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Columella

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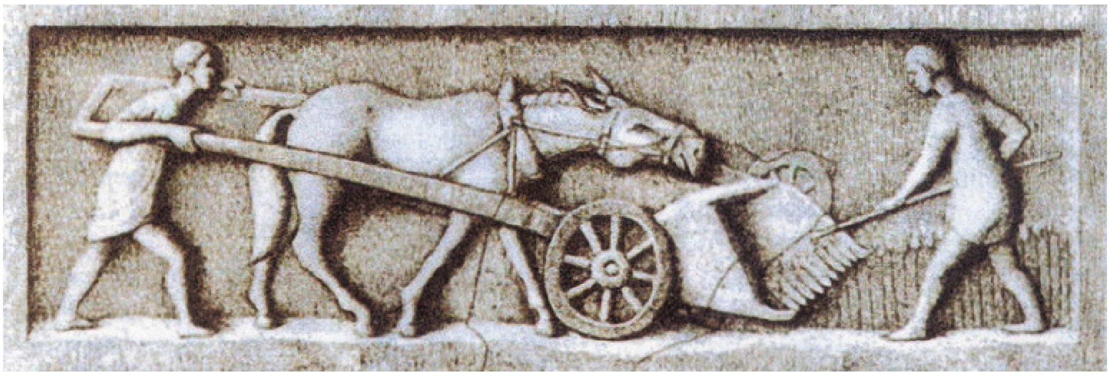

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Editor-in chief:
Dr. GYURICZA Csaba

Edited by:
KENDE Zoltán

Responsible publisher:
LAJOS Mihály

Editorial correspondence:
Faculty of Agricultural and Environmental Sciences of the Szent István University

Páter Károly utca 1.
H-2100 Gödöllő, Hungary
Phone: (+36 28) 522067
Fax: (+36 28) 410-804
web: www.columella.mkk.szie.hu
E-mail: columella@mkk.szie.hu

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Authors of current volume of Columella

László ALEKSZA
Csaba FOGARASSY
Márta FUCHS
György FÜLEKY
Mark Piero GEBEL
Miklós GULYÁS
Ferenc GYULAI
Gábor GYULAI
Annamária HOLES
Bálint HORVÁTH
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FOREWORD

HABENT SUA FATA LIBELLI – BOOKS HAVE THEIR DESTINY

Picture: Columella: De Re Rustica cca 1450.
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Really, books have their own fate, and so do journals let them be scientific periodicals or volumes of other kind of literature. This fate is driven by two powers. One is the value of the material published however the other is that this value shall be approved by the satisfaction of the reader.

Mark Twain describes a peculiar situation in one of his evergreen short stories titled "How I edited an agricultural paper". He realised two facts: "I did not take temporary editorship of an agricultural paper without misgivings. Neither would a landsman take command of a ship without misgivings". Of course, this bitter joke of him has a message for all of us. He points to the responsibility of authors and editors. Reading any press articles we meet mistakes regularly. Some of them are of accidental origin while some others were born to drive the readers' mind to definite directions. This latter is a sort of disinformation. A scientific periodical has to be honest, precise, thorough and reliable. We believe that Columella may face these expectations. That is why the cooperation of authors and editors is supported by a third party; the reviewer. We try to keep in mind the old rudiment of wisdom; the quality of any book depends on the strength of the reviewer.

The present volume provides the reader with a patchwork of scientific information. A journal, which is dedicated to agricultural and environmental sciences has to cover a broad field of research. So does the present issue. There are papers dealing with novel results obtained in the field of soil science. Also, we may get acquainted with results of new plant protection and crop production techniques. A series of papers inform us about the archeobotanical features of a field crop. And there are various papers dealing with questions of plant physiology and genetics.

We do hope, that the readers of this volume will benefit from the papers presented. Also, we have the hope, that a reader of this volume may be author of further volumes of Columella.

Márton Jolánkai
guest editor

ACCUMULATION AND DEPLETION OF FERTILIZER ORIGINATED NITRATE-N AND AMMONIUM-N IN DEEPER SOIL LAYERS

György FÜLEKY

Szent István Unversity, Institute of Environmental Sciences, Gödöllő, H-2103 Páter K. u. 1. Hungary
e-mail: fuleky.gyorgy@mkk.szie.hu

Abstract
The leaching process of nitrate was studied in a long-term field experiment at Gödöllő on a brown forest soil started in 1969. 0, 90, 180, 270, 360 kg ha^{-1} y^{-1} N doses have been broadcasted as basal fertilization in the form of ammonium-nitrate for 16 years. The test plant was maize in monoculture. In autumn 1989 fertilization was discontinued and alfalfa was sown on the experimental area. In 1995 pseudoacacia was planted into the field. In 1986, 1989, 1994 and 2003 the soils of the treatments were sampled in 6 replications from 0 to 3 meters depth at every 20 cm. The nitrate-N and ammonium-N content of the sample was determined.

Increasing the dose of nitrogen fertilization nitrate content in the soil profile increased, as well. About the half of fertilizer nitrogen can be found in the 3 meter layer of the soil profile after 16 years fertilization. Using deep rooting plants the accumulated nitrogen could be used to prevent the leaching losses.

Results prove the necessity of a suitable technology preventing nitrate losses. As the experiment shows 100 kg N ha^{-1} fertilizer nitrogen enough for the maximum crop yield in this area. The amount of nitrate-N exceeding plant demand could be leached into 1-3 meter depth of soil or into deeper horizons.

Keywords: ammonium-N, fertilizer, field experiment, nitrate-N, soil

Introduction

Nitrate leaching was studied for a winter leaching period in a layered calcareous silt loam with tile-drains at about 1-m depth. Measured NO_3 leaching was 11 kg N ha^{-1} y^{-1} in the relatively dry, winter leaching period 1991-1992 (De Vos et al., 2000). Paramasivam et al. (2001) evaluated NO_3-N distribution in soil solution at various depths in the vadose zone, and N leaching below the root zone for two cropping seasons. The treatments included 112, 168, 224, and 280 kg N ha^{-1} y^{-1}. At the 60- or 120-cm depths, the NO_3-N concentrations occasionally peaked at 12 to 100 mg L^{-1}, but at 240 cm NO_3-N concentrations mostly remained below 10 mg L^{-1}. The careful irrigation management, split fertilizer application, and timing of application contributed to the low leaching of NO_3-N below the root zone. Calculated NO_3-N leaching losses below the rooting depth increased with increasing rate of N application and the amount of water drained, and accounted for 1 to 16% of applied fertilizer N. Results showed that well and moderately well drained fields had consistently higher ground water NO_3 compared to more imperfectly drained fields receiving comparable N inputs (Young and Briggs, 2007).

A long-term (1982-2004) field experiment was conducted to investigate the effects of nitrogen fertilizers on accumulation of nitrate-N in the soil profile (0-210 cm). Annual applications of N fertilizer and manure for 23 successive years had a marked effect on NO_3-N accumulation in the 0-210 cm soil profile. Accumulation of NO_3-N in the deeper soil layers with application of N fertilizer and manure is regarded as a potential danger, because of pollution of the soil environment and of groundwater (Yang et al., 2006).

The magnitude of nutrient accumulation and its distribution in the soil profile varies with soil-climatic conditions. The objective of the study was to determine loading and distribution of manure-derived nitrogen in the soil profile as influenced by repeated manure applications.

Lower crop removal and reduced leaching of NO_3-N due to drier conditions contributed to greater accumulation of nitrate-N in the top 60 cm. At large manure rates, excess N from the balance estimates could not be accounted for in soil organic N and was assumed to be lost from the soil-plant system. At the Dixon LHM site, deep leaching of NO_3-N was observed at the excessive rate up to the 150 cm depths compared to the control. To prevent loading, rates of applied manure nitrogen should be reduced when crop N removal potential is diminished by high frequency of drought (Stumborg et al., 2007).

A long-term fertilizer experiment on dry land of the Loess Plateau, northwest China, has been conducted since 1984 to study the distribution and accumulation of NO_3-N down to a depth of 400 cm in the profile of a coarse-textured dark loess soil after continuous winter wheat cropping. Annual N and P (P_2O_5) rates were 0, 45, 90, 135 and 180 kg ha^{-1}. After 15 successive cropping cycles, the soil samples were taken from each treatment for analysis of NO_3-N concentration. The application of fertilizer N alone resulted in higher NO_3-N concentration in the soil profile than the combined application of N and P, showing that application of P could greatly reduce the NO_3-N accumulation. With an annual application of 180 kg N ha^{-1} alone, a peak in NO_3-N accumulation occurred at 140 cm soil depth, and the maximum NO_3-N concentration in the soils was 67.92 mg kg^{-1}. The amount of NO_3-N accumulated in the soil profile decreased as the cumulative N uptake by the winter wheat increased (Fan and et al., 2003).

Nitrate leaching occurs when there is an accumulation of NO_3-N in the soil profile that coincides with or is followed by a period of high drainage. Therefore, excessive nitrogen fertilizer or waste effluent application rates or N applications at the wrong time (e. g. late autumn) of the year, ploughing pasture leys early in the autumn or long periods of fallow ground, can all potentially lead to high NO_3-N leaching losses. N returns in animal urine have a major impact on NO_3-N leaching in grazed pastures (Di and Cameron, 2002).

Nitrate leaching from agricultural soils can increase groundwater nitrate concentrations. Ammonium nitrate was applied only to the percolation lysimeters. Leachate from the lysimeters was extracted from a depth of 2.1 m and soil samples were collected from field plots in 0.3 m depth increments to 2.1 m on a periodic basis. Determining accurate yield expectations under deficit irrigation conditions, correct scheduling of irrigation and the use current best management practices for N management can help minimize nitrate losses in leachate (Tarkalson et al., 2006).

Very low NO_3 concentrations were found in the rooting zone at most sample positions, indicating that crop demand during recent growing seasons matched or exceeded supply. Accumulations of NO_3 below the rooting zone indicated that deep percolation of NO_3 has been an important process over the longer term throughout the upper and mid slope positions of the landscape. A lack of NO_3 accumulation in one lower-toe position and the depression indicated that excess NO_3 in these profiles may have been leached into the groundwater and/or removed via denitrification or simply may not have accumulated (Whetter et al., 2006).

A long-term (1982 to 2000) field experiment was conducted - under wheat - wheat-corn rotation to determine the effects of N, P, and K chemical fertilizers and farmyard manure accumulation on nitrate (NO_3-N) in the soil profile (0-180 cm).

Fertilizers (N, NP, and NPK) led to NO_3-N accumulation in most subsoil layers. Combined applications of fertilizers and manure reduced soil NO_3-N accumulation in soil compared with fertilizers alone. In conclusion, the findings suggest that it is important to use balanced application of chemical fertilizers and manure at proper rates in order to protect soil and underground water from potential

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NO_3-N pollution while also sustaining high crop production (Yang et al., 2003).

Four variants of a leaching experiment were conducted at 2 sites to parameterise and check the theory. The experiment involved the application of ammonium chloride to an area of 25 m^2, and then from 6 days to 5 months taking soil samples at 200 mm intervals down to 2 m depth and analysing them for chloride, ammonium, and nitrate. Background concentrations were obtained by contemporaneous sampling nearby. In one variant of the experiment 353 mm of rain in 6 days moved nearly half the applied nitrogen to below 400 mm depth (Banabas et al., 2008).

Drainage and nitrate leaching were simulated using the Water and Nitrogen Management Model (WNMM). Nitrate concentrations in the drainage water and nitrate leaching increased with increasing N application rate. Annual leaching losses ranged from 21.1 to 46.3 kg N ha^{-1} (9.5-16.8%) for inputs between 0 and 150 kg N ha^{-1}. Growth of oilseed rape decreased the nitrate concentration in the drainage water, but growing N fixing peanuts did not. Rainfall had a greater impact on nitrate leaching than crop uptake. The loss of nitrate was low during the dry season (October-February) and in the dry year (rainfall 17% below average) mainly as a result of reduced drainage (Sun et al., 2008).

Under fertilizer treatment, larger quantities of NO_3-N were present in the upper soil layer (0-40 cm) at 7 days after fertilization. From 7 to 37 days after fertilization, NO_3-N decreased, obviously because of the heavy rainfall together with the increase in the capacity of maize to accumulate N in this period and a significant decrease in NO_3-N stock was observed. There was a significant positive correlation between the quantity of NO_3-N stock decrease and the nitrogen fertilizer application rates during this period. And there was more NO_3-N accumulated in the lower layers under fertilization treatment at 76 days after N fertilizer application. Nitrogen fertilizer application increased NO_3-N concentration and stock in 0-100cm soil profile and changed NO_3-N distribution during maize cropping season. Nitrogen fertilizer application promoted movement of NO_3-N down the soil profile and increased N loss (Yin et al., 2007).

