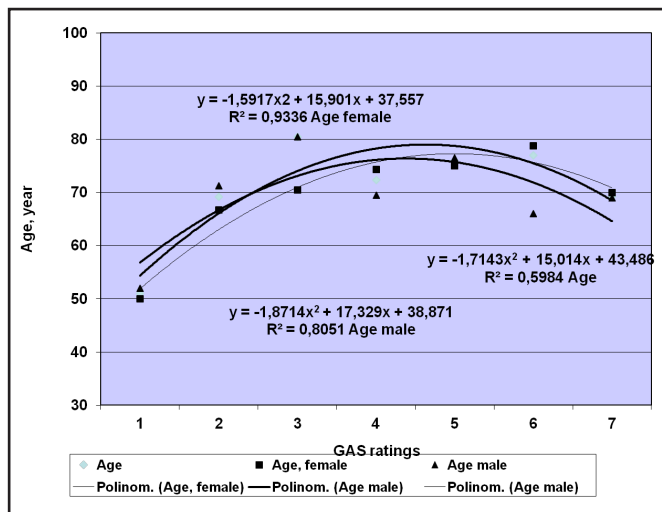


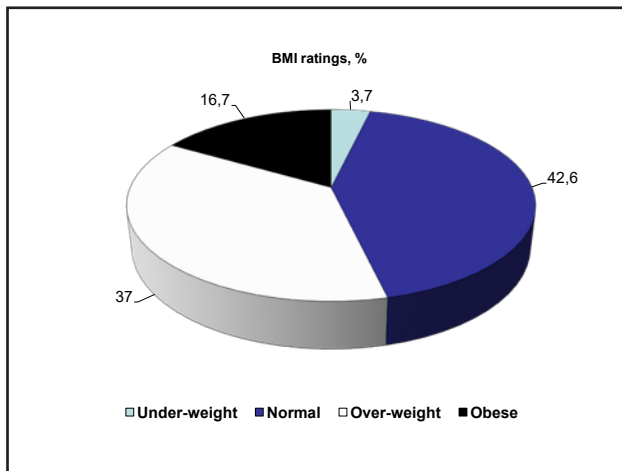*Figure 3. Age and gender impacts on Generel Angiological State GAS performance*



*Figure 4. Distribution of BMI ratings*

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# Appearance of the health – conscious consumer behavior in the V4 countries

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Abstract: In the past two-three decades the concept of sustainability has changed significantly – more and more attention is focused on social welfare, its preservation and increase. The health-conscious consumer behavior has become more important for both individuals and food industry - the realization of the strategy of the domestic food industry increasingly promoting healthy eating for example, consuming natural, domestic, fresh ingredients, prepared foods, in order to improve the overall health. The study is intended to present the change in consumer behavior – how the eating habits of consumers can influence the overall health status of the population in Visegrad countries. Furthermore, the aim was also to investigate the appearance of health awareness, as an increasingly significant factor of sustainability, in eating habits.

Keywords: health awareness, sustainability, food industry, consumer behavior, eating habits

## Introduction

Our research is based on the extensive literature review and Eurostat statistics. To understand the role that health-conscious consumer behavior can play in life quality development it is essential to know the most important definitions – what the health awareness and quality of life means, what kind of health issues (most common chronic diseases) are in the public nowadays, what kind of food categories are in the population's consumption, etc.

Furthermore, the paper also focuses on the different indicators as tools for sustainable development and the consumer-focused health communication as the key of the effective life quality development.

The main objective of the paper is to provide a better understanding of life quality development related to health awareness and its measurement. Moreover, the study also offers a brief introspection into the situation of Hungary in comparison with the European Union and regionally, with the other V4 countries (Slovakia, Czech Republic and Poland).

## Materials and methods

The study based on the extensive literature review and the data analysis related to the regional quality of life. Analyzed data derive from the Eurostat database – health related quality of life metrics between 2005/2010 and 2014 were acquired and analyzed.

The study aims to examine the relevant relationships and differences in quality of life between Hungary and the European Union or the Visegrad countries. Furthermore, the correlation between food consumption and health indicators was analyzed with correlation analysis – based on data from KSH database.

### *Theoretical background*
*Health, health awareness and health literacy – relations and differences*

The most integrated, accepted and commonly used definition of health was defined by the World Health Organization (WHO) in 1948. According to the Preamble to the Constitution of the WHO: "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." (WHO, 1948). It is increasingly recognized fact that health can be maintained and improved not only through the health science and different healthcare services, but also through the smart lifestyle of the individuals or society. Thus, WHO also determined the main elements of health, which include the social and economic environment, the physical environment, and the person's individual characteristics and behaviors. These determinants include the following key factors:

a) Income and social status: the greater the gap in the social status and income level, the greater the differences in health – linear relationship.
b) Education and literacy: low education level likely means more stressful lifestyle, poorer health and self–confidence level.

e) Genetics

f) Personal behavior and characteristics: balanced or unhealthy diet (fruit and vegetable consumption), physical activity, overweight and obesity, smoke, alcohol consumption, stressful work environment all affect health condition.

g) Health services: the accessibility of healthcare services can help preventing certain diseases, treating easily others and or avoiding different threats.

Health awareness or health–conscious behavior is all of the individual attitudes, behaviors and activities in order to live longer and remain healthier. To reach these targets, people:

- keep important and enforce their health aspects during their decisions,
- control consciously their habits (e.g. proper nutrition, physical activity, sexual behavior, avoiding the harmful practices and habits) and thus, they are actively involved in the development of health,
-  learn basic assistance and self–help skills (WHO, 2016)

The diverse resources (Busse et al., 2010; OECD, 2012; Cancer Research UK, 2009) mention the same diseases, as chronic diseases. Traditionally, chronic diseases include the following diseases: cardiovascular disease, diabetes, asthma, chronic obstructive pulmonary disease, cancer. Table 2 shows the deaths as a function of the chronic disease risk factors. It is clearly seen that smoking is one of the most important risk factor, because smoking is the responsible for 4.80 million deaths globally (8.5% of all deaths).

*Table 2. Major chronic disease risk factors*

| Chronic disease risk factors | Low-and middle-income | High-income | Worldwide |
|---|---|---|---|
| | Death (millions) | | |
| High blood pressure | (12.9%) | (17.6%) | (13.5%) |
| Smoking | (6.9%) | (18.5%) | (8.5%) |
| High cholesterol | (6.3%) | (10.7%) | (6.9%) |
| Low fruit and vegetable intake | (4.8%) | (4.2%) | (4.7%) |
| Overweight and obesity | (3.6%) | (7.8%) | (4.2%) |
| Physical inactivity | (3.2%) | (4.8%) | (3.4%) |

Source: Adapted from Busse et al., 2010

Overweight and obesity is also a main problem, and not only in case of adults, but also an increasing number of children is affected. Overweight and obesity are usually derived from the unhealthy diets and the lack of physical activity: both adults and children consume less fruits and vegetables, and do less exercises than they should in order to live healthier.

## Results and discussion

### The correlation between food consumption and health indicators

We can observe the amount of food consumption of households per capita in different regions and settlements on Table 3. The direction of changes of consumption levels between 2010 and 2014 was indicated by different colours. The consumption levels changed minimally on country- and Hungarian regional levels. The fact that in certain regions the consumption of fruits and vegetables increased, while cereal and fat consumption dropped, indicates that the health-awareness of the population is positively changing.

Table 3. The amount of food consumption of households per capita in different regions and settlements (2010, 2014), (kg/capita)

| Name of region | Total cereals | | Total meat | | Total milk, cheese, egg | | Total oils and fats | | Total fruits | | Total vegetables and potatoes | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2010 | 2014 | 2010 | 2014 | 2010 | 2014 | 2010 | 2014 | 2010 | 2014 | 2010 | 2014 |
| | kg/capita | | | | | | | | | | | |
| Hungary | 85 | 78 | 53 | 54 | 17 | 16 | 38 | 39 | 77 | 75 | 14 | 14 |
| Central Hungary | 74 | 64 | 48 | 46 | 16 | 13 | 42 | 36 | 79 | 65 | 12 | 10 |
| Central Transdanubia | 82 | 75 | 52 | 52 | 17 | 15 | 33 | 39 | 69 | 71 | 15 | 14 |
| Western Transdanubia | 87 | 74 | 50 | 49 | 17 | 15 | 32 | 38 | 60 | 67 | 15 | 13 |
| Southern Transdanubia | 92 | **94** | 51 | **64** | 17 | 16 | 39 | **45** | 80 | **99** | 14 | 16 |
| Northern Hungary | 91 | 87 | 53 | 56 | 19 | 18 | 34 | 36 | 81 | 78 | 15 | 15 |
| Northern Great Plain | 86 | 90 | 54 | 60 | 19 | **19** | 35 | 39 | 72 | 82 | 16 | **17** |
| Southern Great Plain | 99 | 84 | 67 | 61 | 18 | 16 | 42 | 41 | 95 | 83 | 14 | 14 |
| Budapest | 60 | 60 | 41 | 44 | 13 | 12 | 42 | **42** | 69 | 64 | 8 | 8 |
| The consumption: | | | | | | | | | | | | |
| decreased | | | | increased | | | | stagnated | | | | |

Source: Authors' compilation based on data from the KSH

### Analysis of quality of life in V4 countries

There are different metrics for measurement of quality of life (for example net income can influence the quality of life). However, this study exclusively examines the following health related metrics based on the Eurostat database:

- life expectancy by age and sex
- healthy life years in absolute value at birth and in percentage of total life expectancy
- body mass index (BMI) by sex
- daily smokers of cigarettes by sex and age

Life expectancy increased in all countries in case of both females (Figure 2) and males (Figure 3) from 2005 to 2014. If total values are compared by V4 countries and EU (28), total life expectancy increased exactly by 1 year between 2010 and 2014.

Among the V4 countries Hungary is in the worst, and Czech Republic in the best situation in all categories. In 2014, total life expectancy was 76 years in Hungary, and 78.9 years in Czech Republic (similarly, males: 72.3 and 75.8; females: 79.4 and 82), the difference is around 3 years. Life expectancy is higher in female population than in males in all countries.
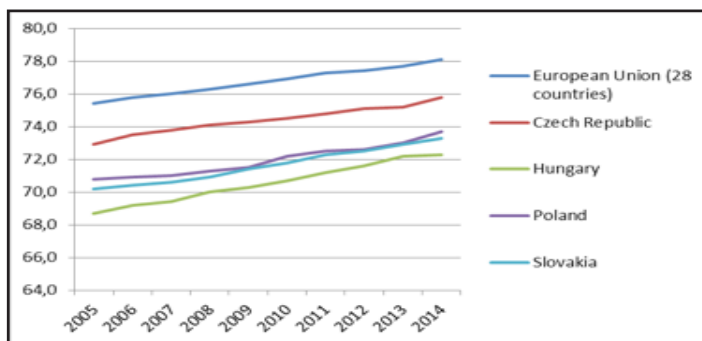


*Figure 2. Life expectancy by age in females between 2005 and 2014. Source: Author's compilation based on Eurostat database*
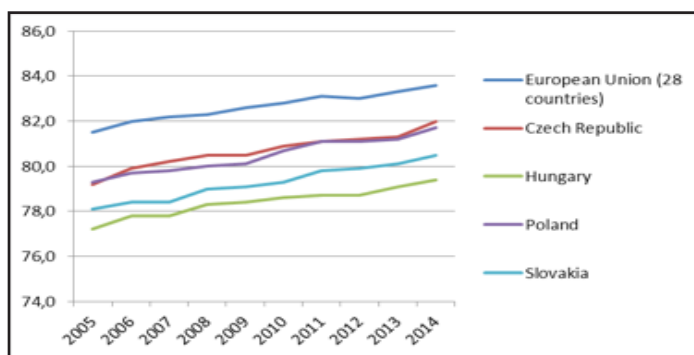


*Figure 3. Life expectancy by age in males between 2005 and 2014. Source: Author's compilation based on Eurostat database*
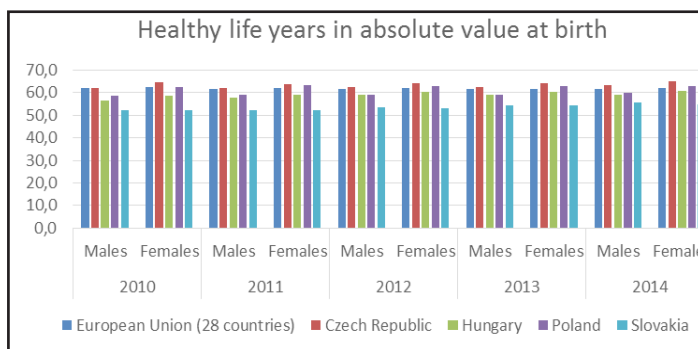


*Figure 4. Healthy life years in absolute value at birth between 2010 and 2014. Source: Author's compilation based on Eurostat database*

Figure 4 presents the healthy life years in absolute value at birth by sex and countries. Healthy life years can be analyzed and evaluated in percentage of the total life expectancy or in absolute value. When it comes to the analysis based on absolute value at birth it is clearly seen that both the male and female population can count the highest figure in Czech Republic among the Visegrad countries. In other words the development of life quality through health awareness would be the most important task in case of Slovakia. The difference was relatively high between the lowest (Slovakia – males: 55.5, females: 54.6) and highest (Czech Republic – males: 63.4, females: 65) value in 2014.

**Conclusions**

Theoretical background summarizes the most relevant scientific literature in relation to the health awareness, life quality development and food consumption. Based on the regional comparative analysis, it is clearly seen the health status of the population in Visegrad countries is not so bad, however, there are several opportunities to improve it with a well-designed and structured life quality development process. In order to improve the quality of life it would be necessary to analyze, evaluate and change the population's consumer behavior related to health awareness – not only the food consumption factors, but also the healthy life factors. The key of change is the appropriate life quality development process by influencing the population's eating habits and reducing the main risk factors – the most important tasks are collected and summarized in the following list:

Food consumption factors for example:

- to decrease the consumption of fat and sugar -> it can reduce the cholesterol level
- to change our eating habits -> the population should reduce the quantity of unhealthy food and consume more quality food instead.

Cronic disease risk factors:

- to reduce the mentioned risk factors (such as smoking, high blood pressure, obesity etc.) in order to avoid the different chronic diseases by
- increasing the physical activity of the population
- decreasing the number of daily smokers or amount of smoking

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# Changes in capacity and utilization of general practitioner care in Northern Hungary since the EU accession

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Abstract: Primary care is an indispensable element of the healthcare system: it creates a link between the patient and the healthcare system, plays an important role in the establishment of the diagnosis and healthcare focusing on prevention, thus decreasing the burden of higher levels of progressive care and costs. The new alternatives of organisational solutions, the horizontal and vertical forms of cooperation of general practitioners' clusters and group practices allow a more efficient organisation of healthcare. The changes that took place in the structure of the economy after the change of the political system were mostly disadvantageous for the socio-economic situation of Northern Hungary. The different components characterizing the marginal situation of the region are widely described in specialized literature, but there are only few scientific articles dealing with the health situation of the region. The present study is based on a previous own research showing that the capacities of the healthcare system of the region are lagging far behind the situation in other parts of the country.

Keywords: health, healthcare, primary care, general practitioner, Northern Hungary

## Introduction

The importance of health care has increased in the past decades: nearly 50% of the increase in life expectancy is due to the extension and improvement of healthcare (Figueras et al., 2008). The way of life chosen plays also an important role, but at the same time access to care and the way of life are influenced by social factors (Chan, 2008). Consequently it is vital to understand the relation between society and the economy and health status coupled with the level of health care. At micro-level, the improvement of health status affects positively economic productivity, workforce offer, propensity to save, as well as learning and creative abilities (Orosz, 2001).

At macro-level, these positive changes result in the improvement of the economy in general, and if the incomes generated are used to consume healthy products and services, develop the public health system, enrich knowledge and maintain democratic principles and institutions, these factors contribute to a better general health status meaning that in this case we are facing a positive feedback (Kollányi, 2013).

### The place of GP care in the Hungarian healthcare system

According to the Health Care Act, the health care system is built on an institutional system which is based on the distribution of work and the principle of progressive care, serving the differentiated care of individuals with diverse health status, in which the required level of care is determined by all the joint parameters of the patient's health status.

The principle of progressive care prevails on every level of healthcare. (CLIV/1997. §75/3) In accordance with the Act CXXIII of 2015 on primary care, patients are entitled to receive in the framework of primary health care long-term, continuous medical treatment based on personal relationship in or near their place of residence, based on the patient's choice, regardless of age, gender and the nature of the disease.

The main areas of primary care defined by law are the following:
– general practitioner (GP), paediatrician
– district nurse care
– school health care
– home nursing and home hospice care related to primary care
– occupational healthcare

Territorial healthcare is the obligation of the maintaining institution or the owner of healthcare services, or the healthcare providers, to provide health services for persons who are eligible for healthcare financed by compulsory health insurance, in areas of care determined in specialized healthcare, using contracted capacities. (CXXXII/2006. § 1/1/n) Regarding the mandatory health care tasks, the Act on Local Governments stipulates that municipal governments have to provide primary health care, while specialized healthcare exceeding primary care has to be provided by county and capital governments. (CLXXXIX/2011. § 13/4)

According to paragraph 8 (1) of the Act on primary healthcare, "*the general practitioner provides personal and continuous care aimed at the preservation of health, prevention, early diagnosis and treatment of diseases, as well as the promotion of health.*" Pursuant to paragraph 8(2), the family paediatrician provides the care defined in paragraph 8(1) for persons under the age of 19. Paediatric care can also be provided – upon choice – by the general practitioner for persons aged 14-19. The GP is authorized by law to manage health data and performs other medical expert activities which are not financed by social insurance, i.e. releasing medical report, medical opinion for driving license, firearm ownership, etc. According to the law – in addition to territorial limitations – the free choice of GP is the fundamental right of every citizen. (4/2000. EüM regulation)

*Motives leading to the selection of the study area*

In my former research I carried out the territorial comparative analysis of particular elements of the healthcare system in the 19 NUTS 3 counties plus the capital of Hungary using the method of cluster analysis. I applied twenty indicators describing the personal, infrastructural and accessibility characteristics of the health services, representing the individual segments of care. The source of indicators was the Regional Healthcare Database (REA) inside the Online Hungarian Healthcare Database (IMEA) of the National Healthcare Service Center (ÁEEK). Based on the assumption that all segments of health care – represented by the variables – were equally important, and that the supply of certain services in a given area nowhere exceeded the optimum value: I considered the higher values of the indicators as ideal. The counties of Northern Hungary, Borsod-Abaúj-Zemplén (hereinafter referred to as Borsod), Heves, Nógrád were included into the first cluster (together with Somogy and Zala counties). For each variable, the Northern Hungarian counties of the first cluster were ranked at the last or penultimate place, comparing their average values with the elements of the other clusters (Bálint, 2016).

*Changes in the capacities and utilization of the GP and paediatrician care in Northern Hungary*

According to the data of the Hungarian Central Statistical Office, in 2015 the number of adult and child care GPs together was 732 in Northern Hungary, out of which 423 persons

practiced in Borsod county, 188 in Heves county and 121 in Nógrád county. In Borsod, between 2005 and 2014, a continuous decrease of 8.4% occurred, and the value of the indicator measured for 2014 stagnated in 2015. In Heves county, the decrease of 7.6% in the period from 2008 to 2013 was followed by a slight increase of 3.3% by 2015. Between 2004 and 2015, an overall decrease of 9% could be observed, accompanied by fluctuations. For the years 2004-2015, the average number of inhabitants per general practitioner was 1555 in Borsod, 1635 in Heves and 1621 in Nógrád, so – based on this specific indicator – the workload of general practitioners was the highest in Heves county, however, later I will refine this finding by analysing statistical data on consultations and visits.

In the Regional Healthcare Database of the National Healthcare Service Center, the latest data on general practitioners providing adult, paediatric and mixed care are available for the year 2014. In the three counties examined, the absolute number of all the three categories of family care shows a slight decline, basically stagnation since the year of accession to the EU. The tendency is just the opposite for the values projected to the population (due to the continuous decrease of the population observed in all three counties, especially in the 0-14 and 15-64 age groups). The increase in the number of adult general practitioner services per 100 thousand inhabitants aged 20 or older is hardly perceptible, while an average of 5.7% growth in the number of paediatrician services per 100 thousand inhabitants aged 19 or younger was more spectacular between 2004 and 2014. For both indicators, Borsod county produced the highest values, followed respectively by Nógrád county, and Heves county. The number of general practitioner services per 100 thousand inhabitants providing mixed care has been slowly growing (near stagnating) since 2008. Nógrád county, which had produced the best values until 2007, lowered over the years to the level of Heves county, while Borsod county is lagging behind in the specific number of mixed GP services for the entire time series. Within the general practitioner services, the proportion of practices accepting to deliver territorial care in the area showed an average of 98.2% for the period 2004-2014.
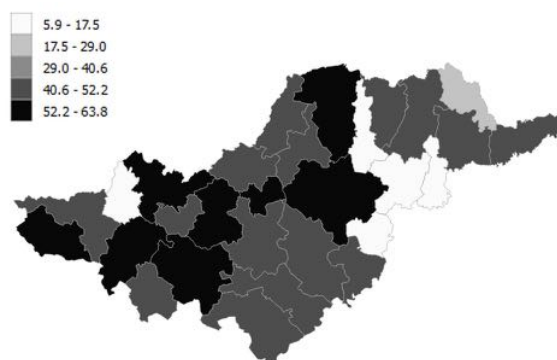


*Figure 1. Number of general practitioners per 100 000 inhabitants in the LAU1 districts of the Northern Hungarian Region*
*Source: own edition based on HCSO (2016)*

According to the stipulations of the government decree 313/2011. (XII. 23.), a general practitioner's district having the obligation to provide healthcare in the area is considered as a permanently vacant district if the services are provided by replacement for a period exceeding six months, except if this is due to work impediment of the GP in charge of the

district, or if – with the exception of replacement – local government is not able to provide health care for a period of six months at least with a person entitled to perform medical activity. Table 1 contains the most important information concerning permanently vacant general practitioner's services by district. We can see that most of these districts belong to the category of mixed services, especially in Borsod-Abaúj-Zemplén county where the length of the vacancy is also the highest.