Numerous studies have shown that 54-72% of mineral nitrogen fertilizer applied is taken up by the plant, 8—21% is bound in the soil organic matter, 2—18% is lost to the atmosphere by denitrification and only 2-8% is lost by leaching (Owen and Jürgens-Gschwind,. 1986). In their opinion the major source of leached nitrate is the nitrogen mineralised from the soil organic reserves. However these values are valid at careful fertilizer application. In general, nitrate movement in the soil follows water movement. Increased leaching loss of nitrate can be resulted by rainfall, and irrigation between the growing seasons when soils are without plant cover. Less water percolates through heavy soils than through light soils, resulting in lower nitrate leaching losses from heavy soils. On average, nitrate leaching losses are 30-40 kg ha^{-1} from sandy soils and 20-30 kg N ha^{-1} from loamy soils. In the absence of a crop leaching losses are extremely high. High groundwater level also favours nitrate leaching from the soil. With very high fertilizer nitrogen application rates the proportion of the applied nitrogen taken up by the plant decreases, and the residual fertilizer nitrogen in the soil will be vulnerable to leaching. Approximately 300 mm annual drainage water at 100 kg N $ha^{-1}y^{-1}$ fertilizer rate has only little effect on nitrate 1eaching loss, but at higher fertilizer rate more than 100 kg N $ha^{-1}y^{-1}$ is the 1eaching loss from a sandy soil. Application of nitrogen fertilizer in spring rather than in autumn avoids leaching of fertilizer nitrogen.

The N_{min} method (Wehrmann and Scharpf, 1979) is based on observations that cereals utilise the mineral nitrogen contents of deeper soil levels. To start with, the mineral nitrogen content of the 0-90 cm soil level was taken into

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account when applying nitrogen head dressing in spring. Later, wide-ranging studies proved that winter wheat is capable of efficiently utilising the mineral nitrogen content of the soil to a depth of 150 cm (Kuhlmann, Barraclough and Weir, 1989). The utilisation of the mineral nitrogen present in deeper soil layers was also confirmed in the case of other crops (barley, sugar beet, maize) (De Willigen and Van Noordwijk, 1987). These nitrogen sources were found to be used by maize varieties with nitrogen requirements in the shoot and large root density in the soil layers (Wiesler and Horst, 1994). The uptake of nitrate-N leached into deeper soil layers is taken into consideration by crop models and nitrogen submodels (SOILN) (Jansson et al., 1991).

Under the climatic and soil conditions in Hungary, the annual application of 100 kg/ha N fertiliser (2000 kg/ha/20 years) does not result in any great increase in the nitrate-N content. At higher rates, however, depending on the climatic and soil conditions, there is an exponential rise in the nitrate-N content in the 0–3 m soil layer, both in the 1 m root zone and in the 1–3 m layer below it.

Nitrate-N migration towards the deeper soil layers can be clearly characterised by the depth of maximum nitrate-N accumulation in the profile, which was determined using a Gauss distribution curve. The nitrate-N concentrations recorded every 20 cm in the 0-3 m soil layer were plotted as a function of depth, after which a Gauss curve was fitted. Linear correlation analysis was applied to determine the correlation between the depth of maximum nitrate-N accumulation and the soil texture or rainfall migration.

The depth of maximum nitrate-N accumulation after various rates of mineral fertilisation was closer to the surface in heavier soils. The depth of maximum nitrate-N accumulation did not exhibit a close correlation with the rainfall. There was, however, a clear tendency for higher quantities of rainfall to cause the nitrate-N to move to deeper layers and accumulate there (Füleky, 2009).

The objective of this study is to monitor nitrate-N and ammonium-N distribution in the deeper soil layers of a brown forest soil effected by annual nitrogen fertilizer application for the 1969-2003 period.

Materials and methods

The experiment was set up on a brown forest soil at the experimental station of GödölIő University of Agricultural Sciences at Szárítópuszta in 1969. The physical soil type in the top 60 cm was sand, fol1owed by sandy loam, loam and, at a depth of 200-300 cm, clay or clayey loam. The thickness of the humus layer was 35 cm, with $CaCO_3$ appearing at a depth of 60 cm. The humus content was 1.3 % in the ploughed layer and less than 1 % in lower layers. The parent material was loess, the groundwater level was below 4 m, with a layer of limestone in the soil profile at a depth of around 2 m.

Nitrogen fertilizer was applied in the experiment from autumn 1969 onwards at rising rates of 0, 90, 180, 270, and 360 kg Nha^{-1} in the form of ammonium-nitrate. Phosphorus and potassium fertilizers were applied together in the rates of 0, 60, 120, 240 kg P_2O_5 ha^{-1}y^{-1} and 0, 50, 100, 150, 200 kg K_2O ha^{-1}y^{-1}, respectively. The experiment was not irrigated except of 3 years when 100 mm of water were applied yearly. Maize was sown on the area in a monoculture for 20 years. Yields and the nitrogen content of stalk and grain were measured. Average corn yields were 3.7, 5.1, 5.2, 5.0, 4.6 tha^{-1}, respectively. In autumn 1989 fertilization was discontinued and alfalfa was sown on the experimental area. In 1995 pseudo-acacia was planted into the field. Each year the hay yield and the nitrogen content of alfalfa were recorded. In 1986, 1989, 1994 and 2003 soil samples were taken every 20 cm in 6 replications to a depth of 3 m from the various fertilizer treatments. Nitrate and ammonium content of the soil samples were determined.

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Table 1. Nitrogen fertilizer rates and the amount of accumulated NO_3-N in 1986, 1989, 1994 and 2003, respectively

Nitrogen application rate, N kg $ha^{-1}y^{-1}$	0	90	180	270	360
Total amount of fertilizer nitrogen, N kg ha^{-1}	0	1440	2880	4320	5760
Total N uptake by the maize crops, N kg ha^{-1}	852	1436	1561	1574	1512
Nitrogen balance in 1986 N kg ha^{-1}, after maize	-852	+2	+1319	+2746	+4248
NO_3-N in 3 m soil layer, N kg ha^{-1}	134	298	1125	1189	2051
NO_3-N in 1 m soil layer, N kg ha^{-1}	41	43	205	198	235
NO_3-N in 3 m soil layer in the % of nitrogen balance	-	>100	85	43	48
Nitrogen balance in 1989 N kg ha^{-1}, after maize	-1070	+14	+1666	+3432	+5294
NO_3-N in 3 m soil layer, N kg ha^{-1}	288	628	933	1634	1787
NO_3-N in 1 m soil layer, N kg ha^{-1}	82	132	256	424	397
NO_3-N in 3 m soil layer in the % of nitrogen balance			56	48	34
Nitrogen balance in 1994 N kg ha^{-1}, after alfalfa	-1710	-791	+842	+2525	+4371
NO_3-N in 3 m soil layer, N kg ha^{-1}	90	183	381	625	885
NO_3-N in 1 m soil layer, N kg ha^{-1}	56	61	85	87	95
NO_3-N in 3 m soil layer in the % of nitrogen balance			45	25	20
Nitrogen balance in 2003 N kg ha^{-1}, after forest	Not calculated				
NO_3-N in 3 m soil layer, N kg ha^{-1}	183	223	195	359	324
NO_3-N in 1 m soil layer, N kg ha^{-1}	88	124	117	117	133

Results and discussion

In a long-term fertilization experiment set up in 1969 on a brown forest soil in Gödöllő, the 0-3 m soil layer under a maize monoculture had accumulated a total of 130-2050 kg nitrate-N by 1986 (Table 1.) depending on the rates of nitrogen applied (Füleky and Debreczeni, 1991). Between 1986 and 1989 the quantity of nitrate-N in the soil continued to increase due to the application of unchanged fertilizer rates. In long-term experiments in other parts of the country a similar extent of nitrate-N accumulation was observed (Németh and Buzas, 1990).

Considering the fact that the Gödöllő soil also contained several hundred kg nitrate-N, it was a natural thought to sow alfalfa, which is more deeply rooted than the crops previously used, and has a high nitrogen requirement, in order to utilise the nitrate-N to be found in the deeper layers of the soil. The vertical distribution of nitrate-N is shown in Figure 1.

In the control plot the amount of nitrate-N is only a few kg N ha^{-1} in the 3 meter soil layer. Increasing the rate of nitrogen fertilization the amount of nitrate-N in the soil profile increases. The maximum of nitrate accumulation is found at about 2 meter. Nitrate distribution usually has a minimum at 40-80 cm depth. Nitrogen uptake of plants usually effects of the nitrate shift by 100 cm depth. Nitrate being in deeper horizons practically is lost for plant uptake and moves downwards with water movement. However further significant nitrate enrichment can be expected below 3 meter at 180 kg N ha^{-1} or higher rates of nitrogen fertilization.

Nitrate accumulation shown in Figure 1. can be compared to the nitrogen uptake of maize plants. Yield and nitrogen concentration of grain and stalk are given in Table 1. Data for nitrate accumulation in the soil can be seen in Table 2. Crop yield was found to be increased by90 kg N ha^{-1} in the field. Additional fertilizer rates increased only the N content of grain and stalk but not the yield. In the control treatment and uptake by stalk and grain together was about 53 kg yearly. In other treatments this is fluctuated about 100 kg N ha^{-1}. In control plots than only source of nitrate-N is nitrogen mineralised from the soil organic reserves

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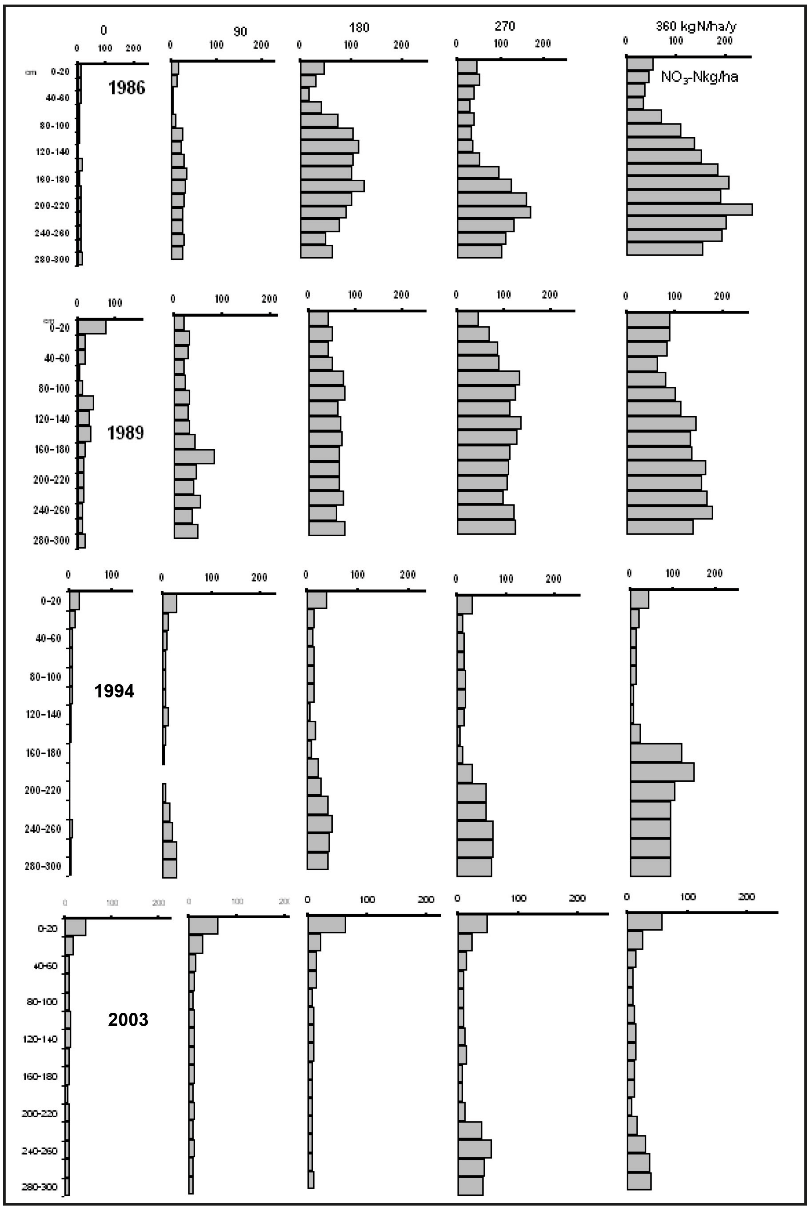
Figure 1. The vertical distribution of NO_3-N in 1986, 1989, 1994 and 2003, respectively



(852 kg N ha^{-1} in 16 years). As it can seen from the nitrogen balance at 90 kg N ha^{-1} fertilizer rata the amount of nitrogen taken up by crop is equal to the nitrogen added in 15 years. Nevertheless, the 3 meter layer of soil profile has remarkable nitrate content (298 kg N ha^{-1}). For this the downwards movement of both the mineralised and fertilizer originated nitrate can be responsible. At 180 kg N ha^{-1} and higher fertilizer rates N balance becomes more positive so a considerable amount of fertilizer nitrogen has to remain after each harvest in the soil. Data for vertical distribution show very significant amount of nitrate accumulation in the 3 meter layer of soil$_1$ at higher fertilizer rates. At 0, 90, 180, 270, 360 kg N ha rates 134, 298, 1125, 1189, 2051 kg nitrate-N ha^{-1} are found in 3 meter soil 1ayer. From these amounts only 41, 43, 205, 198, 235 kg N ha^{-1} are in the upper 1 meter layer which is available for the plant roots. These data suggest that a big amount of nitrate practically lost for plant uptake and its fate is the leaching. The annual rainfall surplus is 116 mm in the average of 16 years and it is enough for leaching the nitrate not taken up by plants.

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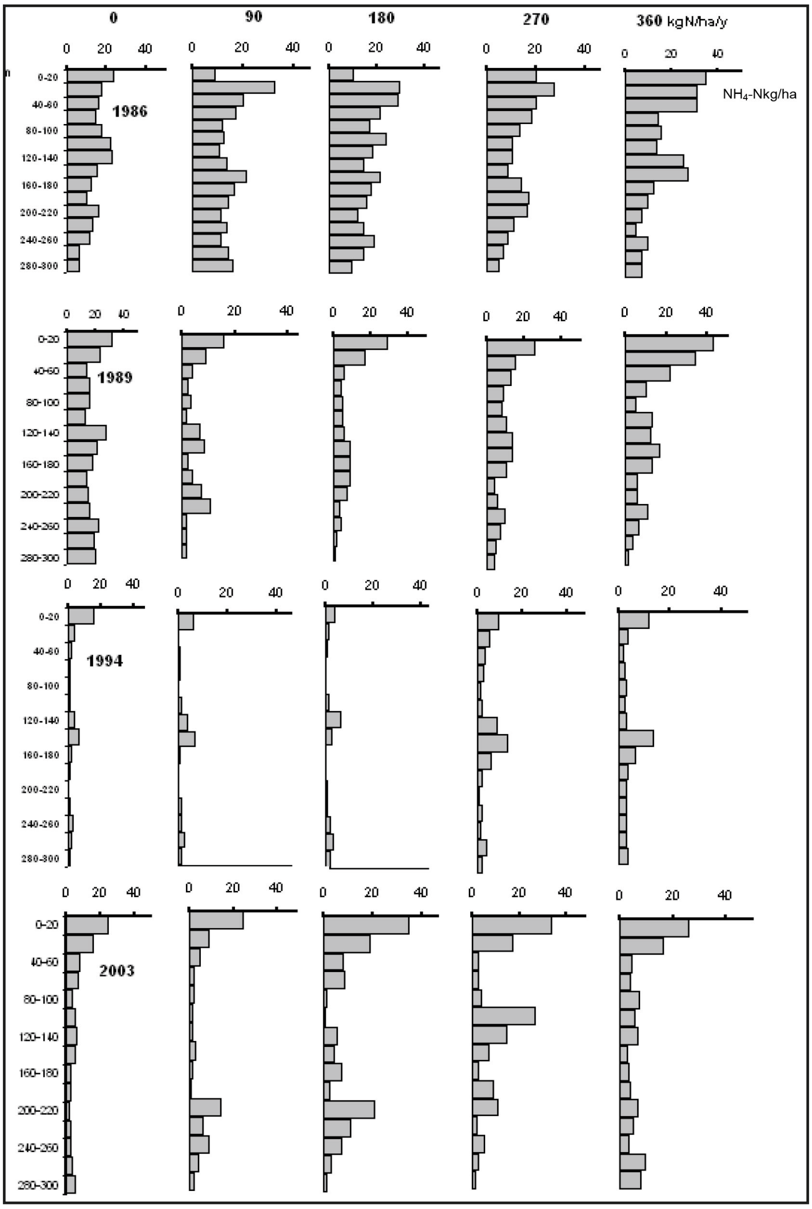
Figure 2. The vertical distribution of NH_4-N in 1986, 1989, 1994 and 2003, respectively



Regarding the nitrate content of 3 meter soil layer in relation to the total amount of applied fertilizer nitrogen, the 20-40% of fertilizer nitrogen can be found in this soil layer. If the amount of nitrate accumulated in the 3 meter 1ayer is related to the "nitrogen balance" .data, at 90 kg N ha^{-1} fertilizer rate the whole amount of nitrogen balance, at 180 kg N ha^{-1} rate 85%, at 270 and 360 kg N ha^{-1} rates 43 and 48% of the "nitrogen ba1ance" can be found in the 3 meter soil layer. As it can be seen in Figure 1 this nitrogen loss is probably not caused by nitrogen volatilization to the atmosphere but by leaching downwards with the drainage water into the deeper soil layers.

At increasing nitrogen rates nitrate accumulation in soil is due to the fact, that at higher fertilizer rates plants take up nitrogen mainly from the fertilizer, so nitrate mineralised from soil organic reserves is exposed to leaching. Application of nitrogen fertilizer in autumn also can be contributory factor tor nitrate leaching into deeper soil horizons.

Table 2. Nitrogen balances and the amount of accumulated NH_4-N in 1986, 1989, 1994 and 2003, respectively

Nitrogen balance in 1986 N kg ha^{-1}, after maize	-852	+2	+1319	+2746	+4248
NH_4-N in 3 m soil layer, N kg ha^{-1}	224	244	285	219	260
NH_4-N in 1 m soil layer, N kg ha^{-1}	90	103	130	110	138
Nitrogen balance in 1989 N kg ha^{-1}, after maize	-1070	+14	+1666	+3432	+5294
NH_4-N in 3 m soil layer, N kg ha^{-1}	288	82	117	150	204
NH_4-N in 1 m soil layer, N kg ha^{-1}	101	35	61	69	114
Nitrogen balance in 1994 N kg ha^{-1}, after alfalfa	-1710	-791	+842	+2525	+4371
NH_4-N in 3 m soil layer, N kg ha^{-1}	40	25	24	65	66
NH_4-N in 1 m soil layer, N kg ha^{-1}	21	7.2	5.1	22	22
Nitrogen balance in 2003 N kg ha^{-1}, after forest	Not calculated				
NH_4-N in 3 m soil layer, N kg ha^{-1}	95	85	133	140	112
NH_4-N in 1 m soil layer, N kg ha^{-1}	58	42	69	60	58

Compared to the average 588 mm precipitation measured over many years, there was a deficiency of 199 mm in 1989-1990, 13 mm in 1990-1991 and 197 mm in 1991-1992, while a surplus of 55 mm was recorded in 1993-1994. Due to this water deficiency and the extremely high water requirements of alfalfa, the available quantity of water was presumably insufficient to allow a greater quantity of nitrate-N to migrate downwards, so the decline in the nitrate-N quantity in the lower soil layers after 4 years of alfalfa production can be attributed to the nitrogen uptake of the alfalfa. As shown by the data on the nitrate-N content of the 3 m soil layer, presented in the Table 1., there was a substantial decrease by 1994 compared with the 1989 figures. In the control treatment the nitrate-N quantity, which was initially already small, decreased to below a value of 10 kg N/ha/20 cm throughout the profile (Figure 1.). In plots previously given rates of 90 and 180 kg N/ha the nitrate-N quantity dropped below 10 kg N/ha/20 cm up to a depth of 220 cm, while this reduction was observed up to 200 cm in the 270 kg N/ha treatment. In the 360 kg N/ha treatment there was a substantial decline in the soil nitrate-N content up to 160 cm. In addition a considerable reduction in the nitrate-N level could be observed throughout the soil profile during the production of alfalfa. In the course of 4 years the alfalfa removed between 550 and 823 kg/ha N from the soil, depending on the original nitrogen rates. At the same time, the loss of nitrate-N content from the 3 m soil layer ranged from 215 to 1040 kg N/ha. In the control plot the available nitrate-N was insufficient to satisfy N requirement of alfalfa, while in plots previously given lower rates of nitrogen fertiliser the quantity of nitrate-N removed from the 3 m soil layer over the 4-year period was roughly equivalent to the quantity of nitrogen absorbed by the alfalfa. In the case of the highest nitrogen fertiliser rates the reduction in the nitrate-N content of the soil exceeded the nitrogen uptake of the alfalfa plants. This fact, observed chiefly in the deepest soil layers after a very large accumulation of nitrate-N, suggests the further migration of nitrate-N to still deeper layers. This is confirmed by examinations on deep-lying roots, showing that alfalfa roots could be found up to a depth of 180 cm, though with a great reduction in mass at lower levels. A gradual reduction in root density is in agreement with the depth of the nitrate-N exhaustion zones previously established. The pseudoacacia trees growing between 1995 and 2003 furthered reduced the quantity of nitrate-N accumulated in the deeper layers. The depletion recorded to a depth of 2 m after alfalfa continued to 2.40 m, and there was also a decline in the nitrate-N quantity in even deeper layers. The ammonium-N quantity

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in the 3 m profile exhibited a slightly different picture to that of nitrate.

In 1986 largely identical quantities of NH_4-N could be found in the 3 m soil profile in all the mineral fertiliser treatments (Figure 2). In the upper layers there was an average of 20 kg N/ha in each 20 cm soil layer, while at lower depths only half this quantity was detected. The total ammonium-N quantity in the 3 m soil profile was around 200 kg. By 1989 this had decreased considerably to 100 kg N/ha, except for the non-fertilised control plot and plots receiving the highest rate of N fertiliser, both of which still had around 200 kg N/ha in the 3 m profile. The depth distribution of NH_4/N was completely different in the two treatments, however, being fairly uniform with depth in the control plot, but with a tendency to decrease with depth at the highest fertiliser rate (Table 2).

In 1994, after the alfalfa crop, hardly any NH_4-N could be detected in the 3 m soil profile, partly due to the lack of mineral fertilisation and partly due to the nitrogen uptake of alfalfa. The pseudoacacia trees grown on the area between 1995 and 2003, however, led to the reappearance of NH_4-N in the profile, though not in large quantities. In all the treatments the quantity of NH_4-N was greatest in the upper 40 cm, derived from the substantial N content of the falling leaves. In deeper soil layers NH_4-N was probably formed via the decomposition of dead roots, and in the surface layers by the mineralisation of the leaves. The soil did not contain sufficient nitrate-N to satisfy the requirements of the trees, so they were forced to supply their needs through nitrogen fixation, the result of which could be detected in the NH_4-N content of lower soil layers.

References

Banabas M, Scotter DR, Turner MA. (2008): Losses of nitrogen fertiliser under oil palm in Papua New Guinea: 2. Nitrogen transformations and leaching, and a residence time model. Australian Journal of Soil Research 46 (4) 340-347 pp. DOI: http://dx.doi.org/10.1071/sr07174

de Vos JA, Hesterberg D, Raats PAC (2000): Nitrate leaching in a tile-drained silt loam soil. Soil Science Society of America journal 64 (2) 517-527 pp. DOI: http://dx.doi.org/10.2136/sssaj2000.642517x

De Willigen P. and Van Noordwik M. (1987): Root plant production and nutrient use efficiency. Ph.D. thesis, Agric. Univ. Wageningen, 282 p.

Di HJ, Cameron KC (2002): Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64 (3) 237-256 pp. DOI: http://dx.doi.org/10.1023/a:1021471531188

Fan J, Hao MD, Shao MA (2003): Nitrate accumulation in soil profile of dry land farming in northwest China. Pedosphere 13 (4) 367-374 pp.

Füleky Gy.. and Debreczeni B. (1991): Nutrient accumulations is the soil of a 17-year maize rnonoculture. (Hun), Agrokémia és Talajtan, 40. 119-130.

Füleky Gy. (2009): Downward movement of fertilizer nitrogen in Hungarian soils. In: Nawozy I nawozenie fertilizers and fertilization, Polskie Towarzystwo nawozowe Polish Fertilizer Society – CIEC., No. 37/2009. 73-89 pp.

Jansson P.E., Eckersten H., Johnsson H. (1991): SOILN model. User s manual. Comn. 91:6. Swedish Agric. Univ. 50 pp.

Kuhlmann H., Barraclough P.B. and Weir A.H. (1989): Utilization of mineral nitrogen in the subsoil by winter wheal, Z. Pflanzenenahr. Bodenk. 152. 291-295. DOI: http://dx.doi.org/10.1002/jpln.19891520305

Németh T. and Buzas I. (1990): Influence of nitrogen fertilization on the N03-N content of soil profiles, Swedish-Hungarian Seminar, Stockholm 1990, 169-1 86.

Paramasivam S, Alva AK, Fares A, Sajwan KS (2001): Estimation of nitrate leaching in an entisol under optimum citrus production. Soil Science Society of America Journal 65 (3) 914-921 pp. DOI: http://dx.doi.org/10.2136/sssaj2001.653914x

Owen. Tr., Jürgens-Gschwind S. (1986): Nitrates in drinking water: a review. Fertilizer Research, 10. 3-25. DOI: http://dx.doi.org/10.1007/bf01073902

Stumborg C, Schoenau JJ, Malhi SS (2007): Nitrogen balance and accumulation pattern in three contrasting prairie soils receiving repeated applications of liquid swine and solid cattle manure. Nutrient Cycling in Agroecosystems 78 (1) 15-25 pp. DOI: http://dx.doi.org/10.1007/s10705-006-9071-5

Sun B, Chen DL, Li Y, Wang XX (2008): Nitrogen leaching in an upland cropping system on an acid soil in subtropical China: lysimeter measurements and simulation. Nutrient Cycling in Agroecosystems 81 (3) 291-303 pp. DOI: http://dx.doi.org/10.1007/s10705-008-9164-4

Tarkalson D, Payero JO, Ensley SM, Shapiro CA (2006): Nitrate accumulation and movement under deficit irrigation in soil receiving cattle manure and commercial fertilizer. Agricultural Water Management 85 (1-2) 201-210 pp. DOI: http://dx.doi.org/10.1016/j.agwat.2006.04.005

Wehrmann J. and Scharpf (1979): Der Mineralstickstoffgehalt des Bodens als Masstab für den Stickstoffdüngerbedarf (Nmin-Methode), Plant and Soil. 52. 109-126. DOI: http://dx.doi.org/10.1007/bf02197737

Whetter DA, Bullock PR, Eilers RG (2006): Long-term solute redistribution in relation to landscape morphology and soil distribution in a variable glacial till landscape. Canadian Journal of Soil Science 86 (5) 827-840. DOI: http://dx.doi.org/10.4141/s06-008