*Table 1. Number of permanently vacant general practitioner's services by district categories in the Northern Hungarian counties*

|  | Adult physician | Paediatrician | Mixed | Average length of vacancy (years) |
|---|---|---|---|---|
| Borsod-Abaúj-Zemplén | 5 | 3 | 33 | 5.71 |
| Heves | 3 | 1 | 9 | 3.38 |
| Nógrád | 3 | 4 | 7 | 4.71 |

*Source: own edition based on ÁNTSZ (2017)*

At present, in 38.2% of the 68 vacant primary care districts (26 districts) adult, paediatric or mixed GPs have been missing for 1 or 2 years, and in 39.6% of the vacant areas the period of the vacancy exceeds 5 years. In areas with the longest vacancy – according the data of 1 January 2017 – general practitioners have been missing for 12 years in one or the other type of care, these areas (9 in total) represent 13.2% of the vacant districts in the Northern Hungarian region. The high number of persistently vacant districts raises the problem of work replacement. In my opinion, in the deficit areas the workload of general practitioners covering larger and larger districts should not rise above a certain level, since it reduces in every district the time and resources that can be spent on each patient. The analysis of statistical data on consultation and visits can give a picture of the utilization and burdens of the general practitioner capacities, though, the data in the REA are only available until 2012.

The change in the number of patients who attended consultations or were visited by the general practitioner can be divided into four phases in the period between 2004 and 2012. Between 2004-2006 this indicator increased by around 6% in all counties of Northern Hungary, while from 2006 to 2007, a sudden decrease occurred: 22.3% in Borsod, 18% in Heves and 19.4% in Nógrád. Between 2007 and 2009 the growth was 11.8% in Borsod, 8.8% in Heves and 9.82% in Nógrád. The period between 2009 and 2012 was characterized by a minimal decline, practically stagnation. The decline, which can generally be observed in 2007 in the utilization of GP care was the consequence of the introduction from 15 February 2007 of the so-called 'visiting fee' (contribution or co-payment to be paid uniformly by the patients above health insurance for each consultation in the framework of GP care, dental and out-patient specialized care), while the increase in the subsequent period was the result of the referendum of 9 March 2008 (which obliged the Parliament to restore the previous full scope insurance by 1 January 2009).

Concerning the number of patients attending paediatric care, the infinitesimal growth of the 2004-2006 period in Borsod was followed by a decrease of 8.2% between 2006 and 2008. After the temporary upturn of 4.1% in 2008-2009, the decline continued until 2012 by 11.5%. In Heves, the value of the indicator increased almost exactly to the same extent

(by 3.3%) as it decreased in the previous period between 2004-2008, but the major change happened between 2009 and 2012 with a decrease of 13.6%. Very similar processes took place in Nógrád: the studied indicator decreased by 3.5% between 2004 and 2006, rose by 3.9% from 2006 to 2009, then lowered by 13.6% in the period 2009-2012.

In addition to the absolute indicators, the utilization of the GP services can be further described with the help of specific indicators. For the complete time series, the number of patients attending GP care and patients visited per 1000 inhabitants is the highest in Heves (7004.4 persons on average in the 2004-2012 period), it is slightly lower in Borsod and Nógrád (on average 6482.7 and 6273.9 persons). The periodic tendencies of growth and decline are all along the same in all three counties, and the impact of visiting fee appears of course in the 2007 values of the indicator projected to the population number (and the effect of the referendum for the 2008-2009 values appears as well).

The number of children attending consultation or being visited in the framework of paediatric care is the highest in Borsod for the whole time series (on average 4795.9 for the period), while Heves and Nógrád 'rotate' their position every 2-3 years with smaller or larger fluctuations (with an average of 4503.9 and 4532.4 persons). Since patients under the age of 18 were not concerned by the introduction of the visiting fee, its effect did not prevail at all in the specific number of consultations and visits in Nógrád, and only to a lesser extent in Heves, however in Borsod, the decrease took place similarly to the adult GP care. In Borsod county the sensitivity of the parents towards visiting fee appeared in relation to paediatric consultations as well.

Between 2004 and 2012, the average number of GP consultations or patients visited per general practitioner – similarly to the values projected on the number of inhabitants – was the highest in Heves (14337.7), 12985.5 in Borsod and 12712 in Nógrád. The indicator shows a relatively strong fluctuation and can be divided into three distinctive phases. After the increase of the 2004-2006 period (Borsod: 7.4%; Heves: 6.9%; Nógrád: 8.9%), the strong decrease from 2006 to 2007 (Borsod: 21.7%; Heves: 17.3%; Nógrád: 16.7%) was followed by a similarly noticeable growth between 2007 and 2012 (Borsod: 23.1%; Heves: 14.6%; Nógrád: 13.5%). In all three counties, the last phase can be divided into two parts: the 2007-2009 period is characterized by a sharp increase (around 20% in Borsod and Nógrád, 11.3% in Heves), while in the 2009-2012 period a moderate growth can be registered in Borsod (2.4%) and Heves (2.9%), and a drop of 5.5% in Nógrád.

In the period examined the average number of paediatric consultations and patients visited per paediatrician was the highest in Nógrád with 8425.4 persons, while this periodic average was 8161.9 in Heves, 8050 in Borsod. The value of the indicator showed a very strong volatility during the period between 2004 and 2012. Compared to the year 2006 – which was the peak – a decrease of 7.2 and 18 percent could be registered respectively in Borsod and Heves counties by 2012. After the outstanding values of the year 2009, there was a 13.6% drop in Nógrád until 2012.

**Conclusions**

Similar processes have taken place in the counties of the region in the period following EU accession. The number of general practitioners' services has decreased in general in adult and child care, but has increased in relation to the number of inhabitants due to

population decline. The number of permanently vacant general practitioner's districts has risen dramatically in the past two years, and in many places the vacancy covers more than a decade. The introduction of visiting fee in the middle of the 2000s caused a temporary decrease in the use of primary care, which nevertheless returned to the same level after the suppression of the fee. In the past years the highest workload in general practitioners' care of adult patients was experienced in Heves county, child care attendance was the highest in Nógrád county, but due to ageing the number of visits per paediatrician decreased everywhere.

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# Detection and quantification of enzyme activity of ericoid fungi under solid-state fermentation

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**Abstract:** Today microbial enzymes are commonly used in many industrial applications and the demand for more stable, highly active and specific enzymes is growing rapidly. The aim of this study was the screening of fungal enzymes. Four fungal cultures were isolated and identified. These fungal cultures were tested on Solid-state Fermentation (SSF). The fungal isolate (S2) was noticed to show maximum of lipase production and the fungal isolate (S4) showed maximum production of protein content and cellulase production.

**Keywords:** Lipase, Protein content, Cellulase, Enzymes, Solid-State Fermentation SSF

## Introduction

Microorganisms are attractive sources for enzyme production because of their rapid growth and the limited space required for their cultivation. The ability to secrete large amounts of enzymes is characteristic of a variety of microorganisms such as bacteria, fungi, yeast and Actinomycetes (Gurung et al. 2013). In Morocco, the *Ericaceae* family is represented by only three genera and 10 species including *Arbutus* L*., Calluna vulgaris* L. *and Erica* L (Hamim et al. 2017). They can establish symbiotic root associations with the group of a distinctive type of mycorrhiza, termed ericoid mycorrhiza (Vohnik et al. 2011) and with the most studied group of fungal root endophytes belonging to the group of Dark Septate Endophytes (Lukešová et al., 2015). Several different enzymatic activities have been detected in ericoid mycorrhizal fungi and dark septate (Bending et al. 1996). The selection of the right organism is essential to obtain high yield of desirable enzymes (Padmapriya et al. 2012). Among the most important enzymes are proteinases, that are the most widespread in nature (Akhtaruzzaman et al. 2012), they possess considerable industrial potential due to their biochemical diversity in tannery and food industries (Punt et al. 2002), medicinal formulations, detergents (Devi et al., 2008) Besides, the cellulases are considered also important industrial enzymes. They hydrolyze β-1,4 linkages in cellulose chains (Zhang et al., 2013). Cellulases are used in the textile industry (Carrasco et al. 2016), in detergents and pulp and paper industry (Kasana et al. 2011). Another important enzymes for the industry are lipases, they are not involved in the lignin degradation, but they produced for fungi and its industrial application is extensive, they are used in wastewater treatment (Senthil Raja et al. 2012). The main draw back with production of fungi enzyme is the requirement of cost intensive procedures for separation of enzymes from cells (Devi et al. 2008). Since these enzymes are a product of industrial interest, their production must be combined with cost reduction, which can be achieved through the use of low cost culture

media (residues) from agro-industry. One way to obtain low cost enzymes is through a process named solid-state fermentation (SSF) (Castilho et al. 2000; Godoy et al. 2011). The SSF process is basically the use of a solid culture medium as a nutrient source and as a support to microorganism growth. That allows to reduce the cost of enzyme production processes and to obtain high enzymatic activity through the optimization of production (Vargas et al. 2008, Azcon et al. 2009).

Currently, there is an ever-increasing interest in the isolation and study of microorganisms, capable of producing enzymes with biotechnological applications and high economic impact. Based on the afore mentioned, the objective of the present study emphasizes on screening of fungal cultures for cellulases, lipases and protein content enzyme secreted by the ericoid fungi collected from root of ericaceous plants indigenous to Morocco, and thereby, be able to select those fungi with the greatest biotechnological potential.

**Materials and methods**

Microorganism: The strains S1, S2, S3, S4 have been isolated from ericaceous roots, and identified through the amplification of the Internal Transcribed Spacer (ITS) region (Hamim et al. 2007).

*Table 1. Sequences of isolated ericoid fungi from the gene bank (NCBI).*

| Strains | Best match | Accession | Host species | Region | Ordre |
|---|---|---|---|---|---|
| S1 | *Ericoid mycorrhizal sp* | AF072301.1 | *Calluna vulgaris* | Melloussa | Helotiales |
| S2 | *Ericoid ycorrhizal sp* | AF072296.1 | *Erica umbellata* | Sahel | Helotiales |
| S3 | *Ericoid endophyte sp.* | AF252845.1 | *Calluna vulgaris* | Cap spartel | Helotiales |
| S3 | *Phialocephala fortinii* | EU888625.1 | *Calluna vulgaris* | Melloussa | Helotiales |

**Cultivation media**: Sugarcane bagasse (50%), a solid residue from sugar cane was used as the solid-state fermentation culture medium supplemented with 30% of wheat bran, 15% of potato mash, 5% of olive oil and 300 ml of distilled water. The medium was inoculated with 15 fungal plugs (1cm$^2$) moisturized to 75% and incubated at 30°C. phosphate buffer (100 mM, pH 7.0, 5 mL/g) was added to each flask containing the fermented solids. The supernatant extraction was carried out in a rotary shaker at 35°C and 200 rpm for 20 min. Afterwards solid-liquid separation was done by pressing followed by centrifugation g for 5 min (Gombert et al. 1999). The supernatant was used for enzyme activity determination. Four sampling dates were identified at T0 (0 day); T1 (9 days); T2 (14 days); T3(27 days); T4(35 days).

**Protein quantification** was estimated as described by (Lowry 1951). Then O.D and concentration was measured at visible range 750 nm by a spectrophotometer. The amount of the soluble protein was calculated from the standard curve as mg of protein per ml of test samples.

**Lipase activity**: Lipase activity was measured using p-nitrophenyl laurate (pNP-laurate) as substrate. The hydrolysis reaction was carried out at 30 °C and measured over time up

to 10 min at 412 nm. The specific activity was calculated as the ratio of lipase activity (U g$^{-1}$).

**Estimation of reducing sugars by dinitrosalicylic Acid** method was determined by the colorimetric method of Miller (Miller 1959) using the DNS-reagent. The O.D of the samples was immediately measured at 575 nm. One enzyme unit was defined as 1 µmol of glucose equivalents released per min. The specific activity was calculated as the ratio of cellulase activity (U g$^{-1}$).

## Stastical analysis

The data are reported as means ± SD (standard deviation) for 3 replications. The results were subjected to analysis of variance (ANOVA) according to LSD test (P<0,05) using the stat-graphics plus version 4.0.

## Results and discussion

Protein content of isolated strains is presented in Table 1 and Figure 1. The statistical analysis has revealed the significant effect of strains and sampling date on protein content (P<0.05).

*Table 1. Protein content (mg/ml) of selected fungi*

| Day | Protein content (mg/ml) | | | |
|---|---|---|---|---|
| | S1 | S2 | S3 | S4 |
| T0 | 0.02c[2] ± 0.01[1] | 0.02bc ± 0.01 | 0.03b ± 0.01 | 0.04a ± 0.01 |
| T1 | 0.02 c[2] ± 0.01[1] | 0.02 c ± 0.01 | 0.04 b ± 0.01 | 0.08 a ± 0.01 |
| T2 | 0.02 c ± 0.01 | 0.02 bc ±0.00 | 0.03 b ±0.00 | 0.08 a ± 0.01 |
| T3 | 0.02 c ±0.01 | 0.03 bc ± 0.00 | 0.04 b ± 0.00 | 0.09 a ±0.01 |
| T4 | 0.04 d ± 0.00 | 0.07 c ± 0.00 | 0.09 b ± 0.00 | 0.12 a ± 0.00 |

Significant effect at the P<0.05 [1] Standard error. [2] The values of each line followed by the same letter are not significantly different according to LSD test (p >0.05).

During the experimentation, the concentration of soluble protein ranged between 0.02 to 0.12 mg/ml. The S4 showed highest protein concentration (0.12 mg/ml) and S1 accounted lowest concentration (0.02 mg/ml). The maximum content protein was 0.12 mg/ml; 0.09 mg/ml; 0.07 mg/ml; observed for S4, S3, and S2 respectively obtained at T4. In the present study, the result showed low protein concentration, that may explained by the inability of strains to degrade protein well, this finding may be explained as well by the composition and volume of medium. Reports in the literature suggested that the protein production varied according to different factors.

Estimation of reducing sugars of selected fungal is presented in Table 2. During the experimentation, different sampled cultures fungal showed varied cellulase activity. This concentration ranged between 5.88 to 17.34 Ug$^{-1}$. For instance, S2 have showed a

continuous increase of cellulase activity form 7.23 to 12.07 $Ug^{-1}$; however S4, S3 have showed a slight decrease of cellulase activity at T2 and T3 respectively. At T4, the S4 showed highest cellulase activity (17.34 $Ug^{-1}$) While, S1 have showed an unexpected decline to reach the value of 5.88 $Ug^{-1}$.

*Table 2. Cellulase activity (Ug$^{-1}$) of fungal isolates*

| Day | Cellulase activity (Ug$^{-1}$) | | | |
|---|---|---|---|---|
| | **S1** | **S2** | **S3** | **S4** |
| T0 | 7.20 b[2] ± 0.01[1] | 7.23 a ± 0.01 | 7.18 c ± 0.01 | 7.23 a ± 0.01 |
| T1 | 7.14 d ±0.01 | 10.03 c ±0.05 | 11.41 b ±0.02 | 12.34 a ±0.02 |
| T2 | 9.69 d ±0.04 | 11.84 a ±0.07 | 10.85 b ±0.01 | 10.12 c ±0.01 |
| T3 | 10.93 c ±0.00 | 11.67 b ±0.01 | 7.31 d ±0.01 | 15.15 a ±0.01 |
| T4 | 5.88 a ±0.05 | 12.07 b ±0.00 | 10.08 c ±0.01 | 17.34 d ±0.01 |

Significant effect at the $P<0.05$ [1] Standard error. [2] The values of each column followed by the same letter are not significantly different according to LSD test (p >0.05).

**Lipase Activity**: Lipase activity produced by selected fungi is presented in Figure 1. During different sampled time, the lipase production varied significantly between strains. This concentration ranged between 1.13 to 18.03 $Ug^{-1}$. We have noticed that lipase activity maintain continuous production level during the experimentation. T4 have recorded the maximum of lipase activity for all fungi. Therefore, S2 showed highest lipase activity at T4 (18.03 $Ug^{-1}$) However S3 (5.72 $Ug^{-1}$) and S4 (10.22 $Ug^{-1}$) have showed a moderate lipase activity, while S1 (2.63 $Ug^{-1}$) had lowest lipase activity.
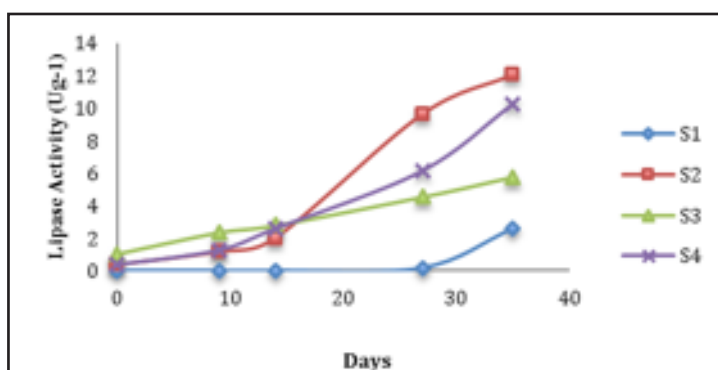


*Figure 1. Lipase activity of selected strains.*

The current study is the first report on cellulase, lipase enzymatic activity and protein content of selected ericoid fungal related to *ericoid mycorrhizal fungi* and to *phialocepha fortinii*. Those fungi are identified as specific to ericaceous plants indigenous to the north of Morocco (Hamim et al. 2017). Our study demonstrated the ability to produce enzyme activity obviously varied from strain to strain. Among the four strains, the ericoid

mycorrhizal fungi related strain (S2) and *phialocephala fortinii* related strain (S4) have a significantly better ability to produce cellulase activity during the experimentation. However, this cellulase activity was lower to that reported for *Trichoderma harzianum* at SSF which varied from 11 to 50 Ug$^{-1}$ (Berne et al. 2013). Furthermore, the ericoid mycorrhizal fungi related strain (S2), has a significantly better ability to produce lipase activity than other strains.. Besides, the DSE related strain (S4), has a significantly better ability to produce protein.

Previous studies suggest enzymatic capabilities of symbiotic fungi such as ecto-ericoid mycorrhizal fungi to produce lignolytic and cellulolytic enzymes (Wagner 2015). Furthermore, (Jumpponen and Trappe; 1998) have reported that the enzymatic capabilities of mycorrhizal fungi require lignolytic and cellulolytic enzymes to facilitate penetration through host cell walls. Likewise, Several different enzymatic activities have been detected in dark septate endophyte (DES) (Ahlich 1997) however, they varied drastically between strains.

Our study demonstrated low level of enzymatic activity content in this experimentation when compared to others in SSF, this low level production might be explained by the slow growth rate for the selected strains compared to *Aspergillus* sp., *Penicillium* sp. or *Trichoderma* sp. strains commonly used in state-solid fermentation or by the composition of the culture medium. Our finding is in agreement with (Lynd et al. 2002) they confirmed that cellulase production depends on many factors such as the fungal species.

**Conclusion**

In the present study, the selected fungal isolates from ericaceous root plants posses enzymatic activities; which has been scarcely studied for ericaceous plants indigenous to the north of Morocco. The enzymatic activity measured in this work is important characteristics for possible biotechnological applications for the biological control. Further research is vital to unravel the full potential of these microorganisms in agriculture.

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# Estimation of different fractions of organic carbon and it's implication to carbon dynamics in agricultural soil

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Abstract: Soil acts as a major sink to atmospheric carbon, thus plays a key role in global carbon cycle. Soil organic carbon (SOC) is composed of recalcitrant and labile carbon pools. Recalcitrant pool of carbon (humin and humic substances) is resistant to microbial degradation due to its complex chemical structure. Labile carbon is an oxidisable fraction of organic carbon. This pool of carbon is mainly composed of three components, i.e. physical fraction (including particulate organic matter), oxidizable fraction ($KMnO_4$-C) and biological fraction (microbial biomass). Recalcitrant fraction does not undergo mineralization easily, so it is not available for plant growth, only labile organic carbon fraction is available to plants and very rapidly responds to changes taking place in soil and surrounding environment. As, composition of total SOC determines the soil quality, it has beneficiary effects on soil quality and productivity. Agricultural practices also affect the soil organic pool as well as composition of SOC. Therefore, quantification of  various fractions of soil organic carbon which is influenced by management practises is necessary  for determining Carbon sequestration and as well as fertility status of soil.

Results indicate that Total carbon (TC), SOC and Total nitrogen (TN) ranged between 8.9-11.9 g/kg; 7.4-9.19 g $kg^{-1}$ and 0.82-1.12 g $kg^{-1}$ respectively. Pool I (Labile carbon) and II (Very labile carbon) together known as the labile pool, which ranged from 3.6 to 4.5 g $kg^{-1}$ and pool III and IV constitute together the recalcitrant/refractory pool, which ranged from 3.2 to 3.8 g $kg^{-1}$.