Wiesler F. and Horst W.J. (1994): Root growth and nitrate utilization of maize cultivars under field conditions, Plant and Soil. 163. 267-277. DOI: http://dx.doi.org/10.1007/bf00007976

Yang SM, Li FM, Malhi SS, Wang P, Suo DR, Wang JG (2003): Long-term fertilization effects on crop yield and nitrate nitrogen accumulation in soil in northwestern China. Agronomy Journal 96 (4) 1039-1049 pp. DOI: http://dx.doi.org/10.2134/agronj2004.1039

Yang SM, Malhi SS, Song JR, Xiong YC, Yue WY, Lu LL, Wang JG, Guo TW (2006): Crop yield, nitrogen uptake and nitrate-nitrogen accumulation in soil as affected by 23 annual applications of fertilizer and manure in the rainfed region of Northwestern China. Nutrient Cycling in Agroecosystems 76 (1) 81-94 pp. DOI: http://dx.doi.org/10.1007/s10705-006-9042-x

Yin F, Fu BJ, Mao RZ. (2007): Effects of nitrogen fertilizer application rates on nitrate nitrogen distribution in saline soil in the Hai River Basin, China. Journal of Soils and Sediments 7 (3) 136-142 pp. DOI: http://dx.doi.org/10.1065/jss2007.04.218

Young EO, Briggs RD (2007): Nitrogen dynamics among cropland and riparian buffers: Soil-landscape influences, Journal of Environmental Quality 36 (3) 801-814 pp. DOI: http://dx.doi.org/10.2134/jeq2006.0270

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ASSESSMENT OF THE ANTIFUNGAL POTENTIAL OF THE ESSENTIAL OIL FROM *Thymus vulgaris* AGAINST *Botrytis cinerea* CAUSATIVE AGENT OF POSTHARVEST GREY MOULD ON STRAWBERRY FRUITS

Mark Piero GEBEL – Franco MAGURNO

Microbiology and Environmental Toxicology Group, Institute of Plant Protection, Szent István University, H-2100 Gödöllő, Hungary
email: Franco.Magurno@mkk.szie.hu, franco.magurno@gmail.com (corresponding author), mark.gebel@hotmail.com

Abstract
Depending on commodity and region, postharvest diseases can cause considerable economic losses. Although effective against postharvest pathogens, synthetic pesticides have gained increasingly negative attention over the past years from the public opinion. Among natural fungicides, plant derived compounds such as essential oils (EOs), have become topic of increasing discussion over the last couple of decades as safe and ecologically friendly alternatives to synthetic pesticides. Grey mould caused by *Botrytis cinerea* is an important postharvest disease of strawberries and many other horticultural products. In the present study the antifungal potential of *Thymus vulgaris* essential oil was tested on individual strawberries artificially inoculated with *Botrytis cinerea.* Two dilutions of thyme essential oil, 200 µl/ml and 500 µl/ml, were applied to strawberry fruits and incubated for a period of 13 days. The dilution 500 µl/ml effectively suppressed fungal growth in strawberries for a period of 7 days, more than twice as long as the control group. Furthermore only one out of five replications in the 500 µl/ml treatment was visibly infected while the others remained free from infection for the entirety of the experiment. At experiment conclusion the strawberries in the 500 µl/ml treatment maintained a healthier shape, firmness, and colour compared to the control and 200 µl/ml treatment. This study has confirmed that the application of thyme essential oil could be an efficient biocontrol tool in postharvest stage.

Keywords: *Botrytis cinerea*, strawberry, thyme, essential oil

Introduction

Due to the perishable nature of fruits and vegetables postharvest handling presents a challenge, in particular when the commodities need to spend time in storage or transit before being available to the consumer. Depending on commodity and region, losses due to postharvest diseases can amount to as much as 50% in developing countries, representing considerable economic damages to plant products (Agrios, 2005). One of the most important factors responsible for degradation of fresh produce is decay by microorganisms, in particular by fungi (Chang et al., 2008). Infections caused during post- harvest conditions shorten the shelf life and adversely affect the market value of fruits (Tripathi et al., 2008). Therefore, most postharvest preserving agents are aimed at inhibiting or suppressing microbial growth. Physical agents, such as refrigeration and modified atmosphere, synthetic pesticides and biological control agents (BCAs) are some common practices used in the control of postharvest pathogens. Although effective, synthetic pesticides are gaining increasingly negative attention due to the concerns of health and environmental nature (Shao et al., 2013). Pesticides have been proven to disrupt the equilibrium of eco-systems, causing disease outbreak and toxicity to non-target organisms. Pesticide residues in the food are more carcinogenic compared to herbicides and insecticides and the accumulations of these residues in the food chain can sometimes reach levels above safety limits (Research Council - Board of Agriculture, 1987). Furthermore the development of pathogen strains resistant to synthetic pesticides can render them useless (Georgopoulos, 1987). To address these growing concerns the trend is to explore new ways to contrast postharvest pathogens efficiently while reducing environmental pollution and public

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health risks. Much attention has been paid to the development of biological control methods of postharvest diseases in order to substitute synthetic pesticides. Some viable biological control methods used in the control of postharvest decay in fresh fruits and vegetables are application of antagonistic microorganisms such as yeasts and bacteria, the use of naturally occurring and/or non-polluting fungicidal agents (Janisiewicz and Korsten, 2002). Among natural fungicides, plant derived compounds such as essential oils (EOs), have become topic of increasing discussion over the last couple of decades. These oils are present as variable mixtures of primarily terpenoids, fatty acids, alcohols, aldehydes, aliphatic hydrocarbons, acyclic esters or lactones, coumarins and homologues of phenylpropanoids (Nazzaro et al., 2013). Extracts and oils alike of plant such as thymus, clove, cinnamon, oregano, lemongrass and many others, have all been proven to possess anti-microbial properties and are able to suppress the development of many postharvest pathogens in vitro and in vivo (Barbosa et al., 2009; Camele et al., 2012; Nuzhat and Vidyasagar, 2013). Furthermore, the use of naturally derived plant compounds, mainly essential oils, can be an efficient method to address areas where pathogens have developed resistance against pre-existing synthetic pesticides (Castillo et al., 2014).

Botrytis cinerea is an important postharvest pathogen of many horticultural crops causing major losses during storage and transport (McFeeters and McFeeters, 2012). Strawberries (*Fragaria* x *ananassa*) are very prone to infection by *Botrytis cinerea*, the causative agent of grey mould, due to their high pH, water content and the large amount of nutrients (Zamani-Zadeh et al., 2014). Due to its enormous reproduction rate by conidia and its high genetic adaptability, *Botrytis cinerea* has been classified as a high-risk pathogen with respect to fungicide resistance (Agrios, 2005) with many strains resistant to multiple (up to six) fungicides (Leroch et al., 2013).

The aim of the present study was to assess the antifungal potential of essential oil from thyme (*Thymus vulgaris*) against the grey mould on strawberry fruits. Thyme essential oil is a common product, easy to produce and cheap. It was reported by numerous investigators to have strong fungistatic and fungicidal effect on several pathogens (Bhaskara et al., 1998; Camele et al., 2012). Therefore it could be considered a valuable candidate in order to provide a postharvest protection in strawberry, where the use of synthetic pesticides is prohibited in Europe by law (Mari et al., 2014).

Materials and Methods

Plant Material

Strawberry fruits were purchased from local market (Gödöllő, Hungary) on the same day they were to be used for experimental purposes. Twenty-five berries were identified based on uniformity of size, ripeness and freedom from injury. They were surface sterilized using 2.5% sodium hypochlorite solution with exposure time of 3 minutes, washed with distilled water for 5 minutes and allowed to dry under sterile conditions.

Fungal inoculum

Botrytis cinerea strain BC4 was isolated from grape in the Plant Protection Institute of the Szent István University, Gödöllő. The strain was re-cultured on tomato-agar for two weeks at room temperature and stored at 4°C prior to use. In order to prepare the spore suspension, culture plates were washed using 0.8% Tween solution. The conidia collected were counted in a Burker chamber and the concentration was adjusted to 10^6 conidia/ml. Prior to inoculation a small wound was inflicted on the strawberry using a needle tip. Each individual berry was inoculated with 10µl of spore suspension. At experiment conclusion *Botrytis cinerea* was re-isolated from infected strawberries in order to confirm its identity under stereomicroscope at 100X magnification.

Constituent analysis and description of the essential oil

The essential oil from *Thymus vulgaris* was produced through steam distillation by Aromax

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Table 1. Constituents analysis of *Thymus vulgaris* essential oil provided by Aromax Inc.

Compound	Composition (%)
2-isopropyl-5-methlyphenol (thymol)	25 – 49.99
p-cymene	20 – 24.99
p-mentha-1,4-diene	5 – 9.99
Linalool	5 – 9.99
1,8 Cineol	2.5 - 5
p-mentha-1,3-diene	2.5 - 5
β-caryophyllene	2.5 - 5
Carvacrol	2.5 - 5
Myrcene	1 – 2.49
Camphene	1 – 2.49
α-pinene	<1
2,6-octadien-1-ol-3,7-dimethylacetate	<1
Dipentene	<1
Terpinolene	<1
3,7-dimethylocta2,6-dien-1-ole	<1
β-pinene	<1

Inc. (Budapest, Hungary). The EO was stored in a dark glass vial at room temperature. The EO dilutions with sterile distilled water were stored at 4°C prior to use. The relative composition of thyme essential oil is reported in the Table 1. The two principal constituents were thymol and p-cymene.

Experimental design

All equipment used in the experiment was disinfected using ethanol and UV radiation to minimize the presence of microorganisms foreign to the inoculum. Two dilutions of thyme essential oil in sterile distilled water, 200 µl/ml and 500 µl/ml respectively, were tested and compared with a control treatment (CON, sterile distilled water instead of EO). For each treatment five single berries as replications were placed after fungal inoculation in a 50 ml plastic container with a cellulose filter applied on the top, in a way to not come in contact with the berry. Totally fifteen berries were used in the experiment. The cellulose filters used were 1.5 cm long and 1.1 cm in diameter. A not-overflowing volume of 300 µl of EO dilution or sterile distilled water was used to saturate the filters in each container. To avoid over exposure of EO and build up of carbon dioxide in the containers the caps of the plastic containers were loosened approximately 8 hours after the inoculation and remained this way for the duration of the trial. The containers were stored at 13°C for the length of the experiment.

Evaluation of infection

Following inoculation the berries were checked every day for a period of 13 days. Documentation was carried out using a digital camera and pictures were retrieved in a standardized fashion in order to use them later for extrapolation of data. Spatial analysis was carried out using Analyzing Digital Images (ADI) suite software (http://www.umassk12.net/adi/). The diameter of the infection was measured across the inoculation point and then recorded in a database.

Statistical analysis

The statistical analysis was carried out using SPSS software version 22 (IBM). The normality of the distribution and homogeneity of variance were assessed using the Kolmogorov–Smirnov test (K–S test) and Levene test respectively. These tests were used as pre-condition tests. If p-value value from the K-S test and p-value from the Levene test were found to be less than 0.05 the data was analyzed using the non-parametric Kruskal-Wallis test, which is better suited for asymmetric distributions and/or non-homogenous data. In addition, the Mann-Whitney U test was used as a post-hoc test for pairwise comparison between treatment groups.

Results and Discussion

In the present assay the effect of thyme oil was tested on the control of infection by *Botrytis cinerea* in garden strawberry (*Fragaria x ananassa*). Thyme oil volatiles proved to be highly effective in reducing gray mould incidence in strawberry fruits (Figure 1).

The development of infection day by day in the two EO treatments and in the control group (CON) is showed in the Figure 2. The table below the graph illustrates the average area (mm²) of infection of each treatment during the course of the experiment. A cut-off point was put in place when the area of the infection reached 706.9 mm² based on the surface size of the strawberries (it was not possible to measure the diameter of infection beyond this point as the mould covered the entire surface of the strawberry).

In the control group the infection by *Botrytis cinerea* started 3 days after inoculation. In the 200 µl/ml treatment infection began on day 5 while in 500 µl/ml treatment the infection began on day 8 of the experiment. At experiment end the strawberries belonging to the 500 µl/ml treatment maintained an overall better condition than the 200 µl/ml treatment and the control group. There was some water logging in a few of the replications by the end of the experiment and clearly the fruits had become paler and lost some firmness. However, this phenomenon was less noticeable in the 500 µl/ml treatment, where half of the replications maintained a fairly health physical appearance without any browning or water soaking at all on the surface of the fruits. Out of 5 replications, in the 500 µl/ml

Figure 1. Appearance of fruits and progression of grey mold infection throughout the experiment (days: 4, 8, 13). In the figure one berry per treatment is shown as an example. Five single berries inoculated with *Botrytis cinerea* per treatments: control (berries not exposed to thyme EO), 200 µl/ml and 500 µl/ml (berries exposed to thyme EO diluted in distilled water 1:2 and 1:5 respectively)

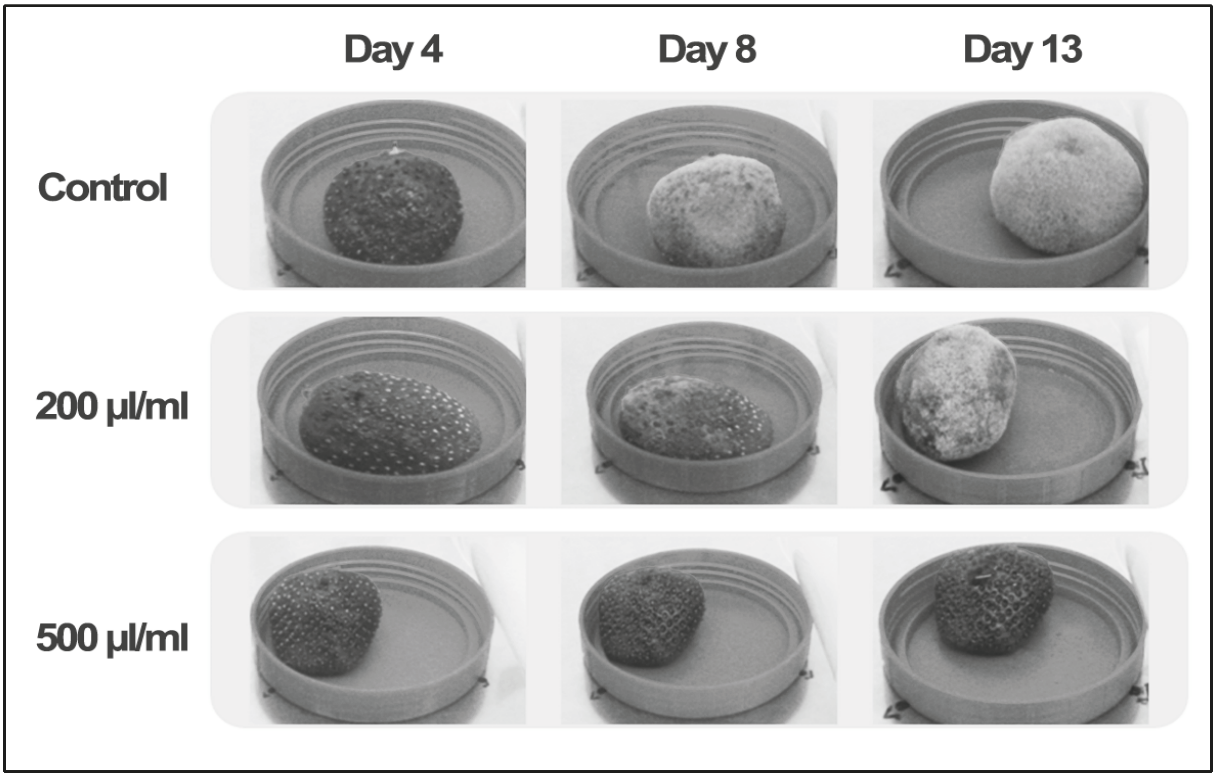

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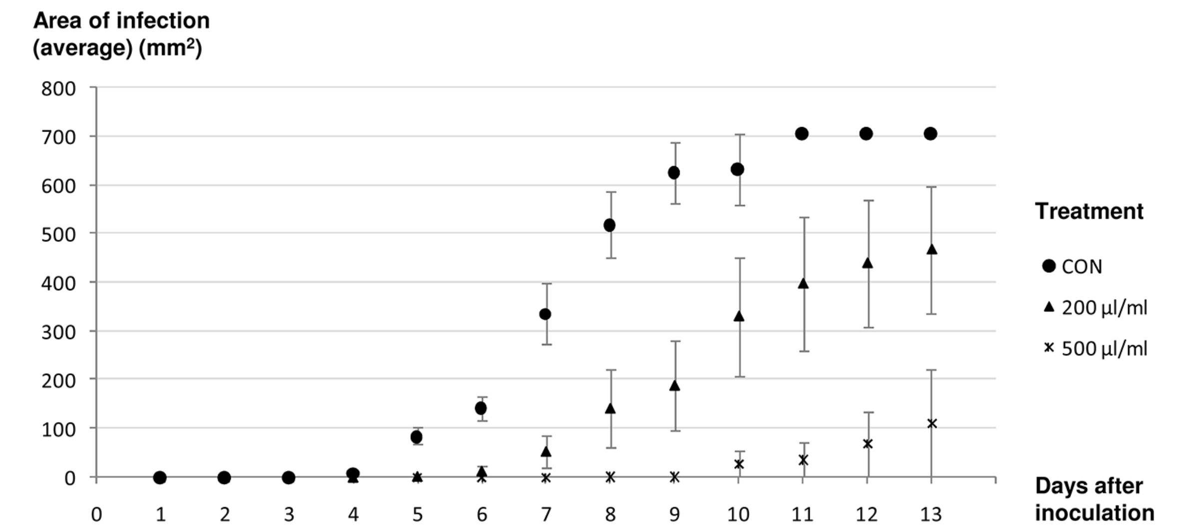
Figure 2. Development of infection by *Botrytis cinerea* in the Control treatment (CON) and in the thyme EO treatments at two dilutions (200 and 500 µl/ml). The average value of area infected in each treatment group is reported per every day of the experiment (the exact values are given in the table below the diagram). The error bars represent the standard deviation.



CON	0	0	1.24	8.76	85.13	142.37	336.45	518.12	625.26	632.80	706.86	706.86	706.86
200 µl/ml	0	0	0	0	1.81	12.68	53.1	141.42	188.39	330.94	397.83	439.77	467.86
500 µl/ml	0	0	0	0	0	0	0	0.63	0.98	26.55	36.29	67.96	110.31

treatment, only one developed mycelium on it, the others remained free from visible infection. Even though one of the replications in the 500 µl/ml treatment developed mycelium the average size of infection was approximately four times less than the 200 µl/ml treatment and seven times less than the control group at experiment conclusion (on day 13). The difference between the 200 µl/ml treatment and the control group was not very substantial. By the end of the experiment most of the berries in 200 µl/ml treatment were completely covered with grey mould. However, the effect of the oil, even though not as remarkable as in the 500 µl/ml treatment, was still visible.

After performing a pre-condition K-S test, the hypothesis of normality was rejected for all three treatment groups (including control group), with all p-values <0.05, and therefore the conditions for applying a parametric test were rejected. This was further confirmed by a second pre-condition test, the Levene test for homogeneity of variances, whose p-value was also <0.05. After performing the Kruskal-Wallis test, for non-parametric analysis of variance, a significant difference was found between the three treatment groups caused by the use of

Table 2. Kruskal-Wallis non-parametric analysis of variance test results. p-value (Asymp. Sig.) = 0.000 < 0.05; null hypothesis (all populations have identical distribution functions) is rejected

Ranks				Test Statistics	
	Treatment	**N**	**Mean Rank**		**Area**
Area	control	65	132.88	Chi-Square	60.895
	200 µl/ml	65	97.85	df	2
	500 µl/ml	65	63.26	Asymp. Sig.	.000
	Total	195			

different concentrations of EO. The p-value was <0.05 (Table 2). Since the effect of EO treatments was significant, a post-hoc Mann-Whitney U test was run on the data in order to compare between the different treatment groups. The p-values of all three comparisons performed by the Mann-Whitney U test were all <0.05 (Table 3), meaning that there was a significant effect of the presence and difference in concentration of essential oil treatment across all three groups.

In conclusion the present study demonstrated the efficiency of a commercial thyme oil volatiles in delaying significantly the occurrence of fungal infection in strawberries inoculated with *Botrytis cinerea*. The mechanism of action was not investigated but probably the oil volatiles operated reducing primarily conidial germination (Bhaskara et al., 1998). Considering the data from single replications across the different treatments (data not shown) the general trend was a significant effect of the oil volatiles on the initial development of the infection. Once the mycelium started to be visible the infection spread with an exponential rate. In particular the dilution 500 µl/ml resulted to be highly effective since the symptoms of infection were visible only in one replication on five. Our results confirmed the antifungal and food preservative properties of thyme oil observed by other authors (Bhaskara et al., 1998; Zamani-Zadeh et al., 2014). Fruits in the 500 µl/ml maintained better physical qualities such as fruit firmness and reduced water loss compared to other treatments, throughout the duration of the experiment. Furthermore, since the inoculum that was used (10^6 conidia/ml) was very concentrated it can be assumed that thyme EO tested could be a perfectly viable method to suppress postharvest development of *Botrytis cinerea*, even if a sensory quality analyses of fruits should be performed in future assays.

Table 3. Mann-Whitney U test for the pairwise comparison of the treatments: Control treatment (Con) and the thyme EO treatments at two dilutions (200 and 500 µl/ml). p-value (Asymp. Sig. (2-tailed)) = 0.000 < 0.05 in all the three comparisons; null hypothesis (populations have identical distribution functions) is rejected. According to the Z value the thyme EO treatment 500 µl/ml was found the most effective in controlling the development of the infection.

Test Statistics	**Con vs 200 µl/ml**	**Con vs 500 µl/ml**	**200 vs 500 µl/ml**
	Area	**Area**	**Area**
Mann-Whitney U	1322.500	635.000	1332.000
Wilcoxon W	3467.500	2780.000	3477.000
Z	-3.825	-7.677	-4.655
Asymp. Sig. (2-tailed)	.000	.000	.000

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References

Agrios, G.N. (2005): Plant Pathology's Contribution to Crops and Society, in: Plant Pathology, fifth ed., Academic Press, San Diego, CA, 67.

Barbosa, L.N., Rall, V.L., Fernandes, A.A., Ushimaru, P.I., da Silva Probst, I., Fernandes, A. (2009): Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathogens and Disease. **6**: 6. 725-728. DOI: http://dx.doi.org/10.1089/fpd.2009.0282

Bhaskara Reddy, M.V., Angers, P., Gosselin, A., Arul, J. (1998): Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochemistry. **47**: 8. 1515-1520. DOI: http://dx.doi.org/10.1016/s0031-9422(97)00795-4

 DOI: 10.18380/SZIE.COLUM.2014.1.2.17

Camele, I., Altieri, L., De Martino, L., De Feo, V., Mancini, E., Rana, G.L. (2012): In vitro control of postharvest fruit rot fungi by some plant essential oil components. International Journal of Molecular Sciences. **13**: 2. 2290-2300. DOI: http://dx.doi.org/10.3390/ijms13022290

Castillo, S., Pérez-Alfonso, C.O., Martínez-Romero, D., Guillén, F., Serrano, M., Valero, D. (2014): The essential oils thymol and carvacrol applied in the packing lines avoid lemon spoilage and maintain quality during storage. Food Control. **35**: 1. 132-136. DOI: http://dx.doi.org/10.1016/j.foodcont.2013.06.052

Chang, H.T., Cheng, Y.H., Wua, C.L., Chang, S.T., Chang, T.T., Su, Y.C. (2008): Antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* Florin leaf against plant pathogenic fungi. Bioresource Technology. **99**. 6266-6270. DOI: http://dx.doi.org/10.1016/j.biortech.2007.12.005

Georgopoulos, S.G. (1987): The development of fungicide resistance, in: Populations of Plant Pathogens: Their Dynamics and Genetics, Blackwell, 239-251.

Janisiewicz, W.J., Korsten, L. (2002): Biological control of postharvest diseases of fruits. Annual Review of Phytopathology. **40**. 411-441. DOI: http://dx.doi.org/10.1146/annurev.phyto.40.120401.130158

Leroch, M., Plesken, C., Weber, R.W., Kauff, F., Scalliet, G., Hahn, M. (2013): Gray mould population in German strawberries fields are resistant to multiple fungicides and dominated by a novel clade closely related to *Botrytis cinerea*. Applied and Environmental Microbiology. **79**. 159-167. DOI: http://dx.doi.org/10.1128/aem.02655-12

Mari, M., Di Francesco, A., Bertolini, P. (2014): Control of fruit postharvest diseases: old issues and innovative approaches. Stewart Postharvest Review. **10**: 1. 1-4. DOI: http://dx.doi.org/10.2212/spr.2014.1.1

McFeeters, H., McFeeters, R.L. (2012): Emerging approaches to inhibit *Botrytis cinerea*. International Journal of Modern Botany. **2**: 5. 127-144. DOI: http://dx.doi.org/10.5923/j.ijmb.20120205.02

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V. (2013): Effect of Essential Oils on Pathogenic Bacteria. Pharmaceuticals. **6**: 12. 1451-1474. DOI: http://dx.doi.org/10.3390/ph6121451

Nuzhat, T., Vidyasagar, G.M. (2013): Antifungal investigations on plant essential oils. A review. International Journal of Pharmacy & Pharmaceutical Sciences. **5**. 2-9.