Keywords: Soil organic carbon, Carbon sequestration, soil quality, Organic matter, carbon pool

## Introduction

Soil organic carbon (SOC) pool is a measure of fertility of agricultural soil. Organic matter is a rich pool of nutrients required by plants for their growth and sustenance. But due to increasing population and thereby it's increasing demand for food have exerted a great pressure on agricultural lands, which resulted in degradation of soil quality and increased mineralization of soil organic matter. To improve the soil quality and nutrient availability a sustainable agricultural practice is required which can reduce the increasing pressure on agricultural soil. Recalcitrant fraction does not undergo mineralization easily, so it is not available for plant growth, only labile organic carbon fraction is available to plants. Composition of total SOC determines the soil quality.

The sedimentary/soil organic matter is widely distributed over the Earth's surface occurring in almost all terrestrial and aquatic environments *(Singh and Kazuo, 2004)*. Sediments contain a large variety of organic matter ranging from simple sugars and carbohydrates to more complex proteins, fats, waxes, and organic acids. Important characteristics of the organic matter include their ability to form water-soluble and water- insoluble complexes with metal ions and hydrous oxides; interact with clay minerals and bind particles together; absorb and desorb both naturally occurring and anthropogenically introduced organic compounds; absorb and release plant nutrients; and hold water in the soil environment. Naturally occurring organic carbon-forms are derived from the decomposition of plants and animals. In soils and sediments, a wide variety of organic carbon-forms are present and range from freshly deposited litter (e.g., leaves, twigs, branches) to highly decomposed forms such as humus. Sorption of dissolved organic matter (DOM) on to settling particles

is considered to be a major process in the preservation of organic matter (OM) in marine sediments. Evidence for this hypothesis includes the close relationship between sediment particle surface area and organic carbon (OC) concentrations and strongly reduced biological degradability after DOM has adsorbed to mineral surfaces *(Kaiser et al., 2000)*.

In this paper an attempt has been made to determine soil organic carbon (SOC) along with other linked constituents and a comparative investigation have been carried out on the influence of organic amendments in differential manner not only on the nature and properties but also fertility issues, productivity etc and to ascertain its important linkage with complex soil carbon dynamics using standard methods.

**Materials and Methods**

Soil samples (0-30 cm) were collected from wheat crop field with the help of PVC pipes. Soil samples (0–30 cm depth) were taken randomly in triplicate during August-September 2015-16, from the agricultural fields near Lucknow city, and analyzed individually. After initial soil identification other experiments were performed. In this investigation oxidizable-total organic carbon (SOC) was determined by using Walkley-Black method (Walkley and Black, 1934). Total N was determined by Gerhardt Kjeldhal method (Misra, 1968) that consists of three steps namely digestion, distillation and titration. The SOC content was calculated into different pools by the modified Walkley–Black method as described by Chan et al. (2001). All the fraction of SOC was estimated by using 12.0, 18.0 and 24.0 N $H_2SO_4$, respectively. Total SOC was divided into four different pools according to their order of stability against oxidation. Soil organic carbon, oxidized by 12.0 N $H_2SO_4$ was termed very labile pool (pool I). Labile pool (pool II) was calculated by taking difference in SOC oxidizable by 18.0 N $H_2SO_4$ and that by 12.0 N $H_2SO_4$, the difference in SOC oxidizable by 24.0 N $H_2SO_4$ and that by 18.0 N $H_2SO_4$ was pool III. The difference between total SOC and SOC oxidizable by 24.0 N $H_2SO_4$ was termed refractory pool (pool IV). Hence, it involves mixing 1N dichromate solution with $H_2SO_4$ in different proportions. The comparative analysis of physico-chemical properties of soil was done by different analytical methods. Soil pH and electrical conductivity (EC) were analyzed by pH and conductivity meter respectively**.** Phosphate – P and total Nitrogen – N were determined by Olsen's sodium bicarbonate method (Mackereth, 1963) and Micro-kjeldahl distillation assembly (Misra, 1968), respectively. Available potassium $K^+$ was estimated with the help of a flame photometer. Exchangeable sodium percentage was calculated as follows: ESP = (exchangeable sodium concentration (cmol/kg)/cation exchange capacity (cmol/kg))×100.

**Results**

*Soil Analysis*

Samples were analyzed individually for Soil type etc it revealed to be sandy, clay, loam, with pH ranged between 8.5 to 9.06. Electric conductivity (Ec), Phosphate (P) and potassium (K) are ranged between 0.60 – 0.96 (dSm$^{-1}$); 21.2 – 46.4 (kg ha$^{-1}$) and 167 -258 (kg ha$^{-1}$) respectively. TC ranged between 8.9 - 11.9 g kg$^{-1}$, SOC ranged between 7.4 - 9.19 g kg$^{-1}$ and TN ranged from 0.82-1.12 g kg$^{-1}$ (See Graph 2, 1 and Table 1 respectively). Pool I and II together known as the labile pool, which ranged from 3.6 to 4.5 g kg $^{-1}$ and pool III and IV constitute together the recalcitrant/refractory pool, which ranged from 3.2 to 3.8 g kg $^{-1}$(See Graph 1).
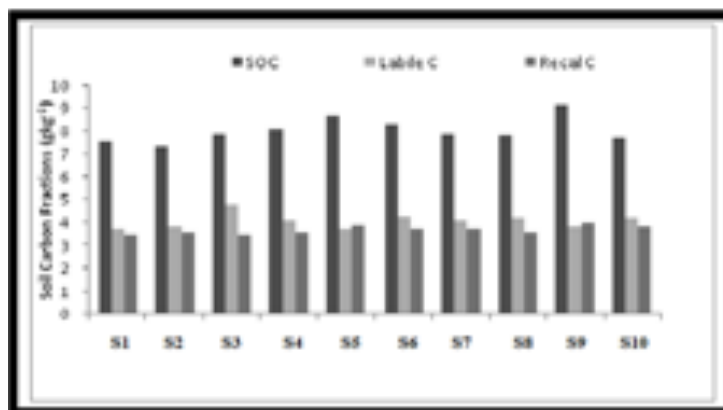
Figure 1. Soil carbon analysis

Table 1. Soil Analysis data

| Sample | Soil organic C Pools (g kg⁻¹) | | | | | Total nitrogen (g kg⁻¹) |
| --- | --- | --- | --- | --- | --- | --- |
| | Very labile (Pool I) | Labile (Pool II) | Labile (Pool I + Pool II) | Labile (Pool III) | Labile (Pool IV) | |
| S1 | 0.18 | 0.19 | 0.37 | 0.35 | 0.938 | 0.83 |
| S2 | 0.2 | 0.18 | 0.38 | 0.36 | 0.892 | 0.82 |
| S3 | 0.25 | 0.23 | 0.48 | 0.35 | 1.04 | 1.03 |
| S4 | 0.2 | 0.21 | 0.41 | 0.36 | 0.940 | 1 |
| S5 | 0.16 | 0.21 | 0.37 | 0.39 | 1.02 | 0.89 |
| S6 | 0.23 | 0.2 | 0.43 | 0.37 | 0.934 | 0.95 |
| S7 | 0.22 | 0.19 | 0.41 | 0.37 | 1.19 | 1.12 |
| S8 | 0.23 | 0.19 | 0.42 | 0.36 | 1.01 | 0.9 |
| S9 | 0.21 | 0.17 | 0.38 | 0.4 | 0.923 | 0.9 |
| S10 | 0.24 | 0.18 | 0.42 | 0.38 | 1.13 | 1.02 |
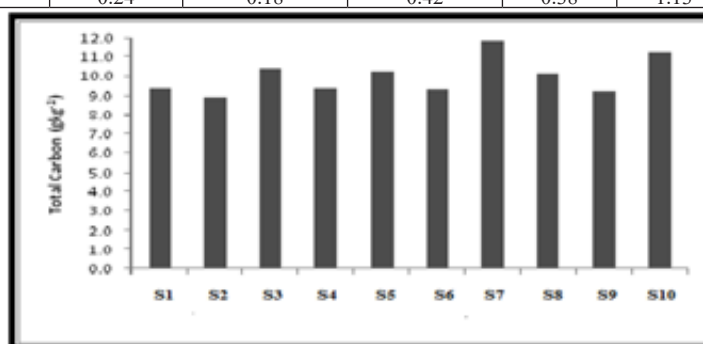


Figure 2. Total Carbon Analysis

## The comparative analysis of physico-chemical properties of sodic soil as influenced by organic amendments

The highest decrease in soil pH, EC and ESP were observed in FYM and VC (T8) treated

*Table 2. Chemical properties of sodic soil as influenced by organic amendments in sodic soil. Values are in means and range. Values in parenthesis represent % deviation w.r.t. pre harvest values.*

| Properties | pH | EC (dS/m) | N (kg/ha) | P (kg/ha) | K (kg/ha) | OC (%) | ESP |
|---|---|---|---|---|---|---|---|
| Pre harvest | 9.05 | 1.08 | 21.7 | 2184 | 184.25 | 0.85 | 78.5 |
| T1 | 9.03 (-0.11) | 0.95 (-0.15) | 22.5 (+3.69) | 2282 (-6.96) | 182.64 (-0.76) | 0.54 (-4.6) | 75.7 (-3.56) |
|  | 9.01-9.06 | 0.90-0.96 | 21.2-23.8 | 2174-2358 | 174.23-0.25 | 0.43-0.72 | 74.3-77.2 |
| T2 | 9.03 (-0.11) | 0.94 (-0.14) | 24.4 (+12.4) | 2489 (+12.2) | 189.29 (+2.7) | 0.29 (+16.6) | 81.3 (+3.56) |
|  | 9.00-9.05 | 0.90-0.95 | 23.3-25.7 | 2367-2597 | 167-2196.33 | 0.24-0.33 | 78.4-83.2 |
| T3 | 8.64 (-4.42) | 0.65 (-42.5) | 33.6 (+54.8) | 3306 (+54.1) | 205.53 (+11.3) | 6.53 (+192.3) | 63.3 (-19.3) |
|  | 8.62-8.66 | 0.62-0.69 | 25.5-39.7 | 2588-3918 | 188-211.55 | 6.53-6.63 | 61.3-66.3 |
| T4 | 8.64 (-4.42) | 0.64 (-43.3) | 35.3 (+62.6) | 3314 (+62.6) | 214.55 (+16.2) | 6.53 (-1.20) | 66.4 (-15.4) |
|  | 8.62-8.68 | 0.62-0.68 | 27.4-42.8 | 2787-4238 | 187.52-0.56 | 6.54-6.56 | 63.4-68.5 |
| T5 | 8.72 (-3.50) | 0.72 (-32.6) | 30.6 (+41.0) | 3096 (+41.6) | 196.43 (+6.7) | 0.43 (-52.2) | 74.4 (-5.22) |
|  | 8.71-8.74 | 0.71-0.78 | 23.7-34.6 | 2378-3416 | 178.42-0.44 | 0.42-0.74 | 71.6-77.4 |
| T6 | 8.65 (-5.42) | 0.55 (-53.0) | 36.8 (+69.5) | 3325 (+52.2) | 225.66 (+22.2) | 6.56 (+30.4) | 54.1 (-30.1) |
|  | 8.55-8.66 | 0.54-0.66 | 27.4-39.3 | 2799-3940 | 199.51-0.58 | 6.55-6.58 | 51.6-57.3 |
| T7 | 8.53 (-5.64) | 0.54 (-53.0) | 38.1 (+75.5) | 3311 (+52.5) | 230.64 (+25.6) | 0.54 (+159.3) | 47.68 (-39.2) |
|  | 8.52-8.55 | 0.53-0.65 | 26.8-49.4 | 2695-4258 | 195.62-0.65 | 0.42-0.66 | 44.8-50.6 |
| T8 | 8.50 (-5.97) | 0.51 (-54.7) | 38.7 (+78.3) | 3838 (+79.5) | 238.63 (+29.5) | 0.68 (+152.2) | 40.8 (-48.0) |
|  | 8.50-8.51 | 0.50-0.62 | 28.6-46.4 | 2897-4248 | 197.62-0.64 | 0.65-6.66 | 38.5-52.6 |

*T1=control; T2=NPK; T3=FYM (farm yard manure); T4=VC (Vermicompost); T5=NPK+VC; T6=FYM+VC (1:1); T7=FYM+VC (1:2); T8 =FYM+VC (2:1)*

plots respectively. The soil nutrients N, P, K and SOC contents were higher in organically amendments sodic soils than soils amended with chemical fertilizers (T3; NPK amended soil) and control soil. The highest soil nutrients were observed in FYM and VC treated plots (T8). The increased values were N (2.62% - 33.2%), P (3.69% – 78.3%), K (2.72% - 29.5%) and OC % (16%-152%) in comparison to pre harvest values. Various physico-chemical properties of used organic amendments such as FYM and VC were also analyzed and given in Table 2. It further indicates that organically amended soil have a potential to reduce the sodicity and enhance fertility. Further FYM and VC in 2:1 can act as boosting element to improve soil structure by facilitating the growth of microbial population and in turn carbon assimilation.

## Discussion

Labile organic carbon has been proved to be sensitive indicators of changes in SOC pool due to changes in agricultural management practices (Ghosh et al., 2013). The soil organic carbon pool and the total amount of labile carbon fraction has direct control over physiochemical and biological properties of the soil system and also influences self organization capacity of soil (Addiscott, 1995; Blair and Crocker, 2000; ). In 1995 in a report the Carbon lability was defined as the ratio of labile C to non-labile C (Blair et al., 1995).

Changes in labile C pools occur within a short period (one to two years) and this labile pool can be used to assess land management effects. Walkley Black C (WBC) or oxidizable soil organic C mostly represents the entire labile C pool and some portion of long-lived C pools, which takes longer to change, due to land management effects (Six et al., 1999). Hence, several workers reported that the $KMnO_4$- oxidizable SOC or labile carbon is a more sensitive SOC indicator compared with total SOC or WBC (Moharana et al., 2012; Liu et al., 2014). This fraction of carbon is a crucial part of global carbon cycle. In the present analysis it is indicative of its complex dynamics. In the other observation of organic amendment the pH may reduce primarily due to high production of $CO_2$ and

organic acids in soil followed by solubilisations of $CaCO_3$ and even neutralization of sodicity is reported (Shiamma et al., 2012) .

## Conclusion

Thus, quantification of soil carbon (C) cycling which is influenced by management practices is necessary for determining carbon sequestration dynamics as hypothesised is justified.

## Acknowledgements

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16th Alps-Adria Workshop

Synergism in science

# Water deficit irrigation strategy and arbuscular mycorrhizae application in field crop production

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Abstract: Most of tomato production areas are limited in water during its growing season, therefore irrigation is needed to optimize the productivity, but higher yield results in Brix losses and adversely affect fruits quality. In this field-based study processing tomato (*Lycopersicum esculentum* Mill. 'Uno rosso F1') seedlings inoculated with arbuscular mycorrhizae (AM) compared to non-inoculated (Control) were subjected to three irrigation regimes: Amply irrigation ($IR_{100}$), water deficit irrigation ($IR_{50}$), and non-irrigated ($IR_0$) depending on crop daily water requirement and by adjusting the irrigation water amount. Effects of irrigation water amount, AM inoculation, and their interaction on plant growth, water use efficiency (WUE), fruits quantity, and fruits quality were determined. AM plants performed better growth and increased the WUE remarkably compared to Control plants. In Control plants marketable yield decreased by (10%) when irrigation water reduced by half, and by (30%) in unirrigated compared to the potential yield in amply irrigation. Mycorrhizal inoculation improved marketable yield by (11%) in $IR_0$, (9%) in $IR_{100}$, and the highest yield increase was by (59%) in $IR_{50}$. More mycorrhizal contribution (37%) to fruit production was observed in $IR_{50}$ water regime. Water supply increased the yield from (65 t ha$^{-1}$) to (85 t ha$^{-1}$) and resulted in a (0.5) °Brix lose, but mycorrhizal inoculation slightly enhanced the °Brix in marketable fruits.

Keywords: *Lycopersicum esculentum* M., water use efficiency, yield, carotenoids, °Brix.

Changes in climate patterns disturbed the global water cycle especially precipitation aspects, where regional differences in precipitation amount is evidently observed and many areas faced more intense drought (Trenberth et al., 2014). Water restriction especially in water limited environments affects the production of processing tomato negatively, due its summer growing season and high water demand (Patanè et al., 2011). The fact that higher tomato paste from processed tomatoes, and lower energy cost for volatilization during the processing as a result of high soluble solid content in produced fruits makes °Brix the most important ingredient in processing tomatoes (Barrios-Masias and Jackson, 2014). Carotenoids are organic pigments that human body cannot create them and they are playing an important role in cell function.

Arbuscular mycorrhizal fungi (AMF) settle symbiosis relationship with most plant families (Smith and Read, 2008), and processing tomato can establish this symbiotic relationship with AMF too. This symbiont relationship backs up tomato plants to avoid water lack stress (Bakr et al., 2017; Candido et al., 2015), while the host plant supplies the fungus with photosynthates and an ecological niche. The improvement direct and indirect water uptake by AMF (Ruth et al., 2011), is also accompanied by enhancement in nutrient acquisition and uptake especially phosphorus (P), leading to better water retention in plant, enhanced physiological functions, and better growth (Augé, 2001; Smith and Read, 2008) more efficiently under dry soil conditions (Neumann and George, 2004). Further

inoculation with commercial inoculum did enhance crop productivity and colonization rate, with no quality improvement (Bakr et al., 2017; Candido et al., 2015). In tomato, antioxidants composition is affected by abiotic factors such as irrigation, temperature, and light (Pék et al., 2014), but mycorrhization could improve lycopene and β-carotene in fruits (Ulrichs et al., 2008).

This field based experiment is to evaluate effects of the commercial bio-inoculant Symbivit®, and different irrigation regimes on growth, production, fruit nutritional content, agronomical water use efficiency of Uno Rosso processing tomato.

**Materials and methods**

Seeds of processing tomato UNO ROSSO F1 (United Genetics Seeds Co. CA, USA), were sown on April 13 in (Klasmann TS3) substrate and inoculated with the arbuscular mycorrhizae (AM) or not (Control). The commercial inoculum Symbivit® (mixture of G. mosseae, G. etunicatum, G. claroideum, G. microaggregatum, G. geosporum, and R. irregularis) produced by Symbiom Ltd. (Czech Republic, www.symbiom.cz) was used by adding 25 grams of the inoculum to each litter substrate.

The experimental farm arranged in a randomized block design with three irrigation regime blocks: Amply irrigation ($IR_{100}$), water deficit irrigation ($IR_{50}$), and unirrigated ($IR_0$) depending on crop daily water requirement and by adjusting the irrigation water amount through a dripping system. Seedlings were arranged in double (twin) rows with 1.1 m and 0.4 m inter rows distance and 0.2 m between plants. Depending on air temperature (data taken from the National Metrological institute) plants daily water requirement calculated (daily water demand mm = average daily Temperature ºC x 0.2 mm ºC$^{-1}$) after Pék et al. (2014). The experimental site had a brown forest soil, loamy in texture (41% sand, 47.5% silt, and 11.5% clay) with a bulk density of 1.49g cm$^{-3}$, 25% field capacity, neutral in pH, free from salinity (0.212 dS m$^{-1}$), low in organic matter 1.4%, and contains [($NO_3^-$ -N (8.6 g kg$^{-1}$), $P_2O_2$ (8 g kg$^{-1}$), $K_2O$ (56.7 g kg$^{-1}$)] super elements. After one month of growth and during transplanting AM seedlings were re-inoculated by adding 20 grams of Symbivit® to the planting hole. According to Helyes and Varga (1994) plant nutrition requirements and plant protection were regulated throughout the growing season. After 100 days of growing the seedlings in the field, fruits and total biomass were harvested on August 23.

Plants roots were stained by Trypan Blue after Phillips and Hayman (1970). A stereomicroscope at × 100 magnification was used to determine internal fungal structures (hyphae, arbuscules), and gridline intersect method (Giovannetti and Mosse, 1980) was used to calculate root length colonization in percentage.

Water use efficiency (WUE) was calculated depending on total marketable fruit (WUE = kg marketable fruit per hectare/ water consumed per hectare m$^{-3}$).

**Extraction of carotenoids and ºBrix determination:** The pigments from raw tomato were extracted according to a previously described procedure with slight modification (Abushita et al., 2000). A Hitachi Chromaster HPLC instrument, which consists of a Model 5110 Pump, a Model 5210 Auto Sampler, a Model 5430 Diode Array detector, and a Model 5440 Fluorescence detector, was used for the determination of all compounds. Digital Refractometer Krüss DR201-95 (Küss Optronic, Hamburg, Germany) was used to

estimate the soluble solid content (ºBrix).

**Statistical analysis**: SPSS Version 22.0. (IBM Hungary, Budapest, Hungary) was use to perform statistical analyses. Effects of mycorrhizae, irrigation level, and their interaction were determined by two-way ANOVA. Means (n=4) with different letters are significantly different at (P<0.05) as determined by Tukey test. Capital letters represent mycorrhizal inoculation effect; small letters represent irrigation effect.