Research Council, Board of Agriculture (1987): Regulating Pesticides in Food – The Delaney paradox, National Academy Press, Washington, DC, 288. DOI: http://dx.doi.org/10.1017/s088918930000165x

Shao, X., Wang, H., Xu, F., Cheng, S. (2013): Effects and possible mechanisms of tea tree oil vapor treatment on the main disease in postharvest strawberry fruit. Postharvest Biology and Technology. **77**. 94-101. DOI: http://dx.doi.org/10.1016/j.postharvbio.2012.11.010

Tripathi, P., Dubey, N.K., Shukla, A.K. (2008): Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*. World Journal of Microbiology and Biotechnology. **24**. 39-46. DOI: http://dx.doi.org/10.1007/s11274-007-9435-2

Zamani-Zadeh, M., Soleimanian-Zad, S., Sheikh-Zeinoddin, M., Goli, S.A.H. (2014): Integration of *Lactobacillus plantarum* A7 with thyme and cumin essential oils as a potential biocontrol tool for gray mold rot on strawberry fruit. Postharvest Biology and Technology. **92**. 149-156. DOI: http://dx.doi.org/10.1016/j.postharvbio.2014.01.019

ARCHAEOBOTANICAL OVERVIEW OF RYE (*Secale cereale* L.) IN THE CARPATHIAN-BASIN I. FROM THE BEGINNING UNTIL THE ROMAN AGE

Ferenc GYULAI

Szent István University, MKK Department of Nature Conservation and Landscape Ecology, H-2103 Gödöllő;
E-mail: Gyulai.Ferenc@kti.szie.hu

Abstract
It seems that rye is a twice domesticated cereal. Then, in the course of the Early Iron Age in Central or Eastern Europe, it is again transformed from weed to crop plant. We summarized the development of rye and his spread in the Carpathian Basin based on archaeobotanical data. The grains of the rye were found always in charcoal form. The rye appeared in Carpathian Basin during the Neolithic Age. Sporadic occurrences of rye were also found in Early Bronze Age Bell Beaker-Csepel Group and Early Iron age Scythian cultures. The prehistoric grains were small and longshaped. In the Prehistoric Ages rye should be exist as weed of hulled wheats. His cultivation started in the Roman Age. The grains found in roman sites are always big and reel shaped like recently. Only a few grains were found in the Migration Period. Among cereals of the conquering Hungarians rye is present. Rye became common product in the Late Medieval Ages. It was grown in a big quantity everywhere, but not independently, but mixed with the wheat.

Keywords: rye, archaeobotany, macroremains, Carpathian-Basin

Origin of rye

Rye (*Secale cereale* L.) is a cereal of the temperate zone of the Old World. It is primarily grown in the cooler regions of Northern and Western Europe. Compared to wheat, it is less sensitive to cold and to drought. Moreover, it can survive even in acidic and sandy soils, conditions that would be unsuitable for wheat. It is an allogamous, diploid (2n=14) grass pollinated by wind (Evans 1976). Its seeds have high protein content and are suitable for making dough. It was grown as a single crop in Northern and Western Europe, as it is less sensitive to cold and dry winters, survives on acidic soils and it is the grain of sandy soils. In other words, it endures conditions that are not suitable for wheat. The protein content of bread grains is high, therefore suitable for making dough.

The primary gene centre of the *Secale* genus is central and West Asia. Despite the large number of studies, the taxonomical organizing of the genus still open. The earlier ancestor *S. sylvestre* Host. and *S. montanum* Guss. do not cross well with *S. cereale*, so they are unlikely to have been genome donors. Based on cytological, ecological and morphological studies the cultivated rye developed with introgressive hybridization of *S. montanum* into *S. vavilovii*. The species *Secale vavilovii* derived from *S. sylvestre*. Other wild species: *Secale africanum*, *S. dalmaticum*, *S. ciliatoglume*, *S. kuprijanovii* modified isolated from *S. montanum*. Also known as *S. anatolicum* which is weedy forms of *S. montanum* (Stutz 1972). In fact, *Secale cereale* contains various wild and cultivated subspecies that may produce fertile hybrids with each other.

Within the species *Secale* many subspecies and variety are located, therefore, a generic name. Four main races are distinguished (Hopf et al. 2012):

- cultivated plant: its earspindle is not fragile, its seeds are large (*S. cereale* subsp. *cereale*).
- weed-type wild plant: its earspindle is not fragile, it is formed from a weed, *S. segetale* (Zhuk.) Roshev. It also invades bread wheat fields, but in bad years it is the only harvest in Turkey, Syria, Iraq, Iran and the countries of the Balkans.
- weed-type wild plant with semi-fragile earspindle (only the top part of the ear is fragile). This race includes various populations.

S. afghanicum (Vav.) Roshev. occurs in NE-Iran, Afghanistan and Transcaucasia.

- weed-type wild plant with fragile earspindle: *S. ancestrale* Zhuk., and *S. vavilovii* Zoh. occurs in various areas of the Middle East.

From the relative numerous macroremains outlined the history of rye. Rye is indigenous to Near East and migrated to Central and Eastern Europe as weed together with other cereals during the early Neolithic. It first appeared in the early Neolithic in Anatolia but disappeared during the Bronze Age (Behre 1992). Rye appears in Western Europe during the Bronze Age Urnfield culture (Chambers & Jones 1984), but was still cereal weed. At this time, the small rye grain was general. The long period of time as weed in the cereal cultivation and the accompanying processes of selection have resulted the cultivated rye. Rye changed from weed to crop probably in the Iron Age here in Europe. Probably the earliest period of rye cultivation was the late Celtic Age. In the La Tène sites in both large and small grain rye were found. It was cultivated in Roman Age on the conquered territories. In the provinces also appeared on the big grain. Period of intense spread of rye is the Middle Ages rye.

Neolithic and Bronze Age finds of rye in Near East are very rare:

- Tell Abu Hureyra/Northern Syria, epipalaeolithic layer (9000 BC) yielded remains with fragile earspindles, probably *S. montanum* Guss. (Hillman 1975),
- Can Hasan III/Turkey, pre-pottery Neolithic layer yielded primitive cultivated form with nonfragile earspindle (Hillman 1978),
- Alaca Höyük/Northern Middle Anatolia/Turkey: Bronze Age, cultivated rye remains were found (Hillman ibid.).
- Asvan Kale/Eastern Anatolia/Turkey: Early Iron Age, cultivated rye (Hillman ibid.).

Most important rye remains in prehistoric European sites:

Neolithic

- Marbach/Württemberg, Linearbandkeramik (4440 BC) (Piening 1982),
- Bruchenbrücken near Frankfurt, Linearbandkeramik (Kreuz 1991),
- Vösendorf near Vienna Linearbandkeramik (Werneck 1951),
- Several places in Poland, Linearbandkeramik (Klichowska 1975),
- Runnymede in England, Linearbandkeramik (Greig 1991),
- Myrehead in Scotland, Linearbandkeramik (Barclay & Fairweather 1984),

Bronze Age

- Federsee in southern Germany (Hopf & Blankenhorn 1987),
- Nitriansky Hrádok, Rajhrad, Nesovice (Tempír 1966, 1968, 1969),
- Šlapanice near Brno (Kühn 1981),
- Diebeshöhle south Harz, Aunjetitzer culture (2300-1600/1500 BC) (in Behre 1992)
- Mošorin-Feudvár/Tisza, Late Bronze Age Urnfield culture (12th to 9th centuries BC) (Kroll 1990),
- Rhine area, Late Bronze Age Urnfield culture (Werneck 1954),
- Myrehead in Scotland, Late Bronze Age (Barclay & Fairweather 1984),
- Several places in Georgia, Ukraine. Moldavia, Poland (Wasylikowa et al. 1991),

Early Iron Age

- Thunau/Kamp, Austria, Hallstatt Period, (8th century BC) (Werneck 1954),
- Northern Württemberg, Hallstatt Period (Körber-Grohne & Piening 1979),
- Bnin, Hallstatt Period ((Wasylikowa et al. 1991),
- Carrowmore in Ireland (2480 ± 55 BP) (Hjelmqvist 1980),

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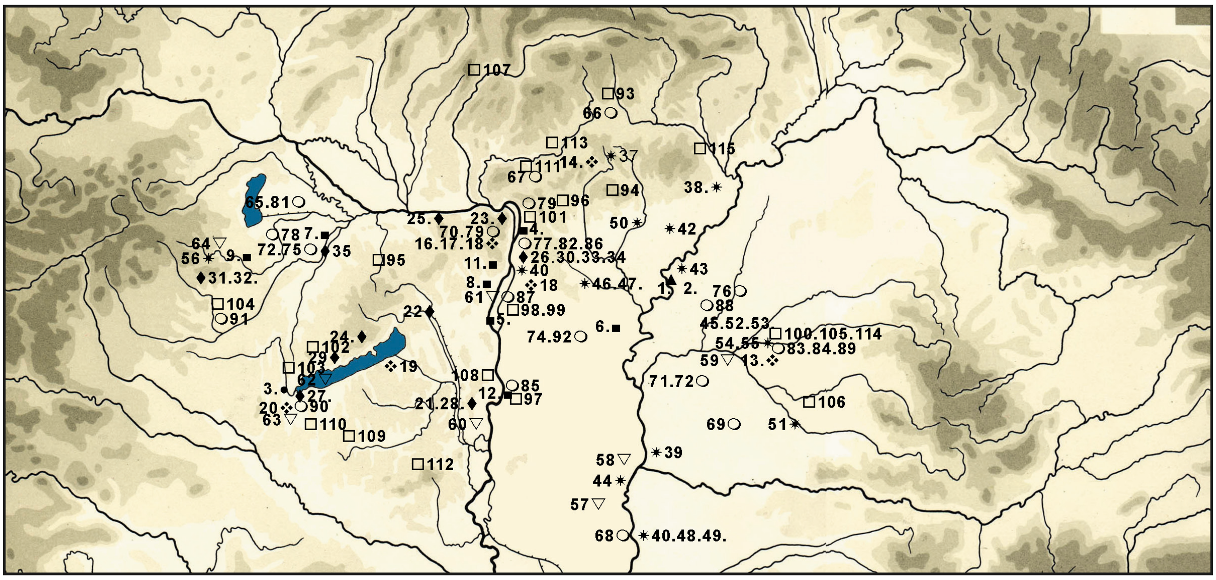
Figure 1. Rye (*Secale cereale*) remains in the Carpathian Basin



Neolithic (6000-4300 BC) ▲

▲1 Polgár-Ferenci-hát
▲2 Polgár-Csőszhalom-dűlő

Eneolithic or Copper Age (4300-3000 BC) ●

● 3 Keszthely-Fenékpuszta

Bronze Age (3000-900 BC) ■

■ 4 Dunakeszi-Székesdűlő
■ 5 Szigetszentmiklós-Üdülősor
■ 6 Ároktő-Dongóhalom
■ 7 Ménfőcsanak-Szeles
■ 8 Százhalombatta-Földvár
■ 9 Gór-Kápolnadomb
■ 10 Ludas-Varjú-dűlő
■ 11 Budapest-Albertfalva Kitérő Street
■ 12 Solt-Tételhegy

Iron Age (900 BC-1st century AD) ❖

❖13 Ebes Zsong-völgy
❖14 Miskolc-Hejő
❖15 Budapest Corvin tér 1.
❖16 Budapest-Nagytétény „Campona"
❖17 Budapest-Nagytétény-Érdliget
❖18 Budapest Hadnagy Street8-10. (Rácz fürdő)
❖19 Zamárdi-Kútvölgyi-dűlő
❖20 Keszthely-Fenékpuszta

Roman Age (1st-5th century AD) ♦

♦ 21 Dunakömlőd (Lussonium)
♦ 22 Tác-Fövenypuszta (Gorsium)
♦ 23 Esztergom-Castle
♦ 24 Nemesvámos-Balácapuszta
♦ 25 Tokod
♦ 26 Leányfalu Móricz Zsigmond Street
♦ 27 Keszthely-Fenékpuszta
♦ 28 Dunaújváros (Intercisa)
♦ 29 Kékkút Basilica No. 2.
♦ 30 Budapest Lajos Street
♦ 31 Sopron Városháza Street
♦ 32 Sopron Beloiannisz Square 6.
♦ 33 Budakalász-Luppa csárda
♦34Budapest-Albertfalva Kitérő Street
♦ 35 Győr St. István Street

Barbaricum (1st-5th century AD) ✷

✷ 36 Garadna
✷ 37 Szirmabesenyő-Sajóparti homokb.
✷ 38 Zalkod
✷ 39 Gyomaendrőd
✷ 40 Kiskundorozsma-Nagyszék (26/72, 34)
✷ 41 Budapest-Paskál park
✷ 42 Mezőszemere-Kismari-Fenék
✷ 43 Polgár-Kenderföld
✷ 44 Felgyő-Kettőshalmi dűlő
✷ 45 Ebes Zsong-völgy
✷ 46 M0 East Pécel 02.
✷ 47 M0 East Budapest 06. Péceli Street
✷ 48 Szeged-Homokbánya
✷ 49 Szeged-Kiskundorozsma-Daruhalom-dűlő
✷ 50 Balmazújváros-Darucsorda
✷ 51 Berettyóújfalu-Nagy Bócs-dűlő
✷ 52 Debrecen-Józsa Klastrompart
✷ 53 Debrecen-Józsa Józsapláza
✷ 54 Debrecen-Repülőtér
✷ 55 Ebes Zsong-völgy
✷ 56 Sopron-Városháza Street

Late Migration Period (8th-9th century AD) ▽

▽ 57 Kistelek
▽ 58 Felgyő-Kettőshalmi-dűlő
▽ 59 Debrecen-Bordás-tanya
▽ 60 Dunaszentgyörgy-Fadd
▽ 61 Budapest-Csepel-Sewage
▽ 62 Fonyód-Bélatelep
▽ 63 Zalavár-Vársziget
▽ 64 Sopron Városháza Street

Hungarian Conquest time and Arpad-Age (9th-13th century AD) ❍

❍ 65 Lébény-Billedomb
❍ 66 Edelény-Borsodi földvár (motte)
❍ 67 Hont-Ispánsági vár
❍ 68 Kiskundorozsma-Nagyszék
❍ 69 Kardoskút
❍ 70 Esztergom-Kovácsi
❍ 71 Gyomaendrőd
❍ 72 Ebes-Zsong-völgy
❍ 73 Győr-ECE
❍ 74 Cegléd-Madarászhalom
❍ 75 Győr-Gabona Square
❍ 76 Hajdúböszörmény-Téglagyár
❍ 77 Rákoskeresztúr-Újmajor
❍ 78 Kapuvár-Feketevár
❍ 79 Esztergom Kossuth Street
❍ 80 Vác Széchenyi Street 3-7.
❍ 81 Lébény-Billedomb
❍ 82 Budapest-Csepel-Sewage
❍ 83 Debrecen Kölcsey Cultural Center
❍ 84 Ebes-Zsong-völgy
❍ 85 Solt-Tételhegy
❍ 86 Budapest-Csepel Rákóczi Ferenc Street (Magnex)
❍ 87 Szigetszentmiklós-Vízmű
❍ 88 Balmazújváros-Darucsorda
❍ 89 Debrecen-Józsa-Józsapláza
❍ 90 Balatonmagyaród-Alsókolon-dűlő
❍ 91 Gencsapáti-sziget
❍ 92 Cegléd

Late Medieval Age (13th-17th century AD) ☐

☐ 93 Torna-Szádelő-völgy
☐ 94 Muhi
☐ 95 Baj-Öregkovács-hegy
☐ 96 Szarvasgede
☐ 97 Solt-Tételhegy
☐ 98 Budapest former Military Headquarters
☐ 99 Budapest Szent György Square ex Teleki palace
☐ 100 Debrecen Kölcsey Cultural Centre
☐ 101 Vác Széchenyi Street
☐ 102 Nagyvázsony-Csepely
☐ 103 Sümeg-Sarvaly
☐ 104 Gencsapáti-sziget
☐ 105 Debrecen-Józsa Józsapláza
☐ 106 Komádi-Gigánytó-dűlő
☐ 107 Lászlófalva-Szentkirály
☐ 108 Dunaföldvár-Öregtorony
☐ 109 Kaposvár-Kaposszentjakab
☐ 110 Pogányszentpéter-Kolostor
☐ 111 Hollókő-Castle
☐ 112 Pécs-Sebészeti Clinic
☐ 113 Szécsény-Plébániatemplom
☐114 Debrecen Kölcsey Cultural Centre
☐ 115 Sárospatak Castle Cannon Foundry

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- Malaya Rublevka, left bank of the Dnieper, Scythian, (6th-5th century BC) (Yanushevich 1976),
- Iwano-Puste, Scythian (Yanushevich 1976),
- Lubimovka on the Dnieper, later Scythian period (4th-3rd century BC) (Pashkevich1983),
- Maslini, Panskoye, Ust-Alminskoye and Alman-Kermen from Crimea (Yanushevich 1986),

Late Iron Age

- Kyffhäuser, Frankleben Kr. Merseburg, La Tène (Celtic) (Hopf 1982),
- Manching, Southern Bavaria, Middle and Late Celtic Periods (Küster 1991),
- Vlineves/Melnik, Bohemia, La Tène (Celtic) (3rd-2nd century BC) (Tempír 1968),
- Stanz/Schwaz, Tirol, La Tène (Celtic) (Werneck 1961),
- Magdalensberg near Klagenfurt, La Tène (Celtic) (1st century BC) (Werneck 1969),
- Steinbühl near Northeim (Willerding & Wolf 1990),
- Vlineves/Melnik, Bohemia, La Tène (Celtic) (3rd-2nd century BC) (Tempír 1968),
- Porz-Lind near Cologne, La Tène (Celtic) (approx. 100 BC) (Knörzer 1987),
- Krivina, Bulgaria, La Tène (Celtic) (1st century BC) (Hajnalová 1979),
- Svetjina in Serbia (Borojević 1987),
- Popesti in Romania, La Tène (Celtic) (Cârciumaru 1983),)
- Geto-Dacian culture site in Romania, end of 1st century BC (Wasylikowa et al. 1991),
- Čuberi and Eceri in Georgia (Schultze-Motel 1988).

Above mentioned macroremains of rye also numerous pollen data are available. Single rye pollen was detected in the Neolithic Linearbandkeramik site of Luttersee near Göttingen (Beug 1986). Several pollen data of rye are known from the Bronze Age, Roman Age and Medieval sites of Europe (Behre 1992).

Prehistory of rye in Carpathian-Basin

The rye originated on the Fertile Crescent area. From there arrived in the Carpathian Basin. Therefore the Carpathian Basin to play a bridging role in the spread of rye cultivation know-how from the region of the Middle East through the Balkans to Central Europe (Fig. 1.). According to palynological data the earliest occurrence of rye in the Carpathian-Basin is the Late Neolithic (VIIth pollen zone, late Atlantic phase, 4500-3000 BC, medium late, Lengyel culture). Already its presence in the Bronze Age (VIIIth pollen zone, Subboreal phase, 3000-800 BP, Bronze Age–Early Iron Age) is continuous. From the later ages (IXth pollen zone, Subatlantical phase, 800 BC–800 AD, Late Iron Age, Roman Age, Migration Period) is already present in higher amounts. More and more rye pollen was found in the sites of Xth pollen zone (cultural phase, from 800 AD) (Zólyomi 1971). Rye pollens were found in drilling settlements of Early and Late Iron Age, Roman Age and Migration Period (Medzrihradszky & Járai-Komlódi 1996). In 2002-ben rye pollens were showed by Elvira Bodor from Early Bronze Age Bell Beaker-Csepel group sites in Budapest-Albertfalva, Hunyadi János Street. But the archaeobotanical macroremains however, much earlier (Fig. 2.). Recently rye pollens were found in Fenékpuszta at Kis-Balatonnál from the beginning of Late Iron Age (Sümegi et al. 2011).

The first occurrence of rye grains in the Carpathian Basin is the Middle Neolithic (Polgár–Ferenci-hát) excavation conducted by P. Raczky in 2001-‘2 (Gyulai 2013). This population of the Alföld Linear Pottery culture which had connections with the Anatolian-Balkans and which conducted farming, animal husbandry, settled in the fertile open lands of the Great Plain near the Danube, avoiding the sandy areas that occur in the region. Here as well in the Late Neolithic Polgár-Csőszhalom-dűlő site (P. Raczky’s excavation

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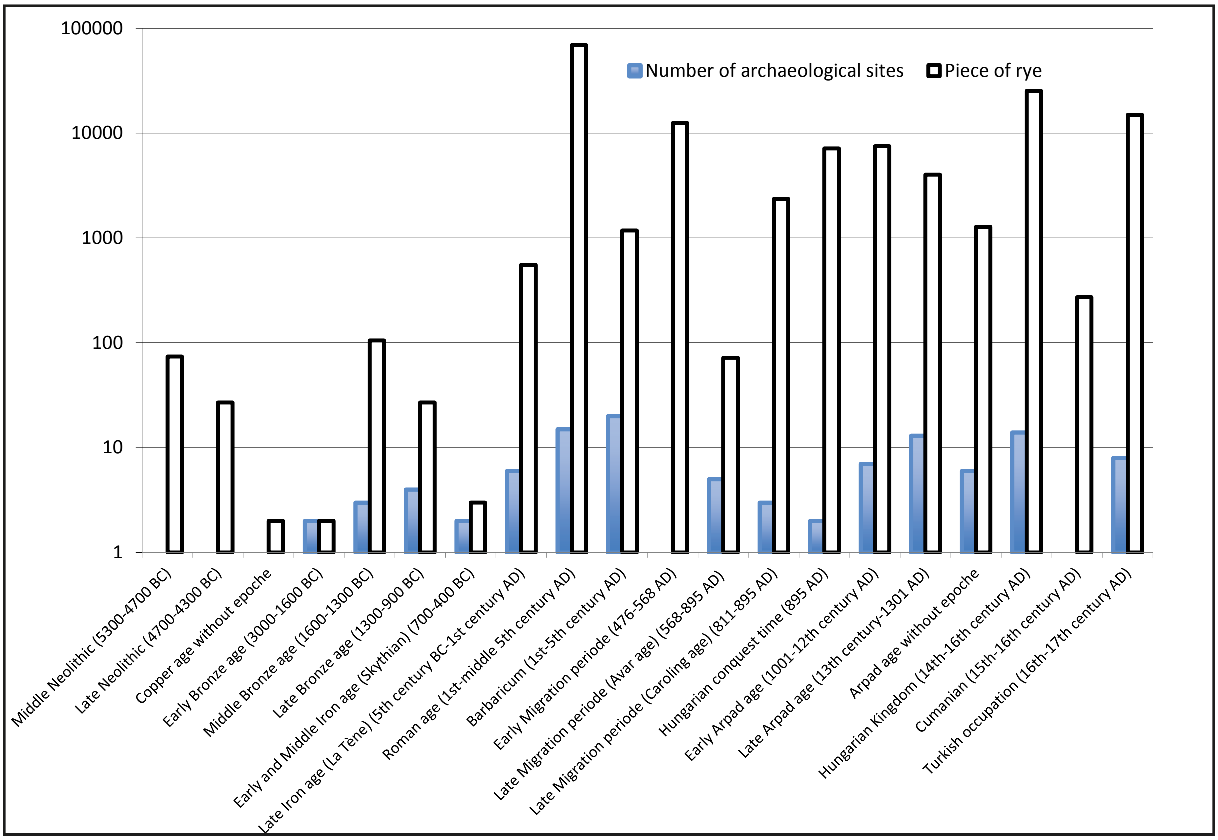
Figure 2. The most important date of rye (*Secale cereale*) in the Carpathian-Basin



between 1994-2005) dozens of rye were found. This prehistoric rye grains found in this area are small and thin (Gyulai ibid.).

Although the valuable archaeological collection of the Balatoni Museum at Keszthely was destroyed almost completely by an air raid during World War II while it was being removed from the building, a small quantity of archaeological material did survive. This includes cereal remains collected by Á. Csák at Fenékpuszta in 1905, within the area of the fort and identified as Bronze Age remains. Due to such circumstances, we can only accept this dating provisionally, and we do not know which era of the Copper Age the remains originate from. Grain remains that survived the war were processed by M. Füzes (1990). According to his findings, cultivated hulled wheats dominared, most of all einkorn and emmer. This place should be the oldest find of rye in the Carpathian Basin. However, but it is uncertain data about rye.

During the archaeobotanical examination of the Early Bronze Age Bell Beaker-Csepel group sites from Dunakeszi-Székesdűlő (excavation by A. Endrődi 2004) and Szigetszentmiklós-Üdülősor (excavation by A. Endrődi 2008-'9) and one small rye grains were found in each settlement-objects (Endrődi & Gyulai in print) (Fig. 3.).

Figure 3. Rye (*Secale cereale*) grain found in Early Bronze Age Bell Beaker-Csepel group sites in Szigetszentmiklós-Üdülősor. Photograph by the author.

At the Middle Bronze Age site of Ároktő-Dongóhalom, in one of the pits of the tell settlement belonging to the Füzesabony culture excavated by T. Kemenczei, rye grains were found (in a ratio of 1:10) alongside common bread wheat (Hartyányi et al. 1967–68). Also some rye grains were found in Ménfőcsanak-Szeles Limedeposit culture site (excavation by E. Szőnyi 1990-'91) and in Százhalombatta-Földvár Vatya culture site (excavation by I. Poroszlai 1989-'90).

From 1989 to 1993, we floated a significant quantity of Late Bronze Age botanical material at an excavation by G. Ilon at Gór-Kápolnadomb. The samples from prehistoric pits proved very rich in plant remains (Gyulai & Torma 1993). We believe it to be extremely significant that a few grains of rye were also found. Only a few grains known from Ludas-Varjú-dűlő site (Kyjatice culture) (excavation by L. Domboróczky 2001-'3) and from Budapest-Albertfalva Kitérő street site (Urnfield culture) (excavation by A. Endrődi 2004) as well.

Between 2007 and 2009, at the Solt-Tételhegy excavation lead by J. Szentpéteri more than one culturlayer was found. From the Early Bronze Age layer were found two small rye grains near barley and hulled wheat (einkorn and emmer) as well (Gyulai 2014).

Up to now are analysed archaeobotanically only three Scythian sites: Ebes-Zsong-völgy excavated in 2003 by J. Dani, and Miskolc-Hejő site (490-440 and 420-390 cal BC) excavated in 2012 by M. Hajdú (Pósa et al. in print). The most important cereal identified was six-rowed barley followed by common millet. Other grain crops were grown but they were by no means significant. Emmer is the dominant wheat cultivar. Einkorn, naked barley and rye grains account for not more than 1% of the total finds. All this demonstrates that the originally nomad Scythians in the Carpathian Basin are settled, although they did use wheats and grew them in a kind of ancient mixed grain together rye, were preoccupied with the production of barley and common millet that better suited their way of life and traditions. In above mentioned site were only three small and elongated rye grains found (Gyulai 2010) (Fig. 4.).

Figure 4. Rye (*Secale cereale*) grains from Scythian site Miskolc-Hejő. Photograph by the P. Pósa and Z. Mravcsik

Climatic deterioration in the La Tène Period brought about changes in the quality of land cultivation and technology as well. In a humid and cold climate farming had to facilitate drainage. At the same time, rye and oats, which adapted well to climatic deterioration increased in quantity and importance, but required deeper ploughing (Balassa 1973).

The rye was in the late Iron Age, if only in small quantities, but it is likely to be grown. Botanical residues taken at Corvin Square in Budapest in 1997–98 (excavation by P. Bertin and T. Hable 1997-'98 and St. Jacomet and O. Dálnoki 2001) dated to the Late Celtic Period (La Tène C/D, cca. 1st century BC.) confirm the picture drawn about the advanced agriculture of the Celts. The botanical assessment of the samples taken from the Celtic structures at the excavation led by T. Hable was made by the author, then by St. Jacomet and O. Dálnoki. The overwhelming majority of the seeds and crop yields found at the settlement inhabited by the Eraviscus are cereals (Dálnoki 2000; Dálnoki & Jacomet 2002).