## Results and discussion

The first month of the growing season experienced regular with sufficient amount of rain, therefore irrigation induced after 5 weeks of transplanting. Throughout the growing season the experimental field supplied 296 mm of rain with couple heavy rain events in the mid of July. Irrigation resulted in 480 mm in IR100 and 388 mm in IR50 including 296 mm of rain, while IR0 block received only 296 mm of rainfall (Fig. 1).
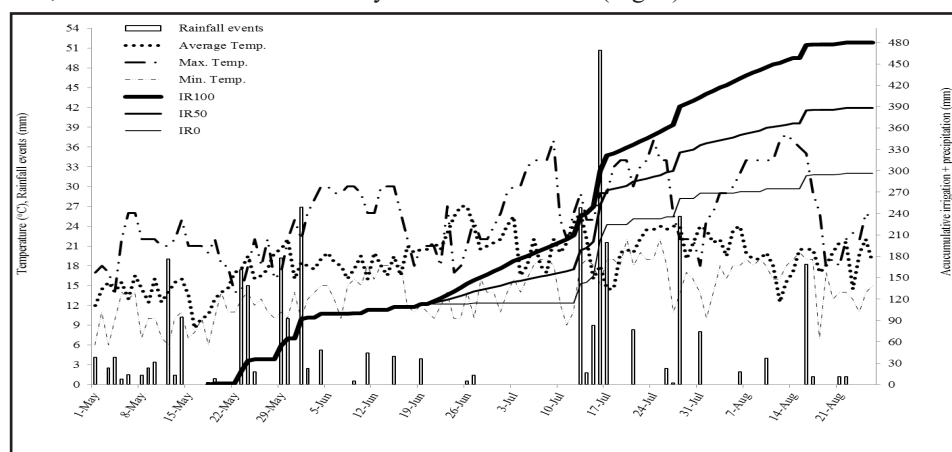


*Figure 1. Average daily temperature, precipitation, and accumulative irrigation amount in 2016.*

Control plants showed relatively high colonization rates (51, 58, and 53%), but mycorrhizal inoculation raised the root colonization in AM plants to (70, 73, and 71%) in IR0, IR50, and IR100 respectively (Fig. 2). The relatively high root colonization levels in control plants is due to natural occurrence of AMF in most of the agricultural soils in Hungary, where Glomeraceae family is dominating the mycorrhizal community (Magurno et al., 2015), and the fact that even the agronomical practices such as winter tillage and bar fallow did not reduce AM colonization potential (Bakr et al., 2017). Mycorrhizal inoculation increased the above ground total biomass in AM plants by 4%, 34%, and 9% in $IR_0$, $IR_{50}$, and $IR_{100}$ respectively compared to Control plants (Fig. 2), and this in compatible with (Lekberg et al., 2005; Ortas et al., 2013) additional mycorrhization enhances the growth in field crops, and in processing tomato (Bakr et al., 2017; Candido et al., 2015). The growth enhancement is related to better nutrient (Augé, 2001) and water (Ruth el at., 2011) uptake from the soil.

Better water uptake in AM plants improved the water use efficiency slightly in $IR_0$, and $IR_{100}$ with most efficient use of water (29.6 kg/m³) in $IR_{50}$ (Fig. 3); more efficient water use by mycorrhized tomatoes was also observed in previous field grown tomatoes (Bakr et al. 2017; Bowles et al. 2016).
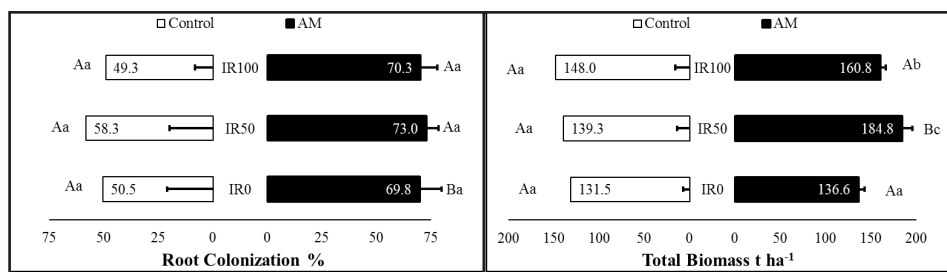
Figure 2. Root colonization rate (%), and total biomass (ton per hectar) of Control plants (empty bars), and AM plants (filled bars) under different irrigation regimes.
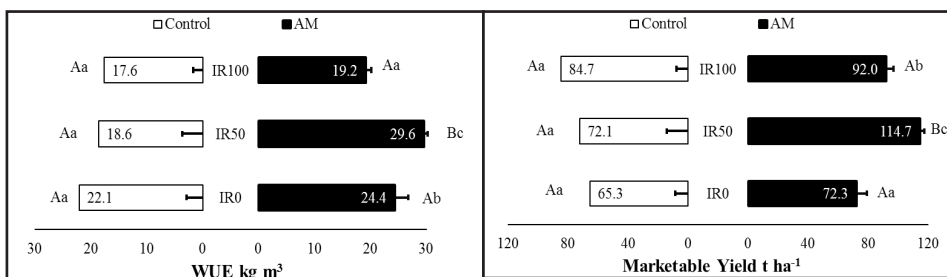


Figure 3. Water use efficiency (WUE) (yield kg / water consumption m3), and total biomass (ton/ hectar)

Irrigation did increase marketable yield from 65 to 72, and 85 tons per hectare in $IR_0$, $IR_{50}$, and $IR_{100}$ in non-inoculated plants, but not reaching significant levels statistically. AM plants raised the yield by 9% when fully irrigated, and by 59% under deficit irrigation regime similar to that of Bakr et al. (2017) on a sandy loam field using the same commercial inoculum *Symbivit®*, and exceeding results of Bowles et al. (2016), who recorded an increase of 28% in mycorrhized tomatoes on a very fine sandy loam field under deficit water conditions.
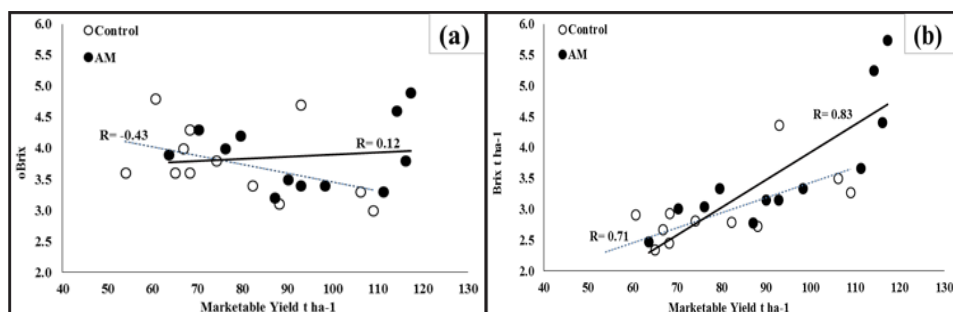


Figure 4. Yield impact on (a) soluble solid (°Brix) content, and (b) soluble solid production per unit area.

In Control plants a loss of a half unit of °Brix was registered when marketable fruit raised from (65 t ha⁻¹) to (85 t ha⁻¹) due watering increase to optimal level (Fig. 4a). Despite losses in soluble solid content in non-inoculated plants, yield increase recompensed this loss and soluble solid was increased as a mass production per area, while this trend was more pronounced in AM fruits with a very strong positive relation (r = 0.83) between soluble solid production and yield production per unite area (Fig. 4b). Unlike Control plants, mycorrhizal inoculation slightly enhanced the °Brix content in marketable fruits (r = 0.12) along with the yield increase.

*Table 1. Fruit and antioxidant concentrations (µg g$^{-1}$)*

| Irrigation | Treatment | °Brix | Total Carotene | Lycopene | β-Caroten | Ascorbic Acid |
|---|---|---|---|---|---|---|
| IR$_0$ | Control | 3.7$^{Aa}$ ±0.1 | 313$^{Ac}$ ±32 | 205$^{Bb}$ ±10 | 13.5$^{Bc}$ ±2 | 331$^{Aa}$ ±23 |
| | AM | 4.1$^{Ba}$ ±0.2 | 282$^{Aa}$ ±32 | 165$^{Aa}$ ±23 | 9.7$^{Aa}$ ±1 | 293$^{Aa}$ ±61 |
| IR$_{50}$ | Control | 4.5$^{Bb}$±0.4 | 233$^{Ab}$±26 | 188$^{Ab}$±26 | 10.1$^{Ab}$ ±1 | 418$^{Aa}$±39 |
| | AM | 4.2$^{Ba}$ ±0.7 | 281$^{Aa}$ ±42 | 185$^{Ab}$ ±23 | 9.7$^{Aa}$ ±.2 | 374$^{Ab}$ ±45 |
| IR$_{100}$ | Control | 3.2$^{Aa}$ ±0.2 | 181$^{Aa}$ ±11 | 95$^{Aa}$ ±19 | 5.4$^{Aa}$ ±1 | 334$^{Aa}$ ±74 |
| | AM | 3.4$^{Aa}$ ±0.1 | 437$^{Bb}$ ±50 | 273$^{Bb}$ ±30 | 17.2$^{Bb}$ ±4 | 236$^{Aa}$ ±72 |
| Significant of Source of variation | | | (ns= not significant, | * P≤0.05, | ** P≤0.01, | *** P≤0.001) |
| Mycorrhizae (M) | | ** | *** | *** | ** | ** |
| Irrigation (IR) | | *** | * | ns | ns | * |
| M * IR | | ns | *** | *** | *** | ns |

In control plants, decreasing irrigation positively affected and increased significantly the total carotenoids concentration in marketable fruits from 181 µg g$^{-1}$ in IR$_0$ to 233 µg g$^{-1}$ in IR$_{50}$, and 313 µg g$^{-1}$ in IR$_{100}$. The same was observed for lycopene, and β-Carotene and along gradients of decreasing irrigation amount lycopene, and β-Carotene concentrations were increased gradually, but this enhancement is accompanied by a decrease in marketable fruit (Table 1). Despite, slight changes within the same treatment and between the treatments, ascorbic acid levels have not shown a clear trend.

In addition to the increase in fresh fruit biomass by 11% and 59% in both IR$_0$, and IR$_{50}$ regimes, mycorrhization could preserve the high antioxidant levels. Moreover, beside the yield improvement in AM plants, mycorrhizal inoculation did increased the total carotenoids by 2.5 folds, and tripled both lycopene and β-Carotene content in ripened fruits in IR$_{100}$ compared to Control plants (Table 1). This scored for the mycorrhizal fungi effects in optimizing the yield with respect to the quality that not achieved in a previous study (Bakr et al., 2017). The enhancement in nutrient acquisition from the soil due to mycorrhization may resulted in better accumulation of the antioxidants in tomato fruits which also reported by Ulrichs et al. (2008).

## Conclusion

This study illustrated field based evident that water deficit irrigation and arbuscular mycorrhizae field inoculation can be followed as an effective strategy in field crop mass production especially in limited water environments. AM enhanced the water uptake and did make considerable improvement in water use efficiency, production, and guarantee high quality when it is combined with deficit irrigation. More studies needed to standardize the application of AMF, and irrigation strategy as well especially under field condition where many environmental and biological aspects are interacting.

## Acknowledgement

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# Status of plant available phosphorus in Nisava area of the South and Eastern Serbia

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Abstract: In the period 2010-2014, total 12447 soil samples (0-30 cm depth) covering 9027 ha of cultivable lands of Nisava area (town Nis and six municipalities: Svrljig, Gadzin Han, Razanj, Doljevac, Aleksinac and Merosina in South and Eastern Serbia region) were collected with the aim to test their agrochemical properties. In this study, status of plant available phosphorus (P) evaluated by the AL-method was shown. About 23% of the samples were very low supplied (<5.0mg $P_2O_5$ 100g$^{-1}$) by P. By the addition of low supplied group (the range is 5.1-12 $P_2O_5$), the rate rose up to 61.3% of samples. These categories of soils were recommended to fertilize more than consumed P (K) by crops. Only 12.4% of soil samples were high and very high supplied with available P (>20mg $P_2O_5$100g$^{-1}$). Recommend fertilization of these categories of soils by P could be maximal up to range of their removals by crops.

Keywords: soil, phosphorus, AL-method, ranges of availability, Nisava district.

## Introduction

Yields of field crops in Serbia are considerably lower compared to the yield possibilities of the high-yielding cultivars and degree of climate and soil potential. Average grain yields of two main field crops; maize and wheat, in a 3-year period (2010-2014) were 5.33 t ha$^{-1}$ (maize) and 3.80 t ha$^{-1}$ (wheat). In this period, in Serbia 1 2137 374 ha year$^{-1}$ (36.8% of used arable land) and 513 098 ha (15.6%) were covered by these two crops, maize and wheat, respectively (SY, 2014). Low yields of main field crops in Serbia could be in close connection with low consumption of mineral fertilizers, less than 80kg ha$^{-1}$ calculated on active ingredients (N + $P_2O_5$ + $K_2O$) basis.

Weather characteristics are considerably affecting the yield of maize (Kovacevic et al., 2014) and wheat (Marijanovic et al., 2010). With that point of view, drought and high temperatures, especially in spring months, are oft in connection with the lower yields of maize and other spring crops, except the sunflower (Kovacevic and Kaucic, 2014). In addition, yields of field crops in the Southern and Eastern Serbia region (SES) is considerably lower in compare with those in Vojvodina region (VR). For example, yields in SES for 2010-2014 period were for 34% (maize) and 23% lower (wheat) in compare with yields of these crops in VR (4.00 and 6.03 t ha$^{-1}$, 3.23 and 4.17 t ha$^{-1}$, for maize and wheat, respectively – SY, 2014). These differences were caused mainly by the lower soil fertility in SES due to unfavorable physical and chemical characteristics. It were found in Croatia and Bosnia and Herzegovina, that low plant available phosphorus (P) and potassium (K) levels are main limiting factor of field crop yields and these phenomena are mainly in combination with additional less favorable soil characteristics, for example, acid reaction and domination of either sandy or clay texture (Kovacevic et al., 2006; Komljenovic et al., 2010).

Aim of this study was testing P nutritional status in soils of the Nisava district of SES and making recommendations on improving fertilization practice in accordance with corresponding soil properties.

**Materials and methods**

This study was carried out during five years period (2010, 2011, 2012, 2013 and 2014) on different soil types. Total 12447 soil samples were analyzed from 2010 to 2014. By these samples, the covered area was 9027 ha in seven municipalities of the area (mean 0.725ha sample$^{-1}$). Majority of samples (8431 or 67.7%) were taken in the area belonging to municipalities Svrljig (24.8%), town Nis (23.0%), and Aleksinac (19.9%). Rest of soil samples (4016 or 32.3%) were taken in municipalities Doljevac (15.6%), Gadzin Han (8.1%), Merosina (5.3%) and Razanj (3.3%). The soil samples were taken by auger up to 30 cm of depth. Soil analyses were made in the agrochemical laboratory of Extension Service Agrorazvoj (eng. Agrodevelopment) Nis. Evaluation of plant available P was made by AL-method (Egner et al., 1960).

*General soil and climate characteristics of the Nisava area*

Majority of agricultural lands in Central Serbia are unfavorable in physical and chemical properties. Those are mostly lowland or hillside types of pseudogley or its leached variants, acid vertisols, podzolic eutric cambisols, diluvial, brown, or leached brown soils of mountainous regions. Those soils are rather poor in bases, medium to heavily acidic, have very poor texture and poor organic content and more or less ill; suited for cultivation of most cereal and maize crops (Đalović et al., 2010). The acidity of these soils, their high contents of $H^+$ ions and low contents of essential plant nutrients, primarily P and Ca, are limiting factors for high and stable yields of cultivated cereals and maize crops (Kovačević et al., 2006).

Recent investigation world widely have shown that massive deterioration of small grains on acid soils caused by elevated concentrations of mobile forms of some toxic elements (Al, Fe, Mn), whose contents become especially evident when no phosphorus nutrition is performed or the Ca components in missing from nitrogen fertilizers (Jelić, 1996).

*Table 1. Precipitation and mean air-temperatures (mean 1961-1990) in Nis (the data of the State Hydrometeorological Institute in Belgrade)*

| Precipitation and air-temperatures: long-term means 1961-1990 in Nis (Serbia) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan. | Feb. | Mar. | Apr. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Year |
| Precipitation (mm) | | | | | | | | | | | | | |
| | 41.3 | 40.3 | 45.3 | 51.3 | 66.7 | 69.7 | 43.6 | 43.3 | 43.6 | 34.1 | 56.8 | 53.6 | 590 |
| Mean (x) air-temperature (°C): m = minimal values; M = maximal values | | | | | | | | | | | | | |
| m | -3.5 | -1.3 | 1.8 | 6.1 | 10.4 | 13.4 | 14.5 | 14.4 | 11.1 | 6.5 | 2.4 | -1.4 | 6.2 |
| M | 3.8 | 7.1 | 12.3 | 18.0 | 22.9 | 25.9 | 28.0 | 28.5 | 24.8 | 18.9 | 11.7 | 5.4 | 17.3 |
| x | 0.2 | 2.9 | 7.0 | 12.0 | 16.6 | 19.6 | 21.2 | 21.4 | 18.0 | 12.7 | 7.0 | 2.0 | 11.7 |

The climate of Nisava area is moderate continental, with an average temperature of 11.7°C. July and August are the warmest months of the year, with the average of 21.3°C. The coldest month is January, averaging at 0.2°C. The average of the annual precipitation is 590mm (Table 1).

**Results and discussion**

In general, soils of Nisava area were less favorable properties regarding their plant available phosphorus in compare with their plant available potassium status. For example, even about 23% of the samples and the same level of covered areas sampled were very low supplied (less than 5.0 mg $P_2O_5$ 100g$^{-1}$) by phosphorus (Tables 2). By the addition of low supplied group, the situations with P status changed to 61.3% (very low + low P supplies). These categories of soils are needed to be fertilized more than the removed P by crops with the aim to improve their nutritional P status.

*Table 2. Plant available phosphorus (AL-method) ranges in level of municipalities of the Nisava area of SES region (sampling in the 2010-2014 period)*

| P ranges (AL-method) mg $P_2O_5$ 100 g$^{-1}$ | The municipality of Nisava district: town Nis (a), Svrljig (b), Gadzin Han (c), Razanj (d), Doljevac (e), Aleksinac (f) and Merosina (g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | e | f | g | Sum |
| | Percentage (%) of soil samples (N) | | | | | | | N |
| < 5.0 | 18.5 | 24.3 | 22.9 | 28.6 | 29.2 | 20.0 | 23.3 | 2847 |
| 5.1-12.0 | 32.1 | 43.8 | 48.0 | 34.0 | 38.5 | 36.2 | 37.3 | 4780 |
| 12.1-20.0 | 33.5 | 22.8 | 19.8 | 23.9 | 22.9 | 27.6 | 22.0 | 3232 |
| 20.1-30.0 | 6.8 | 5.2 | 5.8 | 7.4 | 4.4 | 5.7 | 8.0 | 726 |
| > 30 | 9.1 | 3.9 | 3.5 | 6.1 | 5.0 | 10.5 | 9.4 | 862 |
| Total (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| Total (N) | 2866 | 3089 | 1009 | 406 | 1939 | 2476 | 662 | 12447 |
| | Percentage (%) of covering area (ha) by soil sampling | | | | | | | ha |
| < 5.0 | 18.4 | 22.0 | 22.3 | 36.4 | 30.8 | 20.0 | 22.2 | 2065 |
| 5.1-12.0 | 32.0 | 44.6 | 48.7 | 31.1 | 36.2 | 35.0 | 40.0 | 3459 |
| 12.1-20.0 | 33.7 | 23.0 | 19.6 | 20.7 | 23.4 | 32.6 | 23.5 | 2430 |
| 20.1-30.0 | 7.2 | 5.9 | 5.4 | 5.9 | 4.7 | 6.6 | 7.8 | 554 |
| > 30 | 8.7 | 4.5 | 4.0 | 5.9 | 4.9 | 5.8 | 6.5 | 520 |
| Total (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| Total (ha) | 1767 | 2678 | 555 | 387 | 1417 | 1917 | 306 | 9027 |

Only 12.4% of soil samples (11.9% of area covered by sampling) were high and very high supplied with available phosphorus (>20mg $P_2O_5$ 100g$^{-1}$). Recommend fertilization by P of these categories of soils could be maximal up to range of their removals by crops.

Soil P status in individual municipalities of Nisava area are less or more specific in comparison with the regional level (Tables 2). With that regard, soils of Nis municipality were mainly more favorable P status, as 51% of samples were in the groups of very low and low P supplies. However, soil P status in Gadzin Han municipality was considerably less favorable because 71% of samples of this municipality was very low and low in plant available P. In addition, distribution of high and very high P ranges (>20mg $P_2O_5$ 100g$^{-1}$) were considerably different in soils of these two municipalities. For example, 15.9% of Nis municipality samples and 9.3% of G. Han samples were very low and low supplied with P. Milivojevic et al. (2012) tested plant available P in arable land of Sumadija province of Serbia. Majority of soil samples were very acid and acid reaction with low levels of plant available phosphorus (57% very low and 19% low P status). Petosic et al. (2003) tested P availability (AL-methods) in 480 soil profiles representing an area of 31227 ha of hydromorphic soils of Sava valley area in Croatia. P availability in the surface layer (0-30 cm) in about 30% of the tested agricultural land (9440 ha) was very low P (until 5 mg $P_2O_5$ 100g$^{-1}$ of soil), other 32% (9897 ha) was in the range low P availability (from 5.1 to 10 mg), while only 17% (5445 ha) has good or very good P availability (above 20 mg). Especially a high frequency of low P availability was found in vertic gley, amphygley and hypogley soils (total 8680 ha or 28% tested agricultural

land). By combination of lime, farmyard manure and NPK fertilizers applications during the long-term period, considerable increases of plant available P in acid soils (vertisol and pseudogley) in Central Serbia, were found (Jelic et al., 2006, 2011, 2013).

## Conclusions

Inadequate supplies of plant available phosphorus are considerable factor of yield limitation in Nisava area because about 60% tested samples were in levels of very low and low group of phosphorus availability. We presume that considerable increases of field crops yield is possible by adequate phosphorus fertilization. Banding fertilization of part of planned quantity of P fertilizers, for example together with sowing or interraw cultivation is our recommendation for improving P availability, especially on soil unprovided with phosphorus.

## Acknowledgement

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# Integration of the traits of life history "fitness" of the black bupreste in his environment

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Abstract: Each organism is characterized by a set of traits closely related to its reproduction and survival, called life history traits (size and age at sexual maturity, growth rate, mortality rate, longevity) (STEARNS, 1992). Thus to understand the adaptive strategy (distribution and abundance) of *C. tenebrionis*, it is necessary to know its history. The present study is conducted at the heart of the Tellian Atlas in the region of Médéa, a potential area for the production of rosaceae on prunus orchards heavily infested by *Capnodis tenebrionis*. Our investigations took place from the end of spring to the beginning of autumn of 2014, with the aim of understanding the possible relationship between the nutritive support and the biological performance of *C. tenebrionis*. This trophic relationship is estimated via the fitness of the *C. tenebrionis* individuals evolving in favor of the nutritive potential. The results were significant in terms of variability in the phytochemical quality of the two hosts studied, in particular for sugars and water-soluble proteins. The relationship between variation in metabolite composition and fitness of *C. tenebrionis* individuals has been examined and shows a trophic orientation strongly oriented towards the plum. These changes affect the development of weight and size of the xylophagus.

Keywords: Capnodis tenebrionis, fitness, phytochemistry, Prunus, history traits

## Introduction

In theory, evolution tends to select the organisms that present the best fitness, in order to optimize the selective value of its representatives, in order to optimize survival and optimize the fitness (ROFF, 2002). All organisms are confronted with the biotic and abiotic fluctuations of the environment (VUARIN et al., 2012). PHILLOGENE et al.(1984) report that the secondary compounds of the plant have various actions on the insect, so we took as fitness measure the body weight and body size of the black Bupreste *Capnodis tenebrionis* in reference to the nutrients allocated by its host rosaceous host plants that suffer severe damage following attack

## Materials and methods

Our study took place at the heart of the Tellian Atlas. Benchicao is located at 2 ° 51 'east longitude and 36 ° 12' north latitude, it has altitudes sometimes reaching 1200m, The EMBERGER index classifies the region in the subhumid bioclimatic stage with fresh winters, with rainfall Average of 600 to 700mm. In a place called HAOUCH CHANAS, samples of capnodis and transplant cortex were collected from *Prunus domestica* (Stanley variety, Amandier myrobolan) and *Prunus cerasus* (Bigarreaux burlat variety, Saint Lucie transplant) The experimental period from July 2014 to February 2014. On two randomly selected plots (EAS) among 6 nights of well-defined plots (Frontier, 1983). In order to ascertain whether the growth averages of the different fitness parameters, ie weight, body size and cephalic capsule, vary significantly according to plant metabolite concentrations, we have recommended an analysis of the variance of the global linear model (GLM). Followed by multiple regression analyzes that were performed using the SYSTAT 12 version (Hammer, 2001) to indicate the metabolites involved in the growth of the pest.

**Results**

*Effect of the nutritional quality of the larvae of history traits of C. tenebrionis in time*

The general linear model (G.L.M.) shows a highly significant evolutionary disparity between the various parameters (Figure 1A) and that the season and species parameter has a significant effect on the fitness evolution (Figures 1B and 1C).
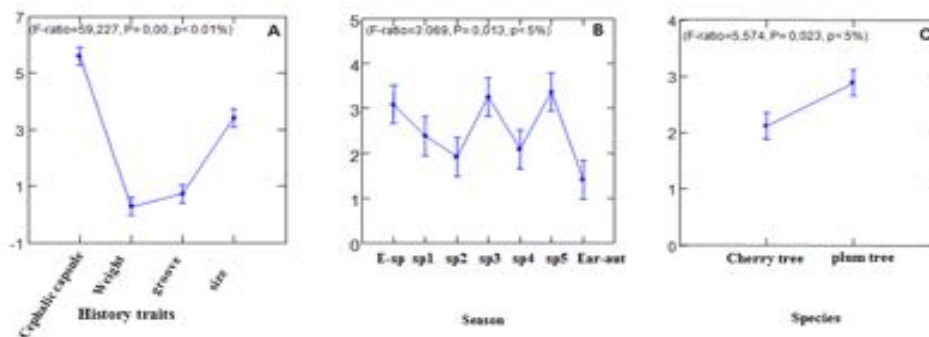


*Figure 1: Graphical representation by G.L.M. Of the evolution of the packaging of C. tenebrionis. E-sp=Early spring, Sp= Spring, Ear-aut= Early autumn.*

The chemical compounds produced by plants are probably the most important factor controlling the behavior of insects in nature. Similarly, the results of larval weight evolution (Figure 1) show a sucking profile of the larvae on Plum tree with the exception of mid August, which shows low weights by intake of their previous ones, assumed to be linked to completion Of the life cycle of the pest at that time on the host plant, and that the proclaimed weight is that of the first larval stages of the new generation. Unlike the Plum tree, the Cherry tree contains small measurements of weight supposed to be related to the poor phytochemical quality contained. Nonacs (2000) shows that when the internal state of the individual is not constant, the optimal behavior will not be the same at all times. For anybody, the increase in fitness associated with a change in a trait is often counterbalanced by a decrease in fitness associated with a change in another trait, at least one of which will be superior to others in an environment particular. This should be selected (Moiroux et al., 2012). In the same idea, it is interesting to note that the results obtained in the study of the analysis G.L.M. Carried out on the various fitness measures (fig. 1) and supplemented by multiple regression (fig. 3 and fig. 4) show a balanced growth between the different life history traits of *Capnodis tenebrionis* evolving on Plum tree with a significance for each conditioning parameter weight, body size and size of the cephalic capsule as opposed to the larvae on Cherry which evolve in a non-harmonious manner where no significance was noted between the different parameters of the conditioning

*Key parameters influencing evolution of life history traits of C. tenebrionis larvae*

The different fitness measures obtained from larvae *C. tenebrionis*, there is a multiple regression of the metabolite concentrations of the two *Prunus* host species.

Table 1: Multiple regressions applied to changes in weight and size as a function of secondary metabolites on plum tree by contribution to carbohydrates and water-soluble proteins.

| size | Coeff. | Std.err. | t | p | $R^2$ |
|---|---|---|---|---|---|
| Constant | 5,5539 | 2,1458 | 2,5883 | 0,060796 | 0 |
| carbohydrate | 0,79532 | 0,31103 | 2,557 | 0,062835 | 0,012267 |
| Water-soluble Protein | -86,955 | 18,747 | -4,6383 | 0,0097468 | 0,59201 |

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The evaluation of weight and size development in relation to secondary metabolites (Figure 2) reveals very marked differences between the observed values and the predicted maximum values.
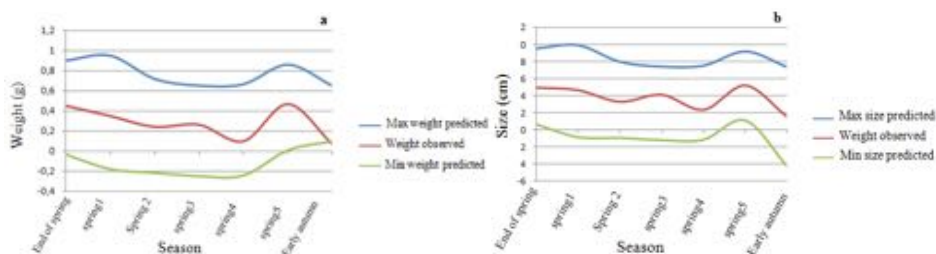


Figure 2: Amplitude of weight growth (a) and size (b) of larvae of C. tenebrionis on plum tree by intake of sugars and water-soluble proteins

Table 2: Multiple regressions applied to changes in weight and size as a function of secondary metabolites on Cherry tree by contribution to proline and proteins

| weight | Coeff. | Std.err. | t | p | $R^2$ |
|---|---|---|---|---|---|
| Constant | 0,92549 | 0,21306 | 4,3437 | 0,012217 | 0 |
| Proline | -3,1027 | 1,2161 | -2,5514 | 0,063213 | 5,6206E-05 |
| Water-soluble protein | -2,3396 | 0,69452 | -3,3686 | 0,02808 | 0,31526 |

| size | Coeff. | Std.err. | t | p | $R^2$ |
|---|---|---|---|---|---|
| Constant | 16,168 | 2,9403 | 5,4987 | 0,011837 | 0 |
| Proline | -33,136 | 9,3634 | -3,5389 | 0,038393 | 0,13886 |
| Soluble protein | -33,54 | 10,429 | -3,2159 | 0,048735 | 0,23575 |
| Water-soluble protein | -43,712 | 8,272 | -5,2844 | 0,013218 | 0,59182 |

The multiple regressions applied for the phytochemical constituents of the two *Prunus* (Plum tree, Cherry tree) and the curves obtained revealed that in Plum tree, the weight evolution and the growth of its size related to the accumulation of sugars and proteins Water soluble. These results are in agreement with Rhoades (1976), who consider that the

favorable conditions for the development of certain phytophages are created during sugar initiation, which is followed by depression of protein levels and accumulation of protein, amino acids. Similar observations of Bidon (1993), confirm that the low sugar level increases susceptibility to some parasites. Sugar is the main source of energy for insects. On the other hand, some species are capable of completely replacing carbohydrates with lipids or proteins (Guessous, 1948); hence the positive correlation with the water-soluble proteins. The work carried out by Bezzala (2005) indicates that protein synthesis is closely linked to the metabolism of sugars during the respiration process from the Krebs cycle, which gives the carbon skeleton for the synthesis of proline. However, in Cherry tree, weight evolution and size growth are related to the accumulation of proline and soluble and water-soluble protein, according to Ramanjulu and Sudhakar (1997) in Bezzala (2005). At the cellular level, increase in the concentration of amino acids, especially proline, has been observed in several species of plants subjected to a water stress, these words confirm the remarks of Martinez (2008), who considers that the capnode is a pest of weakness, and develops only on trees weakened by diseases or by a bad physiological state of the attacked subject.

It is essential to relate these life history traits to each other to highlight the evolutionary trade-offs that may link them (VUARIN et al., 2012). According to ROFF (2002) compromise appears when the increase in the value of one trait occurs to the detriment of another trait. This is also confirmed by VUARIN et al. (2012) who finds the traits of life history not being independent of each other.

The prediction of deviations over time over plum tree shows maximum growth in late July and early September in relation to size and cephalic capsule growth (Figure 14), due to a maximum accumulation of reserves at the last larval stage, enabling a better transition to the pupal stage. Similarly, predictions of size differences over time in relation to the cephalic capsule and weight show homogenization and near growth intervals (Figure 16). The larval growth on cherry tree does not present a large difference between the maximum and minimum values of the size, but they know very small measurements at the beginning to be connected to the dormant early entry of the Cherry tree comparing to the Plum tree, which can be reflected. On the course of the life cycle of Capnodis tenebrionis.

## Conclusions

As a sum of all results, it can be argued that developmental variability is strongly related to the phytochemical quality of host plants, whose metabolites are the main tools of plant-living co evolution that applies to all levels of organism pest, and which is expressed at all stages of the development of the plant

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# Reviews & project communications

16th Alps-Adria Workshop

Synergism in science

# Cultural and economic aspects of the Roman limes route in Hungary

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Abstract: The frontier of Pannonia, which stretched from Klosterneuburg to Belgrade, was one of the most important borderlines of the Empire. This is proved by the strong military power concentrated in Pannonia from the second half of the 1st century onwards. A 400-km-long section of it lies on the territory of present day Hungary. There is a large archaeological area in Budapest (Aquincum), with quite a few monuments, such as parts of the legionary fortress, the military amphitheatre and the whole quarter of a preserved civil town (Figure 1.). The watch-towers are – especially from the late Roman period and around the Danube Bend – at a distance of 1 to 2 km apart from each other (ripapannonica.hu). During the first century AD some auxiliary forces were stationed along the main routes leading across the country to the fortifications at the Danube bank (Moschek W. 2010).

Keywords: Limes, the frontier of Pannonia, world heritage, tourism

## Introduction

The Roman Empire was surrounded by the limes which had three components, the first was the fortification and the hedge fence, in more modern form, the stonewall, the second was the ditch, and the third was the watch tower (Fields N., Dennis P. 2008).

The word, 'limes' originally meant path. The border regions were meshed by roads, especially military roads which were connected to Rome, but sometimes they reached some territories of the enemies as well. The artificial hills and barriers were combined with some natural obstacles, like rivers too. The best example for this is the Hungarian border area which was by the line of River Danube (Visy Zs. 1988).

In the beginning the limes meant to be military paths with some military facilities. Its priority task was to help the soldiers to get to enemy areas, and to insure their successful actions. Defending it got harder and harder in the period of early migrations as more and more warrior tribes wanted to find their shelters inside the borders of the empire. The limes stretched along 382 kilometres from Germania, from River Rhein to River Danube. On the Scotland-England border they built up the 128 km long Hadrianus Wall (now a world heritage site) (Fields N. 2008).

The fortresses from the Roman Ages were also army barracks and administrative centres. The soldiers served at the commands, buildings, workshops and guard duties, on the other hand they could use the spas for their convenience. It was especially true at the camps of legions where the soldiers were Roman citizens and their command was a noble from the Order of the Senators, whereas his utilities was the youth of the order of the knighthood (Klee M. 2006). The fortresses had two main streets and where they crossed there was a forum of the camps. The camp sanctuary, the administrative rooms, the warehouses and the hall of assembly were all located here. Along the main street there were houses of the officers, the bath, warehouses for the grains and behind them the hospital, workshops and barracks. The stone houses were covered with tiles, and people could walk around them on paths made by stone. The defensive walls of the huge fortresses were followed by an artificial hill and a ditch (Hungarian Limes Association 2014).

Along the right side of the Danube, the limes was perfect for transportation even in the Middle Ages, because of its thick stoned grounding and pebble cover. The distances from the legion camps were singed by labelled milestones. From the first century only a few ruins of the wooden watch towers got well-known. The second century's famous memories are the construction signs of the watch towers. Around all watch towers there were ditches, and by the sides of the ditches there were fixed fences (Visy Zs. 2003).

**Materials and methods**

We made a questionnaire and were researching in the Contraaquincum, at the Március 15. tér, in Budapest. This is the place where the Duna Limes Visitors Centers will be built. The square is not big, but it was renovated 2 years ago, and tokens got very important roles. We asked a lot of people from different ranges. One of the main topic was the limes. Firstly, I wanted to know, what do people know about that. Otherwise do they know where are these remains of the limes and whether they visit them. The other main topic was connected to the world heritage sites. What do people think about this and how we can support them?

We asked 120 people. The conclusion: more than half of the people do not know the limes. Some people tried to say something or bluffed, but when they saw my stunned face, they said they do not know. After that, the half of people could answer for the question of „Where you find the remains of the limes?" Somebody could set out Aquincum, Pécs and Szombathely. They were middle-aged or university students. Persons, who know something about the limes, already visited those (Figure 3.).

The most popular place is the Aqiuncum Museum, because it is situated in Budapest, so it is close to their home; and the Early Christian Burial Sites in Pécs, because nowadays it is really popular (Figure 4.).

In conclusion, it is due to the rank of World Heritage and title of European Capital. However, I met such a respondent who took part in the Dunaszekcső's Camp two years ago, and he – according to his telling – got a detailed insight to the stories of Romans. During my questions, I mentioned separately the Savaria Carnival because in my opinion, this is the most known event connected to the Roman Era. This event was very well-known, I did not have any respondent, who had never heard of it. One third of my respondents went to see this Carnival – they were mainly middle-aged people.

People supported the idea of Duna Limes being a World Heritage site and they think this entry is a good idea. 10 people said that our country would do better if we care about more important things. More optimistic people believe in this and they see the possibilities in it to popularize our country and attract foreign people. The mentioned history teacher was who mostly showed and explained his opinion about this topic. He said, it would be necessary to have a lot of support for the development, which is essential to be worthy to be ranked.

Consequently, I suppose my hypothesis is true. Most of the people do not know exactly the definition of limes. People who are interested in the culture and history, they look up the vaults in the Necropolis, the Savaria Carnival, or the Museum of Aquincum (Figure 1.). From my point of view, most of the people would not visit a town only to see the relics of the limes. It would be advisable for those settlements, who would like to show their

relics of roman era, to cooperate with other attractions or features. If we look back to the mentioned settlements, there is minimum one other feature everywhere, because of tourist, who would like to visit that place. The success of Aquincum is due to that this place is located in Budapest. There are several tourist attractions in Pécs, besides the relics, there are crypts as well. There is a camp in Dunaszekcső, while in Szombathely a colourful carnival takes place. These are good examples for the future. If they care more about the marketing plans, some other places might also become popular.



*Figure 1. The old museum building inside the archaeological park at Aquincum (Budapest) is surrounded by the ruins. (Remenyik B.-Szabó L.-Tóth G. 2014)*

**Results and discussion**

Winning the Word Heritage title would make a huge difference in the life of the Danube Valley, it would create a thematic network with a united (yet individual) attraction site. The Roman Lines (the section from The Netherlands to Hungary) would become the biggest and the longest site by the Word Heritage of UNESCO in 2018. (Figure 2.)

This would also show an example for the Word Heritage river side businesses to create a common brand and promote unified marketing activities. This is the only river side nomination out of the Word Heritage nominations' list, which insures an advantage for the settlement around the Danube, but the good operation of this network also depends on the readiness and willingness of the settlements.

For this reason our goal was to design a unified network along the Danube which shows the unification of the attraction sites. The long-term planning of tourism concept of the Danube Limes.

The approval of the Danube Limes to the Word Heritage Program would mean a long-term planning of the tourism concept and the connecting projects. The planning of the cultural

activities has introduced possible theatre locations as well. The theatres were suggested to perform ancient-themed plays. We have dealt with tourism networking of the nominated and already accepted heritage sites at those sections, which would introduce the World Heritages along the Danube.
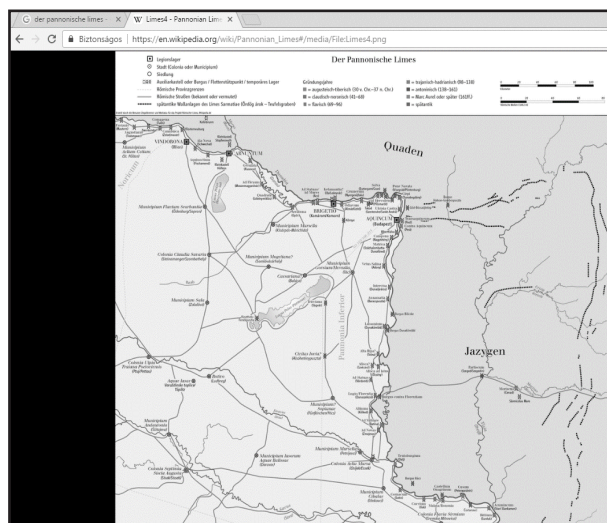


*Figure 2. Map of the Roman frontier in the Middle Danube area. http://mek.oszk.hu/02100/02185/html/1324.html*

During the archaeological demonstration of Roman Limes we divided the Danube to thematic design sections (upper part of the Danube-Early Empire, Danube Bend-Late Empire, under part of the Danube-Golden Age of Pannonia), we attach story matrixes to them and mention the Hungarian heritage parks, which banter to Danube and Roman Empire. The designers agreed about introducing two emphasized Roman locations, as the giant attraction to the tourists, the fortress of Brigetio at Komárom and the fortress of Alisca in Őcsény. The syllabus of active tourism is divided to smaller chapters, the first of these is the bicycle tourism, where the section of EuroVelo bike path and the future World Heritage sites were liaised. At the equestrian tourism there are serious equestrian developments, which are already accomplished along the Danube, but some of these attraction components do not work together, therefore we made a suggestion about the configuration of an EuroHorse 5 route along Danube and renewal of the Kincsem – National Equestrian Programme. As part of the water tourism to help the Kayak-Canoe Association's work, we made a suggestion to build a harbour. With the Hiking Association we drew a route for hike-loving tourists.

At the industrial tourism we would turn the industrial cities' latest developments along the Danube to a thematic route (from AUDI to nuclear power station). At the part of medical tourism, we analysed the baths in the Danube area. At the chapter of gastro tourism, besides the fisherman's soup, the wine regions in the Danube area and Hungaricums were represented. At the part of castles, the castle buildings, which banter to Danube, were thematically introduced. At the National Parks we took the matching and the variant points, and we made the programme suggestions about how we can accept the extent visitors as a result of World Heritage title. At the pilgrimages, we analysed the spots of the different religions, we made a suggestion to build the unitary cardinal's, bishop's, abbot's

pilgrimage. Towards the Danube regions' development, we thought there is a need of creating the Budapest-Duna card.

**Conclusions**

In conclusion, the Duna Limes World Heritage Project is completed and sent to UNESCO and ICOMOS to assess it. The two organizations asked in their reply to get the tender again in 2018, together with the Dutch-German-Austrian-Slovakian-Hungarian participants. We hope that they will judge it positively – because they promised so. Hungary also has another roman heritage, which increased the tourist attendance of the city significantly. The Early Christian Necropolis of Pécs became World Heritage Site of UNESCO in 2000, which was both good for the crypts, and for the city. These crypts came from the Roman Era. Heritage Committee explained, they accepted such cultural historical treasures on the list, because they represent the ancient building structure and the early Christian burial architecture mixed with the arts of the North and West Roman provinces, through the architecture and wall-paintings. The underground crypts and relic chapels show the endurance and faith of the late Roman European Christian communities and also present the eternal roots of civilization and culture which still has effects for nowadays (Jilek S. 2006).

If the UNESCO Heritage title could be awarded to the limes, there would be an economic advantage:

- increment the number of visitors, the durations of stay, the capacity's usage and the purchasing's power,

- developing the attractions, visitors centres and accommodation's,

- building the transport network,

- built up the Danube destination and the Danube industrial area.

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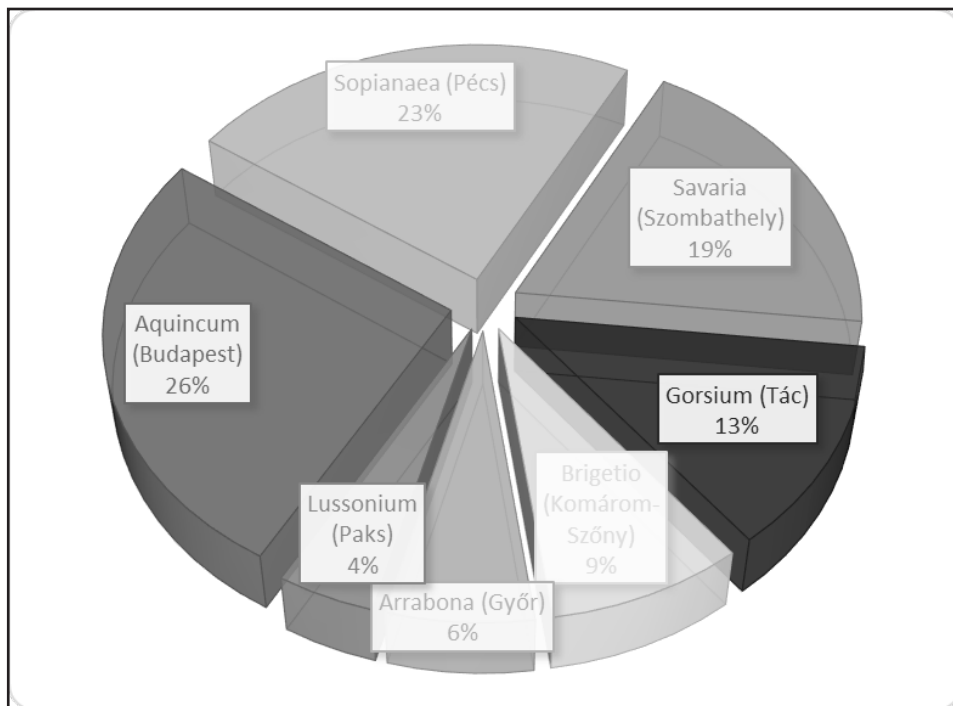
Figure 3. Those who knew something about the limes, already visited it.
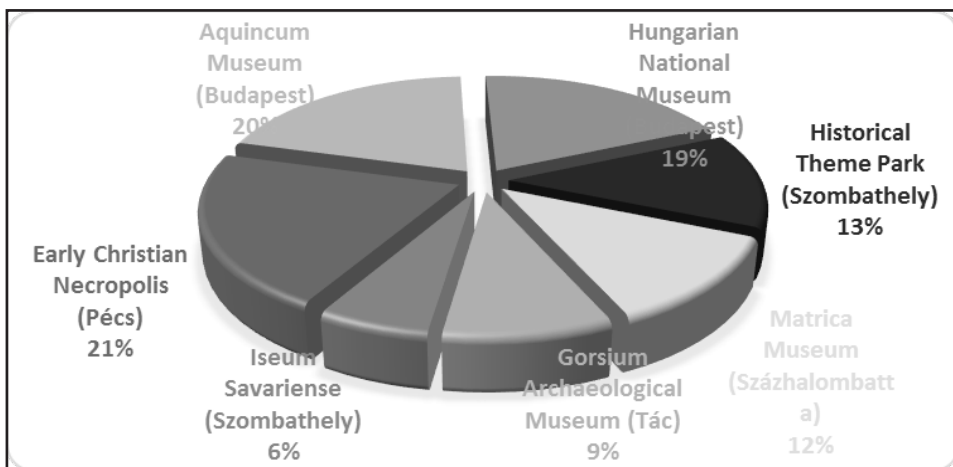


Figure 4. Known Roman museums in Hungary

# Agronomic impacts on the performance of active ingredients of hemp (*Cannabis sativa* L) plant

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Abstract: The presentation comprises a literature review in relation with the application impacts on the cannabidiol content of fibre hemp plants. Since cannabidiol is a highly valuable natural compound used for various purposes from phamaceutical fields to direct chemical applications, there is a need to get acquainted with the agronomic conditions of its crop physiology.

Keywords: Cannabis sativa, cannabidiol (CBD), hemp active ingredients, agronomic impacts, non-psychoactive cannabinoids,

Hemp is the most important of our fibre crops under temperate climate, but the purpose of its cultivation extended in the last few years thanks to its highly valuable natural compounds.

The origin of hemp is in Central Asia. From there the plant came to north and central Europe in the 7th century B. C. but through several ways. It is said that hemp was brought by the Scythians to the regions north of the Black Sea and the mouth of the Danube by the direction via Russia. Hemp has been known since about 450 B. C. in the Mediterranean countries, where it is also spread by way of Asia Minor. (Bredemann et al. 1956)

The oldest information about the plant, its production of bast-fibers from the stem has been known to the Chinese from about 2800 B. C. The seeds were used for human consumption just later. The medical purposes have been discovered in India, where the plant was cultivated for since 900-800 B. C. as a source of a drug. In North-Africa since the mediaeval has been cultivated exclusively for hashish. (Bredemann et al. 1956)

The main varieties performed under varied climatic conditions and developed as a result of natural selection. It was mostly influenced by very different lengths of day and vegetation period. Although hemp is a very old cultivated crop in great part of Europe, it could be described as a wild plant cultivated by man, rather than as a genuine, cultivated crop. (Bredemann et al. 1956)

Thanks to that the varieties were grown for a long time in lack of systematic breeding work it has a number of unfavourable characteristics of wild plants until the breeding started in Hungary in the 1950's. (Bredemann et al. 1956) One chemical constituents of Cannabis was undesirable used as a fibre crop.

Up to 2005 489 natural constituents of *Cannabis sativa* L. have been identified (Rishi 2011), of which 70 are cannabinoids, the others are terpenes, hydrocarbons, sugars, proteins, enzymes, flavonoids, vitamins, pigments, elements (Gambaro 2002). Among the 70 known cannabis cannabinoids, the most important in terms of pharmacological relevance are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN).

THC, the primary psychoactive component of cannabis exists in two isomeric forms known as $\Delta 9$-THC and $\Delta 8$-THC depending upon the position of the double bond in the

C-ring (Holler et al. 2008), and its structure was elucidated by Mechoulam, et al. in 1964. According to Mehmedic (2010) at least 100 different cannabinoids have been described.

Cannabidiol was isolated by Adams et al. (1940), from freshly harvested North American hemp. It is a dihydrophenol, which is not the active principle of cannabis either (Beam 1911).

According to Grlic (1962) CBD constitutes approximately 40% of the cannabinoids in cannabis organic extracts. Cannabinoids with lower importance are cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabielsoin (CBE), cannabinodiol (CBND), cannabitriol (CBT) and other miscellaneous compounds (Rishi 2011).

The most important parts of the plant in terms of the production of the active ingredients are the leaves and bract covered by glandular hair. It is the location where the $\Delta^9$-tetrahydrocannabinol with other cannabinoids and terpenes are synthetizing (Hammond & Mahlberg, 1977).

Nowadays there is a need on the field of medicine, cosmetics and healing for some of the cannabinoids produced by Cannabis plant. In many countries of the World - also in Hungary - nether the recreational use, nor the medical marijuana usage is not allowed due to the psychoactive effect of the THC. Therefore the Hungarian hemp breeders strive to increase the amount of CBD among the valuable, non-psychoactive cannabinoid in the new Hungarian industrial hemp varieties. In parallel the amount of THC may not rise, moreover continuously stay under 0,2% (Implementing Regulation of the Commission 809/2014/EU). With the efficiency of the THC-reductional breeding in the 1960's carried out in Hungary, the other cannabinoids like CBD, CBDA, CBN, CBG also decreased and now should be increased again as required. Bredemann et al. were reporting in 1956, that hashish is the resin produced by the glandular hairs of the female inflorescence, and this resin contains at least three chemically-related substances: cannabinol (CBN), tetra-hydrocannabinol (THC), and cannabidiol (CBD). "Of these, cannabidiol found in the greatest quantity, has no narcotic effect; cannabinol is weak or inactive; but tetrahydrocannabinol, a derivative of cannabidiol, is the principal narcotic agent (Todd 1940, 1942, Bergel et al. 1938, Adams et al 1940a, Adams et al. 1940b, Bose & Muckerji 1943, Wollner et al. 1942)."

Bredemann et al. in 1956 were also reporting about problems of modern hemp breeding, and the need of hemp varieties containing little or no hashish: the elimination or reduction of the hashish content, which has remained the same in the cultivated as in the wild varieties. Along with many others they name the problem of high cannabinoid content causing an essential breeding goal because even the fibre hemps can produce so much hashish on their female inflorescence that they can be misused for producing narcotics.

They suggested that it must be possible to eliminate these undesirable narcotics by breeding. The concept was to look first for mutants which lack only one hashish component and the three different substances that hashish contains has to be followed in breeding. Judging by experience with other cultivated plants, mutants which lack only one of the hashish components can be found more frequently than mutants or combinations which lack all three. After breeding varieties of the Indian hemp plant which have very little or no hashish, the next step towards a non-psychoactive variety is to produce strains which

lack all three by cross-breeding these varieties (Bredemann, G. 1956). *Cannabis indica* is rather used with purpose of the active ingredients than the other species caused by their higher cannabinoid content, but on the hemp seed market is also available some variety containing C. sativa and C. ruderalis strains. Defining taxonomic categories of the genus cannabis is still controversial.

According to Soó (1953), Simon (1992) and Borhidi (1998) the *Cannabis* fall under/ belongs to the phylum of Angiospermae, the class of Dicotyledones, the order of Rosales, and the family of Cannabinaceae.  Besides the *Cannabis* L. genus only the hops (*Humulus* L.) genus belongs to this family.Previously hemp and hops were both classified into the *Moraceae* or *Urticaceae* family (Jávorka 1925).

Today it is generally accepted that hemp and hops together forms a separate family, the *Cannabaceae = Cannabinaceae*. The two related genera, hemp (*Cannabis* L.) and hops (*Humulus* L.) belongs to this family. According to this conception the genus/tribes has not races; only one species named by Linnaeus *Cannabis sativa*, which is divided into multiple varietas (Small 1975, Small & Cronquist 1976, 1999 Ranalli, Iványiné 2005):

- *Cannabis sativa* var. *vulgaris* (common, cultivated hemp)
- *Cannabis sativa* var. *indica* Lam. (Indian hemp)
- *Cannabis sativa* var. *indica* Lam. subvar. *gigantea* (giant hemp)
- *Cannabis sativa* var. *Ruderalis Janisch* (the so-called weed hemp).

According to the opinion of Szizov and Szerebrjakov, there are two species: *Cannabis indica* and *Cannabis sativa*. Within the *Cannabis sativa* species there are two subspecies, *Cannabis sativa* L. Szerebr. subsp. *spontanea* and *Cannabis sativa* L. Szerebr. subsp. *culta*, which are accepted in cultivation.(Mándy & Bócsa 1962)

The majority of the handbooks in Hungary also describe the spontaneous growing hemp as a separate subspecies (*C. sativa* ssp. *spontanea*), and definitely distinguish from the hemp shape that got mad and grow naturalized, which is also classified as subspecies taxa (*C. sativa* ssp. *sativa*) cit in Benécsné (2003). Due to the geographical environment emerged different geographical races: Northern hemp, Central Russian Hemp, Southern (Mediterranean Hemp and Asiatic Hemp race (Láng, 1976). There are significant morphological and physiological differences between geographical races, but despite this, it cannot be broken down into further taxonomic categories, because there is no genetic barriers between the most diverse forms; the dwarf-growing northern hemp and the non-ripening East Asian hemp both have 2n = 20 chromosomes. (Bócsa 2004).

The diversity at the hemp taxonomy can be caused by the genetic and agronomic background as well. Very important questions remain regarding field-scale systems to produce cannabinoids. An optimal agronomical proposal for cannabinoid production at *Cannabis sativa* has not been defined. These should include variety collection, nutrition, soil preparation, sowing, plant care, harvest and crop management of the industrial plant in terms of the newly emerged demand for the cannabinoids. The variety collection in Hungary includes varieties and hybrids exclusively from the *Cannabis sativa* species. All of the Hungarian varieties and hybrids, used for fiber, energy plant, or crop are proper for cannabinoid production as well. The amount of the two most important cannabinoids, the Δ9-tetrahydrocannabinol level is between 0,1% and 0,2%, but definitely under 0,2%, the

cannabidiol is about 1-3% depending on the variety.(http1) The maximum amount of Δ9-tetrahydrocannabinol is regulated by law, and growing any species with higher content of the psychoactive ingredient is forbidden.

Coffman and Gentner (1975) declare that Turkish fibre variant of *Cannabis sativa* did not change cannabinoid profiles when the variants were grown in different environments; but also say, that nitrogen content in vegetative parts of the plant has been thought to correlate positively with its THC content. An experiment was performed in Hungary using Kompolti Hibrid TC variety to get knowledge about the effect of nitrogen fertilization on THC content in hemp leaves. The fertilizer treatments were 150, 450 and 600 ppm N, from $NH_4NO_3$. These experiments show that the THC content of leaves decreases with increasing N doses. The decrease was significant in the case of the highest N dose. There was a significant increase in fresh weight of shoot (80-130%) and plant height (28-39%) due to N supplementation.

This phenomenon is favourable for agricultural production, because nitrogen fertilization will increase stem yield and simultaneously decrease THC content of the plant significantly. (Bócsa, I., P. Máthé, and L. Hangyel 1997) According to the results of further research, the amount of THC and CBD are increasing simultaneously during the ripening (Kempf 2015), so the predictable decrease of both of cannabinoids occurred by high dose N fertilization is not preferred in terms of CBD-usage. As the N fertilization is in a negative correlation with the THC content; it seems to be possible, that higher N amount is also in a negative correlation with CBD. It could be a further aim of our field-experiments. By high density the crops generates rare, unbranched population, at lower density the hemp evolves thick, branched, coarse stems.Agromag Ltd, the biggest hemp propagating company in Hungary offers a 12, 24 or 70 cm row spacing for the Hungarian species depending on the breeding purpose. (Agromag 2015)According to Agócs et al. (1962) the seed yield in rare position will be big thanks to the richly branching: the optimal field size for a seed purpose plant is 0,35-0,49 m² in average of different years. It is practically 50-70 cm plant distance by 70 cm row spacing. However, sowing rates and plant size has a connection, it is unknown if increased production of female flowers as would be expected with decreased plant densities would result in increased yields of CBD, thanks to stress from competition.

- Bill Drake (1986) proposes by transplanting in rows the Southwest-Northeast or Southeast-Northwest row orientation, rather than the North-South or East-West. Because of blocking some of the sun, in the north, where the sun's rays fall slanting upon the earth, it can mean a significant reduction in vigour and therefore in crop yield at harvest.
- The same handbook mention pruning for mass protection, as the most common form to force side growth. Cutting off the head between the fifth and sixth sets of branches cause multiple shoots to develop, one on each side on each node. Due to its outstanding ability to suppress weeds during its vegetation period, the thick-sown hemp requires no herbicides during that time. Weeds are eradicated during soil preparation for sowing. In row separation the plants can not completely overshadow the soil, and may require the use of herbicides or hoeing. (Bócsa I., Karus, M. 1997, Bócsa I. 1996)
- The technical length of the bust fibre is a value measure property in the fiber industry, the branched stem is even not advantageous by dual usage: utilize the fiber and the

seed or fibre and flowering terminal branches. (Agócs P. 1962; Finta Z. 2012)

- According to Geoffrey (2004), the contents of the key constituents of Cannabis sativa L., cannabinoids, terpenoids and flavanoids varies depending on the plant genetics, time of harvest and drying conditions.

- The time of harvesting has a high importance on CBD content according to our latest research connected with the varieties. (Kempf 2015) The early ripening dioecious candidate variety used for seed yield did not show a statistically proven difference in cannabinoid content between the flower and the bract around the seed after ripening. The result of the other, late ripening monoecious variety attended in the experiment differs: the CBD content measured in the bract was higher, than the CBD content in the flower samples. It shows that the amount of this non psychoactive cannabinoid is rising during the ripening in the late ripening monoecious variety. These characteristics are in connection with the harvest time: by this variety the harvesting – also for cannabinoid usage - is recommend after the full ripening of the seed.

The time of harvesting has also significance such as the harvested part of the plant. An experiment by Bócsa et al. (1997) shows, that the amount of THC was significantly different in leaves from various plant regions: leaves of the plant axis, the side branches and the plant top. THC was highest in leaves near the shoot tip and on side branches, and lowest in oldest leaves. (Bócsa, I., P. Máthé, and L. Hangyel 1997). The cannabinoids are in acidic form in the plant part. The decarboxylation, as a postharvest treatment is essential for transforming the acidic form into a neutral form. Carboxylation can be carried out by heating or burning the leaves. The duration and temperature of treatment may indicate different efficiency: the higher, 137 oC / 1 hour treatment is more efficient than the 45 $^{o}$C / 24 hour treatment (Kempf 2015).

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# Support of implementation process of EU directive 2007/60/EC on the assessment and management of flood risks in Georgia

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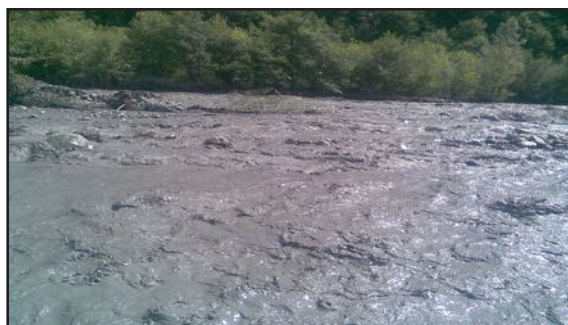
Abstract:The Directive 2007/60/EC on the assessment and management of flood risks entered into force on 26 November 2007. The government of Georgia has been declared for a long time orientation to European Union. This applies also to water sector. In spite of significant progress in this field there is still a challenge to improve legal, personal and technical security, especially in the sphere of flood protection as a part of crisis management. Georgia suffers from floods, landslides and mud every year. In the period 2003 – 2009, damages reached the value of more than 250,000 USD. It is expected that implementation of the EU Directive 2007/60/EC will enable accurate identification of threatened areas as well as adoption of necessary measures, especially development and issuing of accurate hydrological forecasts and warnings. This issue was solved in the framework of the project "Support of support of implementation process of the Directive 2007/60/EC on the assessment and management of flood risks in Georgia" financed from the program SlovakAid. The implementing organisation was the Water Research Institute Bratislava and partner organisation was National Environmental Agency in Tbilisi, Georgia.

Keywords: flood protection, Georgia, EU Directive 2007/60/EC, project

## Introduction

The Directive 2007/60/EC on the assessment and management of flood risks entered into force on 26 November 2007 (Gajdová, 2009). The government of Georgia has been declared for a long time orientation to European Union. This applies also to water sector. In spite of significant progress in this field there is still a challenge to improve legal, personal and technical security, especially in the sphere of flood protection as a part of crisis management. Georgia suffers from floods, landslides and mud every year. In the period 2003 – 2009, damages reached the value of more than 250,000 USD. It is expected that implementation of the EU Directive 2007/60/EC will enable accurate identification of threatened areas as well as adoption of necessary measures, especially development and issuing of accurate hydrological forecasts and warnings.

This requires data from surface water quantity monitoring. There were only 23 operating hydrological stations in Georgia (there are about 430 in Slovakia). Besides review of main legislation it was necessary to formulate a general framework of the Directive 2007/60/EC implementation, get know areas with flood risk and determine conditions for operation of flood forecast service in Georgia.



*Figure 1. Flood on river Kura in March 2010 – mixture of water and mud (NEA, 2010)*

**Material and method**

The issue was solved in the framework of the project "Support of implementation process of the Directive 2007/60/EC on the assessment and management of flood risks in Georgia" financed from the program SlovakAid. The implementing organisation was the Water Research Institute Bratislava and partner organisation was National Environmental Agency in Tbilisi, Georgia.

The project provided following outputs:

1. Elaboration of mechanism for harmonisation and implementation of the Directive 2007/60/EC into Georgia legislation;

2. Elaboration of methodology for development of flood risk maps and supplementing the surface water quantity monitoring according to requirements of flood protection in Georgia in a pilot river basin Alazani;

The Output 1 included activities

1.1 Detail analysis of present situation and specification of needs in the framework of implementation of the Directive 2007/60/EC on the assessment and management of flood risks;

1.2 Elaboration of Roadmap for harmonisation and implementation of the Directive 2007/60/EC in Georgia legislation;

1.3 Elaboration of Draft edict determining details on operation of flood forecast service in Georgia;

The Output 2 included activities

2.1 Elaboration of methodology for development of flood risk maps;

2.2 Elaboration of Proposal of measures against floods and GIS maps of flood risk for river basin Alazani;

2.3 Application of hydrological model in pilot area in Georgia for flood protection purposes

2.4 Purchase and installation of automatic hydrological stations in the river basin Alazani in Georgia;

2.5 Study tour experts from partner institutions in Slovakia

**Results and discussion**

Ad 1.1
There was elaborated a detail analysis of state of implementation of the Directive 2007/60/EC on the assessment and management of flood risks into Georgia legislation. The matter was especially evaluation of existing legislation. Also results and knowledge from

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previous projects on implementation of EU directives in Georgia were taken into account. We cooperated intensively with institutions that are responsible for settling extraordinary situations at present, especially with the Ministry of Environment and Natural Resources Protection in Georgia. Since Slovak experts have been cooperated Georgian partners (especially from Department of Hydrometeorology of the National Environmental Agency for several years the cooperation smoothly proceeded. Results of analysis and specification of needs for implementation of the Directive 2007/60/EC were processed in the form of report (Technical report 1). Those results also provided background for activities 1.2 and 1.3.

Ad 1.2

The result of this activity is the „Roadmap for harmonisation and implementation of the Directive 2007/60/EC on the assessment and management of flood risks into Georgia legislation". This document will help the Ministry of Environment and Natural Resources Protection and other relevant institutions to secure flood protection and settle extraordinary situations in Georgia. It will also help the National Environmental Agency in gradual approximation and integration with EU standards. Experience of Slovak experts both from implementation of the Directive 2007/60/EC in Slovakia and from international projects were fully used (Bacik et al, 2006, Water Research Institute Bratislava 2012, Collection of Acts, 2010).

Ad 1.3

The result of this activity is the „Draft edict determining details of operation of flood forecast service in Georgia". The document especially comes out from needs of Georgia. Specifics of region, tradition in settling of extraordinary situations, and experience from Slovakia and other European countries were taken into account. There was also described form and way of developing and issuing of forecasts and warnings and form of communication with relevant institutions on settling of extraordinary situations including flood protection.

Ad 2.1

The result of this activity was „Methodology for development of flood risk maps for Georgia conditions". A detail recherché of existing approaches in the European Union, especially in countries with landscape relief similar to the Georgian preceded the methodology itself. The methodology is a part of Technical Report 2.

Ad 2.2

Based on the methodology mentioned in the activity 2.1 there were developed „Draft measures for flood protection" and „GIS maps of flood risk" for the pilot river basin Alazani. The Draft measures and GIS maps will serve institutions settling extraordinary situations and also regional and local administration in planning of activities in the area. Maps could be later used in insurance process which is not working properly in Georgia at present. Results of this activity are included into Technical Report 2.

Ad 2.3

Hydrological models are used by hydrological services for various purposes including proper location of surface water bodies monitoring network, water balance, various

scenarios for changes of surface discharge, and development of hydrological forecasts and warnings. Therefore there was done calibration and verification of the hydrological model HRON in the Alazani river basin (Marsalek et al. 2006, Hazlinger et. al, 2011). The activity included also the training for National Environmental Agency workers.

Ad 2.4

In the framework of this activity there was made a detail field survey in the river basin Alazani. Then there were selected localities for installation of 2 automatic hydrological



Figure 2. Field survey, Alazani river
(project documentation)



Figure 3. Field survey, Alazani river
(project documentation)



Figure 4. Field survey, Alazani river
(project documentation)



Figure 5. Location of hydrological station, Alazani river
(project documentation)
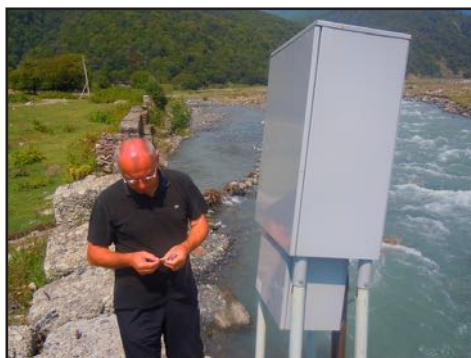


Figire6. Installed hydrological station on Alazani river and Project Coordinator Dr. Roncak
(project documentation)

Ad 2.5

Five experts from NEA Georgia (project partner) visited Slovakia in the period May 19 – May 26, 2013. They were introduced the legal framework of implementation of the Directive 2007/60/EC in Slovakia and acquaint them with system of development and distribution of hydrological forecasts and warnings as a part of flood protection in the Slovak Republic.

**Conclusions**

In implementation of project in a region like Southern Caucasus, an important factor appears political situation in Georgia and the whole Caucasus region. In this case it was identified as a possible external factor which could affect the project implementation. However, even after elections the political situation remained stable and had no impact on the project.

From technical point of view it was necessary to involve experts of the partner into the project and also precise dividing tasks and responsibilities.

Lessons learnt from the project:

• Involve potential partner and final receiver into the project from the very beginning, i.e. from project proposal preparation up to final completing of the whole project;

• Cooperation with existing projects in the region leads to increased quality of results and excluding of duplicities (in case of this project CzechAid, EU, UNDP/GEF);

• In order to secure sustainability of project results it is necessary to intensify the cooperation with other donors (on international and national level) and also with state administration bodies (relevant ministries and regional authorities responsible for flood protection), in order to intensify and make more transparent the assistance provided by them. The assistance should be addressed as much as possible just according to flood protection requirements;

• Cooperation with institutions in Caucasus region (cross-boundary cooperation) may result in receiving finance necessary for improvement of situation in the field of flood protection;

• Such kind of project can be applied also in other countries in Caucasus region, respectively Balkan or Eastern Partnership.

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# Storage proteins in wheat (*Triticum aestivum L.*) and impact of mycotoxins affecting quality and quantity with focus on nitrogen supply

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Abstract: The paper is presenting a work plan and a literature review on storage protein formation and mycotoxin performance in a field trial. Grain quality and quantity parameters will be tested in relation to the impact of nitrogen supply and effects of mycotoxins on it. Nitrogen supply is planned to be given with different timing, doses and means of application. Sown winter wheat varieties Mv Karéj, Mv Nádor, Mv Krajcár, Mv Kolompos and Alföld (*Triticum aestivum* L.) in the experimental field of Szent Istvan University's at Nagygombos will be harvested and examined. Growth and development, yield and grain samples are to be observed, recorded and analysed to determine nitrogen application impacts on quantity and quality.

Keywords: grain protein, gluten, winter wheat, mycotoxin, nitrogen

## Introduction

There is more land planted to wheat in the world than any other crop. It provides 20 percent of the world's caloric consumption and for the world's poorest 50 percent, 20 percent of their protein consumption too (Washington Wheat Facts 2015/2016). The total global wheat output exceeded 749.3 million tonnes in 2016, according to FAOSTAT data (FAOSTAT 2017). Wheat contains vitamins, minerals and essential amino acids, throughout with useful metabolites and dietary fibres. However, such undesired side effects also should be noted, such as, intolerance or allergic symptoms. The goal of wheat production is twofold; provide quantity and quality. Milling and baking quality of wheat are mainly determined by the genetic basis, however, it can be influenced by management techniques (Grimwade et al 1996; Pollhamer 1981; Pepó 2010; Vida et al. 1996). In wheat production, nitrogen plays an essential role, since it affects yield levels and quality; nevertheless, it can exert undesirable effects on the environment due to losses by leaching, denitrification and volatilisation. Storage proteins are more than half of the total proteins in mature cereal grains. Gluten proteins - gliadins and glutenins of wheat determine the quality of the grain for bread making and their amount and composition can be influenced by agronomic impacts leading to changes in dough properties and that of baking quality (Lásztity, 1999; Shewry and Halford 2001, Győri 2008). With the special requirement of end-users, ongoing and prospective investigations involve more features on their projects, such as reduced chemical content, stability, higher/lower ingredient etc. Different types of quality and contents needs for different proposes and different users e.g. different for pasta production and bread or production of biscuits, such as the hardness of grain and protein content. Long-term intake of cereal products (bread, pasta, biscuits, etc.) that are contaminated with these mycotoxins may be the cause of serious developmental and hormonal disorders, chronic poisoning, malignant tumours and other diseases, as well as deformities (Smith et al., 1994; Gregorčič et al., 2009; Casteel and Rottinghaus, 2000). Williams and Hammitt (2001) considered the consumers to be insufficiently aware of the threats posed by the presence of mycotoxins in food. They stated that the consumers were certain that it was primarily the pesticides and not the presence of mycotoxins that put their health at risk, however, the authors' opinion was

just the opposite: human health was exposed to increased risk due to the potential effects of mycotoxins rather than to the residues of fungicides in food. Visible signs of disease (FHB - Fusarium head blight - is a devastating disease of wheat with spikelets exhibit symptoms of premature bleaching shortly after infection by the fungal plant pathogen of genus Fusarium spp.) that may be present in all parts of the plant, especially in the grains and inflorescences (spikes, cobs and wiper), reduce the quantity and quality of crop yields (Tajnsek L. et al. 2014). Fungal contamination causes significant yield decrease, but the losses are even greater because of mycotoxins produced by these fungi (Havlova et al., 2006). Carried out researches clearly demonstrate that mycotoxins have a negative effect on the yield quality and quantity also health impaction on humans and animals, however, linkage with nitrogen (N) applications needs to be investigated.
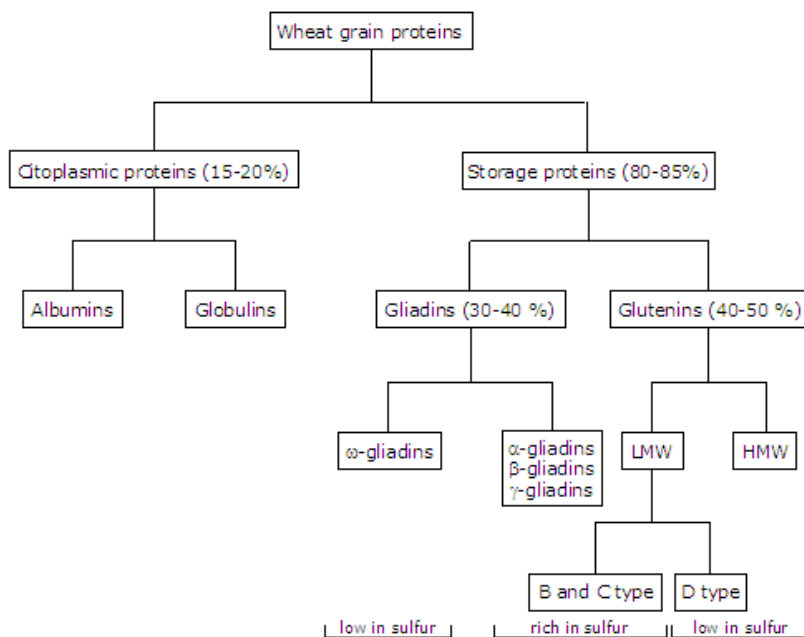


*Figure 1: The classification of wheat grain proteins. Source: Shewry and Halford (2002)*

**Wheat's storage proteins**

Thomas Burr Osborne (1859-1929) categorised grain proteins according to water-solubility: Albumins: water-soluble, globulins: salt-soluble, prolamins (gliadins): alcohol-soluble, glutelins (glutenins): alkali-soluble. The different types of wheat proteins can be separated according to size. In general, wheat flours contain 45% glutenin, 45% gliadin and 10% soluble proteins. Wheat proteins are responsible for most of quality characteristics of flour. Figure 1 is comprises a scheme on the relations of wheat proteins.

**The impacts of fertilisers/manure on plant development, yields and quality**

Studies from various countries show that topdressing with nitrogen fertiliser can effectively increase winter wheat grain yields. According to Zhao et al. (2015) investigation,

topdressing with N fertiliser resulted in significant increases in wheat yield, wheat biomass production, and N concentrations and uptake in wheat grain, straw, and chaff. The genetically determined quality of the various wheat varieties may only be attained by applying appropriate agronomical techniques. In the array of up-to-date agricultural techniques, proper application of fertilisers is the means that can have the most profound impact on the quality and quantity of wheat yields.

A number of authors have come to the conclusion that the improvement of the genetic stock contributes 30-50 %, while agricultural techniques contribute 50-70 % to increasing yields (Jolánkai 1985). Crop growth is influenced by management practices such as cereal crop was grown, variety and planting date, and also by soil and climatic conditions (Kismányoky and Ragasits 2003, Horváth 2014).

The nitrogen supply for a cereal crop comes from fertiliser, but also from manure and mineralisation. Mineralisation is the release of plant-available nitrogen from soil organic matter and crop residues as a result of soil microbial activity. The dosages of N fertilisers have a significant impact not only on protein content but – through the protein content – on the alcohol yield as well.

**Factors influencing wheat response to nitrogen fertiliser**

The utilization of applied nitrogen fertilisers up to several factors such as soil nitrogen and loose from applied fertilisers. However wheat cultivar, available soil nitrogen, delayed or late seeding, weed competition, disease infestation, soil moisture and texture.

**Other factors' impacts on the quality and quantity of the yield**

According to Dupont and Altenbach (2003) when the grains are filling up environmental factors have a major impact on both yield and flour quality. Grain yield and yield quality of winter wheat are highly influenced by the meteorological conditions of the given crop year, especially the amount and distribution of precipitation and the actual temperature (Grimwade et al 1996, Győri 2008, Pepó 2010). Weather conditions are evaluated and labelled favourable or non-favourable in relation to the optimum requirements of the crops' phenophases (Lásztity 1999; Ványiné and Nagy 2012).

Beside of nitrogen, other fertilization activities also increase to parameters of the effect of nitrogen, such as macro-micro fertilisers unless restriction each other. As it also well knows the influence of breeds with suitable environment expects. Disease and insect harms cause up to 20-30% time by time, therefore integrated plant protection applications allow us to keep them under economic loss threshold.

**Influence of nitrogen sources on fungal growth**

According to the Bouras N. et al. (2016) growth of the fungal species, isolates was measured on the synthetic medium in the presence of several nitrogen sources (mineral and organic). Individual isolates exhibited variable growth rates depending on the nitrogen source added to the medium. The mean colony diameter ranged from $6.0 \pm 0.5$ mm to $83.0 \pm 7.9$ mm various media (Fig. 2).
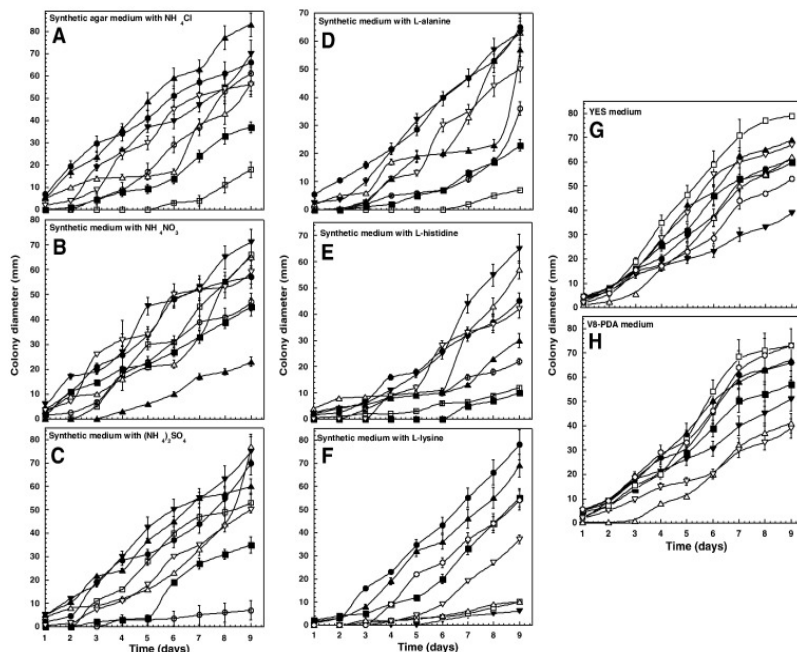
*Figure 2. The Growth of Pyrenophora tritici-repentis on different culture media over a 9 day period. (A–F) Modified synthetic agar medium (SAM) containing: NH4Cl (A), NH4NO3 (B), (NH4)2SO4 (C) (Bourasa N. et al. (2016))*

### Effect of mycotoxin on grain and impactions on health

The harms of fungal diseases on crop products are well known. However, the factors of their interactions with N application and correlations with grain protein quality-quantity have to be studied on. According to Homdork et al. (2000), artificial Fusarium inoculation lowered yield significantly by 24.2-45.0%. Mycotoxins have significant economic impacts in numerous crops, especially wheat, maize, peanuts and other nut crops, cottonseed, and coffee. The Food and Agriculture Organization has estimated that 25% of the world's crops are affected by mycotoxins each year, with annual losses of around 1 billion metric tonnes of foods and food products. Economic losses occur because of: 1) yield loss due to diseases induced by toxigenic fungi; 2) reduced crop value resulting from mycotoxin contamination; 3) losses in animal productivity from mycotoxin-related health problems; and 4) human health costs. Neither of these estimates included human health impacts or crop yield losses. Reduced crop value is a significant component of the losses caused by mycotoxins. Human health impacts of mycotoxins are the most difficult to quantify. It is clear that mycotoxins affect human health, especially aflatoxins in developing countries.

These effects are due to acute (single exposure) toxicoses and immunosuppression by mycotoxins, as well as chronic (repeated exposure) effects. (Zain, 2010). Over then quality-quantity properties effect, it also effects on human and animals health. Mycotoxins may be harmful to human and animal health more than fungicides residues on the crops. Williams and Hammitt (2001) consider the appearance of natural toxins to be influenced by agronomic management practices.

**Materials and methods**

For long-term field trials high milling and baking quality winter wheat varieties Mv Karéj, Mv Nádor, Mv Krajcár, Mv Kolompos and Alföld (*Triticum aestivum L.*) were sown. The small plots trial with four replications run at Nagygombos, experimental field Crop Production Institute of Szent Istvan University, Hungary. The size of each plot is 10 m². Soil type of the experimental field is chernozem (calciustoll). Annual precipitation of the experimental site belongs to the 550-600 mm belt of the Northern edges of the Hungarian Great Plain. Identical agronomic treatment applied to each plot. Supply of N fertiliser planned for single and divided doses. Applications of N topdressing will be done by 6 levels: 0, 80, 120, 160 kg/ha N in single supply and 80+40, 120+40 kg/ha N in two applications. After harvest, samples going to be analysed in the laboratory of Crop Production Institute of Szent Istvan University.

**Expected results and outcomes**

Experimental results (Yield, thousand kernel weight, grain proteins (gliadin and glutenin), baking quality, grain hectolitre weight, fusarium infection and mycotoxin contamination will enable us to compare and find out the correlations for questions below:

Can we find any relationship between single and divided dose application of N fertiliser with regard to yield quality and quantity?

Can we find any relationship between different levels of N supply with regard to yield quality and quantity?

Can we find any relationship between overall soil fertility management strategies, resulting in different overall plant available nitrogen levels and different nitrogen dynamics, and the presence of mycotoxins in wheat and effect on grain parameters?

Can we find any relationship between increased levels of plant available nitrogen during the stages, realized by additional fertiliser application, and the presence of mycotoxins in wheat?

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# Determining the main physical characteristics of fertilisers

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Abstract: Evenly distributed fertilisation is an important demand in agricultural technology. Environment-friendly cultivation technology requires distributing different types of fertilizers more accurately and evenly. For this reason, it is necessary to develop fertilizer spreader machines continuously. In developing this machinery, it is essential to know the physical properties of fertilizers. This study deals with the main factors influencing fertilisation, including surface characteristic, particle (granule) shape, particle hardness, particle size range, specific particle weight. We can determine the following characteristics: particle size and particle size distribution, bulk density, moisture content, and the angle of repose. Six different fertilizer types were examined for their most significant physical characteristics from the aspect of the dispersion of fertilizer particles. The measured moisture content, space filling and fraction characteristics affect storage and transport properties.

Keywords: fertilizer, agricultural, environment-friendly, precision application of fertilizer

## Introduction

Fertilizers are indispensable in nutrient replenishment, which is an essential part of crop production. The precision application of fertilizers is necessary in order to perform modern nutrient management, as it guarantees the stable yield and quality of culture crops without harming the environment. This environmental friendly and cost-effective method is the location-specific "precision" nutrient replenishment, which makes it possible to perform nutrient application adapted to the local endowments and needs of the given plot. The physical characteristics of the used fertilizers greatly differ from each other as they significantly affect the physical distribution of fertilizers applied on the soil. During this research, we examined the physical characteristics of six different fertilizers widely used in nutrient replenishment.

## Materials and methods

Measurements were performed in the material analysis laboratory of the Institute of Land Utilisation, Regional Development and Technology of the Faculty of Agricultural and Food Sciences and Environmental Management of the University of Debrecen at 20°C and a relative humidity of 30-40 %. Fifty kg airtight fertilizer bags were available for each fertilizer type.

The following fertilisers were used for the analyses:
  **I.**  $NH_4NO_3$
  **II.** YARA Mila 13:13:21
  **III.** Genezis NS 21:24
  **IV.** Potassium chloride (60% Potash)
  **V.** Genezis CAN 27%N
  **VI.**  MAP NP 12:52

Based on the sensory evaluation of the particles, it can be concluded that their consistency is representative of each type. No agglutination and friability were observed with the samples.

### Methods used during measurements



Figure 1. Fertilisers after drying



Figure 2. Sieving



Figure 3. Determination of bulk density

### Measurement of moisture content

A 25g sample was heated for 72 hours to $103 \pm 1°C$ temperature in a drying oven. This examination had three replications. Samples were place on aluminium trays **(Fig.1.)** Following heating, samples were weighted using an analytical scale with 0.01g accuracy. Moisture content was calculated from weight measured before and after drying (Csizmazia et. al. 2006).

### Particle composition of fertilisers

A sieve analysis (**Fig.2**) was performed to determine the characteristics of particle size distribution. Using a mechanical shaker, 100g samples of each fertilizer type were shaken for three minutes with three replications. The following sieve sizes were used: 1 mm; 1.25 mm; 2 mm; 2.5 mm; 4 mm. The sample and the leftover after sieving were weighed using an analytical scale with 0.01g accuracy (Battáné et al. 2002; Csizmazia 2008).

### Determining bulk density

A 1000 $cm^3$ measurement pot of 100 mm diameter and 127.4 mm height (**Fig.3**) was used to determine bulk density. Fertilizers were slowly poured into the measurement pot from a height of 150 mm without compaction and with three replications. After the top was skimmed off, the sample weight was measured using an analytical scale of 0.01g accuracy (Gindert-Kele. 2005).

### Globularity

Globularity ($g_a$) was determined as described by Sitkei, (1981). Based on the ratio of the geometric effective diameter and the largest diameter, $g_a = (d_1 d_2 d_3)^{1/3}/d_3$, where $d_1$ is the largest size and $d_2$ and $d_3$ are the two sizes perpendicular to $d_1$. In the case of a regular globe, this value is one unit (Csizmazia 2011).

## Results and discussion

### Moisture content and particle composition of fertilizers

**Table 1** shows the moisture content and particle composition of the examined fertilizers. Based on the performed measurements, sample 2 contained the highest amount of moisture and the lowest amount of pulverized fraction (<1) as a result of the sieved fractioned

measurement, which equals to elated to the examined sample; therefore, it does not cause any significant problem during the application of the fertilizer. Sample 4 (potassium chloride) contained the lowest amount of moisture. As for its structure, this fertilizer type is mostly coarse and irregular shaped and it has the hardest particle. Accordingly, this fertilizer is the least hygroscopic and, as a result, it is the least sensitive of all examined fertilizers to the humidity of the storage environment. Of particle size determination of the pattern uniformity can be inferred. The higher the amount of pulverized fraction is in a sample, the more hygroscopic and uneven the dispersion is. **Table 1** and **Figure 4** show the measurement of the examined samples by fraction.

*Table 1.: The particle composition and moisture content of fertilizers*

| Fertilizer type | I. | | II. | | III. | | IV. | | V. | | VI. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Humidity** | 4.18 | | 5.03 | | 1.36 | | 0.44 | | 2.94 | | 4.44 | |
| **Particle size** | g | % | g | % | g | % | g | % | g | % | g | % |
| < 1 | 9.25 | 9.25 | 0.05 | 0.05 | 9.58 | 9.58 | 0.05 | 0.05 | 0.04 | 0.04 | 0.15 | 0.15 |
| 1 - 1.25 | 3.22 | 3.22 | 0.04 | 0.04 | 1.67 | 1.67 | 0.07 | 0.07 | 0.01 | 0.01 | 0.09 | 0.09 |
| 1.25 - 2 | 6.88 | 6.88 | 0.79 | 0.79 | 7.68 | 7.68 | 3.66 | 3.66 | 4.5 | 4.5 | 1.7 | 1.7 |
| 2 - 2.5 | 70.27 | 70.27 | 9.24 | 9.24 | 15.46 | 15.46 | 16.82 | 16.82 | 7.76 | 7.76 | 5.64 | 5.64 |
| 2.5 - 4 | 9.84 | 9.84 | 81.91 | 81.91 | 49.75 | 49.75 | 76.28 | 76.28 | 77.2 | 77.2 | 82.73 | 82.73 |
| > 4 | 0.1 | 0.1 | 7.9 | 7.9 | 15.87 | 15.87 | 3.28 | 3.28 | 10.1 | 10.1 | 9.68 | 9.68 |
| **Total** | 99.56 | 100 | 99.93 | 100 | 100.01 | 100 | 100.16 | 100 | 99.61 | 100 | 99.99 | 100 |
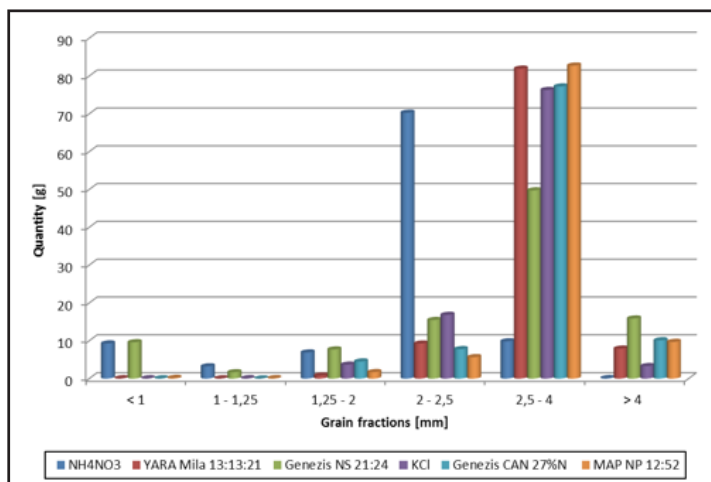


Figure 4. Particle fractions

Of the examined samples, Genezis NS 21:24 contained the highest amount of pulverized (<1) fraction and the highest amount of particles above 4mm, which is considered to be significant value. Therefore, this fertilizer type is rather variable in terms of its water binding capacity and dispersion. There is an unusually high value in the 2-2.5 mm range, which was provided by the $NH_4NO_3$ sample. Therefore, 70.27% of the examined sample belongs into this range, which significantly exceeds the respective values of the other samples. Consequently, it can be concluded that there is a favorable impact on working width in the case of $NH_4NO_3$. That is due to the favorable particle size for the same speed,
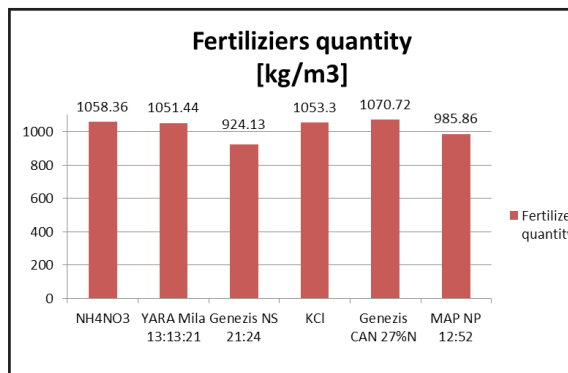
Figure 5. Fertiliser weight per cubic metre

larger working breadth is achieved with the a centrifugal fertilizer spreader.

*Bulk density of fertilizers*

The bulk density of fertilizers is in connection with the capacity demand which arises during transport. The bulk density of the examined fertilizers is shown on **Figure 5**. It can be observed that Genezis CAN 27%N has the highest bulk density which is related to its imperfect globularity (0.871).

### Conclusions

Six different fertilizer types were examined for their most significant physical characteristics from the aspect of the dispersion of fertilizer particles. The measured moisture content, space filling and fraction characteristics affect storage and transport properties. In addition, the change of working width and dispersion plays a very important role in the case of each fertilizer type. The obtained results show these characteristics of the examined fertilizers in a quantified way which leads us to conclude to the existence of even dispersion and even working width as these are indispensable factors in precision technology. Finally, yet importantly, these results may also provide a background for the production characteristics of the examined fertilizers, thereby reducing the pulverization of fertilizers.

### Acknowledgement

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# Methodological approach in rehabilitating coal ash disposal sites from thermoelectric power plants and mitigation of environmental risks

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Abstract: The amount of the ash and cinder that is generated from thermoelectric energy production in Bosnia and Herzegovina exceeded 2.784 Mg for 2015 according to annual report of "Elektroprivreda BiH". Electricity production is the greatest environmental ballast for the city of Tuzla. Excavation of coal and deposition of coal combustion products influence drastic and many times terminate degradation of natural landscapes and soil habitat. There are five coal ash disposal sites in the city covering an area of approximately 170 hectares. It is known that this artificial substrate is the main cause of soil contamination; water/groundwater contamination due to leaching toxins (effluents and process waters); dust dispersion; and toxins entering the food chain. The objective of our research study was to explore the adequate practices of stand remediation through compiling the data on properties of ash from five disposal sites in Tuzla area. Long term study determined that the major characteristics of coal ash and effluents at the disposals sites are high alkaline reaction (pH >10) and presence of metals with high potential for toxicity (Ni, Cr, As and B), which concentrations exceeded permitted limits. Based on study results, how model of site rehabilitation would work and how it can be incorporated by applying green infrastructure approach in spatial planning is discussed.

Keywords: coal ash, disposal sites, environmental risk, remediation, reintegration

## Introduction

Ash and cinder or coal combustion residues (CCR) are normal by-products in the power production at the thermal power plants (TPP), as they are incombustible fraction of coal. At the Tuzla TPP, which is located in northeastern part of Bosnia and Herzegovina, the incineration of coal produces approximately 200 kg of CCR per ton of fuel, which is a specific yield of 0.4 to 0.9 $m^3$ per MWh of energy produced. The annual volume of CCR is approx. up to 800,000 $m^3$, or 1,660 to 2,000 $m^3$/day. The CCR has been disposed of over the years at several disposal sites around the TPP, which are all located in the vicinity of the city of Tuzla. So far, more than 40,000,000 $m^3$ of CCR have been deposited at these locations. Energy production from coal produces large amounts of coal ash that needs to be land-filled if it cannot be used as a raw material for other industrial processes or products. These technogenic deposits, which belong to the category of brownfields since they are industrial land with developed basic infrastructure, just in Tuzla cover more than 170 hectares.

Ash substrate is characterised with predominantly coarse fragments and sand highly erodible, with low sorption capacity, small humus content (<2,1%) and significantly high concentrations of heavy metals (As, B, Cd, Cu, Cr, Pb, Ni, Zn). Average *coal ashes* pH values of the *one year old* ash is *high and varies from 10 to 12 (Čustović* et al*., 2011)*. In general*,* coal ashes taken from disposals in Tuzla were found to be highly alkaline. For exam*p*le at the site Jezero, which had been abandoned in 2003, the mean pH of disposed ash was 9.2 at the surface (0-20 cm) and 9.7 at 40-60 cm depth.

In comparison, water coming from ash disposal sites has very high alkalinity (pH values range from 10-12) (Dellantonio et al*., 2008; Ćerić et al., 2009; Čustović et al., 2016).

Gradual decline of the leachate pH happens over time, but at a very slow rate. The following model was used for the estimate of the leachate pH in time (Čustović *et al.*, 2011):

$$pH_t = (pH_o - pH_n) \cdot e^{-\alpha(t - t_o)} + pH_n$$

where $pH_o$ represents the leachate pH value at the time CCR disposal site was closed ($t_o$), $pH_t$ is the leachate pH measured at the time interval t, $pH_n$ is the pH of the surface water that enters the respective disposal site, $t_o$ represents the initial time, that is, the time when the site was abandoned (in years), t is the time of measurement of pH (in years), and α is a decay coefficient (-). The results of the model are presented on Figure 2.
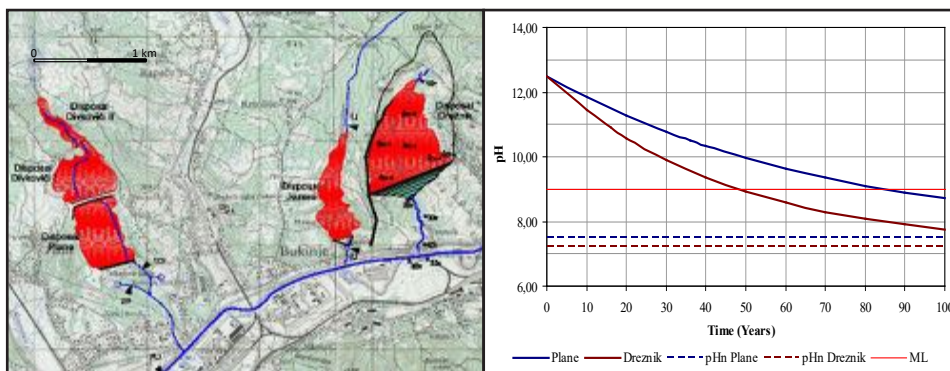


Figure 1. Location map of the CCR disposal sites in Tuzla   Figure 2. Modeled change of pH in time at the Dreznik and Plane disposal sites

Thick solid lines indicate the leachate pH from the two sites, and the dashed lines the surface water pH. The red solid line is the maximum level allowed in the effluent discharged to surface waters according to the water legislation in the Federation of B&H. The model indicates that the natural process of pH decline will require approx. 48 years for the leachate from the Dreznik site to reach national standards. For the Plane site, this process is estimated to be even slower, some 85 years. It has to be considered that the model was developed with very limited data, and the estimates are therefore not perfectly accurate. It was assessed that the model for the Plane disposal site is more realistic, as it is based on a longer observation period of 36 years.

Remediation means decontamination as well as containment procedures to eliminate or interrupt the contaminants pathways to different receptors such as humans, plants and groundwater. Solutions for coal ash disposal remediation are context-dependent and need to be based on the actual documentation on spatial planning, which had already integrated local community needs and technical concerns. Frame methodology for remediation of coal ash disposal sites needs to be designed according to previous risk assessment (Meuser, 2013). In addition, risk assessment should be provided for every planed land use.

**Materials and methods**

„Reintegration of coal ash disposal sites and mitigation of pollution in the West Balkan Area" Project (RECOAL) has developed a basic conceptual structure – a framework – offering a route to structure coal ash management decisions that recognizes the importance of contextual factors. Its objective is twofold:

• provide a framework for researchers and engineers involved in coal ash management to communicate their results to a wider audience of decision-makers and stakeholders; and

• guide policy-makers in the interpretation and synthesis of coal ash-related research for its incorporation into decision-making.

**Results and discussion**

RECOAL has developed a basic conceptual structure – a framework – offering a route to structure coal ash management decisions that recognizes the importance of contextual factors. A four-step procedure is proposed: 1) Problem definition; 2) Short listing of options; 3) Development of remediation strategy; 4) Monitoring program, (RECOAL, 2008). First step consider defining the objectives and motivations behind the remediation strategy.

Remediation palette is a second step and considers evaluation processes in which the best option is chosen as it is presented in the modified flow chart (Figure 3). In some cases one single criterion will be enough to eliminate an option. For instance, a high-cost remediation option may be discarded immediately if there are no available resources for its implementation. Also, ex-situ remediation methods were discarded for the case of Tuzla disposals, because the project's focus was explicitly on locally available low-cost methods. Alternatively, an option may be included even if it does not meet all the criteria. This question arises from the needs of local rural population that needs arable land and pastures for livestock production. The suitability of a remediation strategy) rather than a one-off linear process. The following steps are the development of remediation and monitoring designs. Usually, several remediation proposals will be available to address a particular site problem and these should be systematically compared and evaluated. Different approaches can be used, such as a multi-criteria assessment, scenario analysis, cost-benefit analysis and a variety of recently developed participatory mixed method approaches (Dodgson *et al.*, 2000).

Development of an overall remediation strategy for the setting combining several options from the 'remediation palette' to address the multiple dimension of the pollution problem is the third step of a framework. Fourth step is focused on monitoring and evaluation of strategy in order to ensure its long-term sustainability.

Based on the framework, the case study from Tuzla showed a series of methodological steps for site remediation focusing on three major risks 1) food chain contamination, 2) ash dispersion by wind and water erosion and 3) contamination of ground and surface waters (landfill leachate and ash transport water).

Defined risks for site rehabilitation usually involve only establishment of a soil cover. This is most urgent measure that follows after end of landfilling process which prevents ash dust dispersion. Cover substrate that is usually applied represents a by-product of coal excavation and has low productivity. Uncertainties which need to be addressed are depth of cover layer and the long-term potential harmful effects if the soil cover is unable to neutralise pollution. Ash amendments, usage of adequate cultivars and crop rotation systems are measures that should be explored from technical and financial aspect for successful revegetation. Revegetation with high biomass production is hardly to be
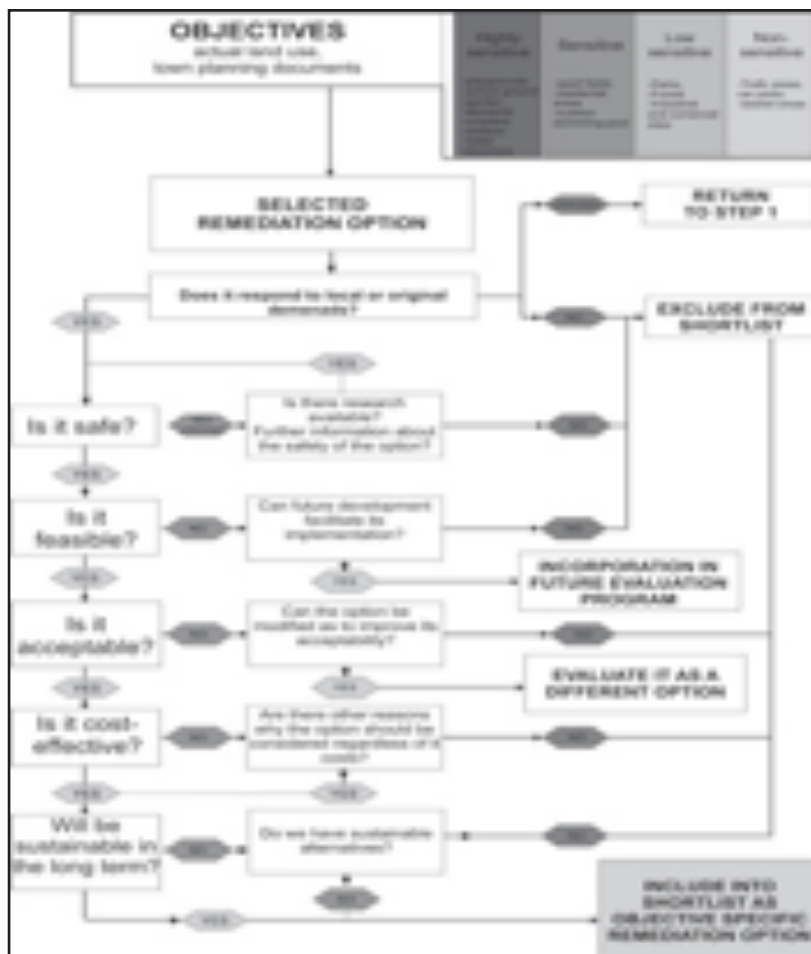
*Figure 3. Modified RECOAL study flowchart for selecting remediation option*

expected in actual cover substrates. Compost amendments improved the fertility of the ash substrates with respect to N, P and K and the microbial activity of the ashes (Dellantonio *et al.*, 2008). To address dust dispersion from active disposal sites - second important issue - wind barriers are proposed as effective measure. The dominant species of trees growing in the areas surrounding the disposal sites are Willows, Poplars, Maple, Beech, Alder, Hazel, Hornbeam, Elder, Ash and Horse Chestnut. Using native species in shelterbelts would help establish a stable wind barrier (species are used to local biotic and abiotic conditions) and preserve/improve local ecosystems. Reforestation of the disposal sites would be an initially costly but potentially highly effective and multi-purpose solution for the long term benefiting local residents and ecosystems.

Third issue considers treatments of water: water-aeration process and filter materials. Aeration of alkaline wastewater for passive remediation was previously suggested by geoscientists from Newcastle University (Mayes *et al.*, 2005). At Drežnik a column system was installed to test different sorbents' capability and capacity to retain pollutants. Locally available material brand (red shist) was able to reduce the pH and was effective in

reducing Arsenic but was not able to filter out Boron (RECOAL, 2008). Constructed wet lands have been designed to purify waste waters with large nutrient and organic loads by microbial decomposition.

Integration of ash disposal brownfields includes preliminary, oriented and detailed risk assessments in the process of town planning (Meuser, 2013). European practices, based on soil protection strategies, indicate that old industrial land can be used as parks, forest land, for biomass production, for new industrial objects, residential housing etc. (Stadt Leipzig, 2008). The area for ash and cinder disposals sites in Tuzla could be used for plant nurseries, forest-parks, recreation, industrial - electricity production from solar panels and the town graveyard. Higher sensitivity use playgrounds, school grounds, arable land, recreational fields demand more intensive remediation, which is due to high cost, reduced to smaller areas of usually <3 ha which are economically feasible. On the other hand, highly contaminated land are usually restricted to low or non-sensitive use.

## Conclusions

RECOAL's research demonstrates that coal ash remediation solutions are context-dependent, i.e. local factors are a crucial influence on decisions. Hence, it is necessary to examine every case independently attending to its local environmental characteristics and social conditions. The decision-support tools are presented within a simple four-step framework that can be used flexibly, according to the needs of the problem and the knowledge available. The results of RECOAL study can serve as a corner stone for the future remediation plans which need to be developed according to Regulatory Plans adopted by Tuzla Town Council in recent period and new land-uses assigned to the space.

## Acknowledgement

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Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones. Lugduni: Apud Ioan. Tornaesium 1559. Centre d'Études Supérieures de la Renaissance - Tours

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### Lucius Junius Moderatus Columella

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His De Re Rustica in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19th Century.