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At the site of the Budapest-Nagytétény "Campona" shopping centre built in Budapest-Nagytétény in 2001 led by G. Szilas, yet another Celtic settlement was excavated. Preponderant in the diasporas was grain crop. It seems from the ratio of different grains that the most important kind of bread wheat must have been emmer, although einkorn was also known. The large amounts of barley are justified by the fact that barley in this period was also used for human consumption. In the Celtic period, one can also presume the production of rye, as it happened here. Three dozens of rye are known from here and two dozen from Zamárdi-Kútvölgyi-dűlő (Dálnoki & Jacomet 2002). Not fare from here in Budapest-Nagytétény-Érdliget (excavation by G. Szilas 2005–'6) where four small and elongated rye were found.

Rye was found recently in Rácz fürdő site (Budapest Hadnagy Street 8-10.) (excavation by B. Maráz 2005-'6) in larger quantities (Gyulai 2011). The nearly hundred grains found here are without exception small and elongated like in other prehistoric site.

They are mostly naked grains, but some hulled grain and spikelet forks are available as well. Between 1976 and 1983, I. Erdélyi conducted excavations at Keszthely-Fenékpuszta and discovered La Tène period finds. The botanical assemblage is associated with the Late Iron Age climate change, an approximately 200-300 year long transitional, cold period. Besides emmer wheat (*Triticum turgidum* subsp. *dicoccum*) and spelt wheat (*Triticum spelta*), there also emerged much barley (*Hordeum vulgare*), common wheat (*Triticum aestivum* subsp. *vulgare*) and club wheat (*Tr. ae.* subsp. *compactum)*, rye (*Secale cereale*), oat (*Avena sativa*), millet (*Panicum miliaceum*) and foxtail millet (*Setaria italica*). Rye adapted well to the worse climate but required deeper tillage. His quantity (three hundred grains and two spikelet forks) is not insignificant, but probably bread wheat and barley were of greater importance besides emmer and spelt wheat and rye (Gyulai & Lakatos 2013).

Rye in Carpathian-Basin at the beginning of the historical ages

The Romans expanded their rule up to the Danube in the first decades of the 1st century AD. Food requirements of the population and military stationed in Pannonia were mainly met by cereals. Therefore, not surprisingly, the overwhelming majority of seeds and harvested materials found here are cereal grains (Hartyányi et al. 1967–68; Hartyányi & Nováki 1973-74). By the late Emperors' Age, the key staple cereal was common bread wheat and rye. Their grains were found in substantial amounts at Budakalász-Luppa csárda, Late Roman watchtower, Kékkút Basilica No. 2., Budapest Lajos Street, Dunaújváros, Esztergom-Castle, Leányfalu Móricz Zsigmond Street watchtower, Keszthely-Fenékpuszta, Sopron Beloiannisz Square and Városház Street, Tác-Gorsium, Tokod. In finds around Keszthely (Keszthely-Fenékpuszta, Keszthely-Mosóház, Keszthely-Vadaskert). The signifycance of rye in provisions is proven by the two litres of carbonised rye grains found in the Late Roman watchtower at Budakalász.

The decorative villa built in Nemesvámos-Balácapuszta in the last third of the 1st century AD was inhabited as late as the 4th century (B. Thomas 1964). Gy. Rhé, who carried out excavations several times here between 1904 and 1912, explored a plastered pool made of

Figure 5. Rye (*Secale cereale*) grains from the Roman fortress at Fenékpuszta. Photograph by M. Füzes

pure limestone in floorless room 19 in the north-western corner of building No I. The plaster of the building walls contained bread wheat-, rye- and barley straw.

From the archaeobotanical point of view, one of the most researched Roman sites is Keszthely-Fenékpuszta. At the area of the fortified Roman settlement (castrum), known since the 18th century. Soil samples of six different excavation periods were analysed during the last century. Altogether near 600 thousand pieces plant remains of 180 taxa were identified. Cereals account for the overwhelming majority of seed and fruit remains coming from the Late Roman Period in Keszthely-Fenékpuszta, naked barley occupying first place among them. There is somewhat less of common bread wheat and rye followed by the rarely seen common millet with common oat least (Hartyányi et al. 1967-68; Gyulai & Kenéz 2009; Kenéz 2014). Three different forms of rye are known from here: some spikelet forks, few small and elongated grains (559 piece) and many large, that "normal" (Fig. 5.). In this case the rye is similar to common wheat were observed morphological differences: small and large-grain varieties. Unfortunately, rye straw was not found. But it can be assumed that the roof of the houses were covered with ryestrow.

The cereals coming from the Dunakömlőd (Lussonium) Late Roman fortress explorations differ in species composition from the Pannonian set of grain remains described above (Gerócs, Kovács & Torma 1995). In terms of number of grains, hulled emmer is ahead of common wheat. One can also find six-rowed barley, rye and oat as well. All these raise the possibility that we might have discovered the plants of another people with different agriculture, maybe those of the indigenous population. However, commercial activities with the Barbaricum might also play a role.

References

Balassa, I. (1973): Az eke és a szántás története Magyarországon. Akadémiai Kiadó, Budapest.

Barclay, G. J. & Fairweather, A. D. (1984): Rye and ergot in the Scottish later Bronze Age. Antiquity 58, 126.

Behre, K.-E. (1992): The history of rye cultivation in Europe. Vegetation History and Archaeobotany. 1/3, 141–156. DOI: http://dx.doi.org/10.1007/bf00191554

Borojević, K. (1987): Analiza ugljenisanog semenja sa pokapiteta Svetinja. Starinar 38, 65–72.

Cârciumaru, M. (1983): Palaeoethnobotanical investigations and contributions to the history of agriculture at Geto-Daces (in Romanian). Thraco-Dacica 4, 126-134.

Chambers, F. M. & Jones, M. K. (1984): Antiquity of rye in Britain. Antiquity 58, 219–224. DOI: http://dx.doi.org/10.1017/s0003598x00056210

Dálnoki, O. (2000): Kelta telepek környezeti képéről a Budapest-Corvin tér keltáinak növénytermesztése alapján. In: Füleky, Gy. (ed.), A táj változásai a Kárpát-medencében a történelmi események hatására. Kulturális Örökség Igazgatósága Budapest–Szent István Egyetem, Gödöllő, 42–50.

Dálnoki, O. & Jacomet, St. (2002): Some aspects of Late Iron Age agriculture based on the first results of on archaeobotanical investigation at Corvin ter, Budapest, Hungary. Vegetation History and Archaeobotany 11, 9–16. DOI: http://dx.doi.org/10.1007/s003340200001

Endrődi, A. & Gyulai, F. (2011): Funerary rituals of Bell Beaker Csepel Group: burials with circular ditches on Csepel Island. International Conference in Sion, Valais, Switzerland, Autour du Petit-Chasseur a Sion, Preactes 50 ans néolithique prehistoire archéologie, Sion, 27-30 october 2011., 67. DOI: http://dx.doi.org/10.1017/s0014479700007821

Evans, G. M. (1976): Rye. In: Simmonds, N. W. (ed.), Evolution of crop plants. London, 108–111.

Füzes, M. (1990): A Dunántúl korai növénytermesztése és növényleletei. A Starčevo kultúra és a "Tapolcai csoport". Bibliotheca Musei Tapolcensis 1, 267–362.

 DOI: 10.18380/SZIE.COLUM.2014.1.2.25

Gerócs, F., Kovács, V. & Torma, A. (1995): További adatok Magyarország fosszilis mag- és termésleleteihez Gór kora vaskori, Dunakömlőd- Lussonium római kori és Szécsény 16–17. sz.-i települések régészeti feltárása alapján. Tudományos Kiákköri Dolgozat. Janus Pannonius Tudományegyetem Bölcsészettudományi Kar Pécs. Manuscript.

Greig, J. (1991): The botanical remains. In: Needham, S. T. (ed): Excavation and salvage at Runnymede Bridge 1978: the Late Bronze Age waterfront site. British Museum/English Heritage, London, 234-261. DOI: http://dx.doi.org/10.1017/s0079497x00003959

Gyulai F. (2014): Solt-Tételhegy régészeti lelőhely archaeobotanikai kutatása. Archaeologia Cumanica 3, 175-207.

Gyulai F. & Kenéz Á. (2009): Mediterrane Landwirtschaft in Pannonien? Makrobotanische Forschung in Keszthely-Fenékpuszta. In: Heinrich-Tamaska, O. & Straub, P. (eds.): Keszthely-Fenékpuszta in Spiegel der Jahrtausende (Keszthely-Fenékpuszta az évezredek tükrében). A Balatoni Múzeum időszakos kiállításának katalógusa. Verlag Yeloprint GmbH, Leipzig/Zalaegerszeg, 2009. 172 p.

Gyulai, F. (2010): Archaeobotany in Hungary. Seed, Fruit, Food and Beverages Remains in the Carpathian Basin: an Archaeobotanical Investigation of Plant Cultivation and Ecology from the Neolithic until the Late Middle Ages. Archaeolingua, Budapest, 479 p.

Gyulai, F. (2011): A Budapest-tabáni késő kelta lelőhely archaeobotanikai vizsgálata. Communicationes Archaeologicae Hungariae (2009), 124-132.

Gyulai, F. (2013): Archaeobotanical Research of the Neolithic Sites in the Polgár Area. In: Anders, A. & Kulcsár G. (eds.): Moments in Time. Papers Presented to Pál Raczky on His 60th Birthday. Ősrégészeti Tanulmányok / Prehistoric Studies 1, L'Harmattan, Budapest, 885-900.

Gyulai, F. & Lakatos, B. (2013): La Tène archaeobotanical remains from Keszthely-Fenékpuszta. In: Heinrich-Tamáska, O. (ed.): Keszthely-Fenékpuszta: Katalog der Befunde und Ausgewählter Funde sowie neue Forschungsergebnisse. Castellum Pannonicum Pelsonense 3. Verlag Marie Leindorf GmbH, Budapest–Leipzig–Keszthely–Rahden/Westf., 647-652. DOI: http://dx.doi.org/10.1017/s1047759413001001

Gyulai, F. & Torma, A. (1993): Az urnasíros kultúra góri településének növényleletei. Nyugat-Dunántúl bronzkora. Pápai Múzeumi Értesítő (Acta Musei Papensis) 6, 277–286.

Hajnalová, E. (1979): Einige Erkenntnisse über die Züchtung und Ausnützung von Pflanzen in der Vergangenheit auf dem Gebiet Bulgariens und Rumäniens aufgrund archäobotanischer Funde. Rapports, Co-Rapports, Communications Tchécoslovaques pour le IVe Congres de l'Association Internationale d'Étude du Sud-Est Européen. Académie Tchécoslovaque des Sciences. Prague, 61–70.

Hartyányi, B. P. & Nováki, Gy. (1973-74): Növényi mag- és termésleletek Magyarországon az újkőkortól a XVIII. sz.-ig II. (Samen- und Fruchtfunde in Ungarn von der Jungsteinzeit bis zum XVIII. Jahrhundert II.). Magyar Mezőgazdasági Múzeum Közleményei, 23–73.

Hartyányi, B. P., Nováki, Gy. & Patay, Á. (1967–68): Növényi mag- és termésleletek Magyarországon az újkőkortól a XVIII. sz.-ig I. (Samen- und Fruchtfunde in Ungarn von der Jungsteinzeit bis zum XVIII. Jahrhundert I.). Magyar Mezőgazdasági Múzeum Közleményei, 5–85.

Hillman, G. C. (1975): The plant remains from Tell Abu Hureyra. A preliminary report. Proceedings of the Prehistoric Society 41, 70–73. DOI: http://dx.doi.org/10.1017/s0079497x00010902

Hillman, G. C. (1978): On the origin of domestic rye – Secale cereale: the finds from aceramic Can Hasan III in Turkey. Anatolian Studies 28, 157–174. DOI: http://dx.doi.org/10.2307/3642748

Hjelmqvist, H. (1980): An Irish cereal find from the transition between Bronze and Iron Age. Theses Papers North Eur. Arch. 9,130-132.

Hopf, M. (1982): Vor- und frühgeschichtliche Kulturpflanzen aus dem nördlichen Deutschland. Kat. vor u frühgesch. Altertümer 22.

Hopf, M. & Blankenhorn B. (1987): Kultur- und Nutzpflanzen aus vor- und frühgeschichtlichen Grabungen Süddeutschlands. Ber. Bayr. Bodendenkmalpflege 24/25, 76-111.

Hopf, M., Zohary, D. & Weiss, E. (2012): The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. Fourth edition. Oxford University Press. 243 p. DOI: http://dx.doi.org/10.1093/acprof:osobl/9780199549061.001.0001

Kenéz Á. (2014): Keszthely-Fenékpuszta római kori régészeti-növénytani leleteinek feldolgozása, különös tekintettel az egykori környezeti állapot jellemzésére. PhD értekezés. Szent István Egyetem Gödöllő. Manuscript.

Klichowska, M. (1975): Die ältesten Getreidearten aus den polnischen Ausgrabungen Archeol. Po1. 20, 83-143.

Knörzer, K.-H. (1987): Geschichte der synanthropen Vegetation von Köln. Kölner Jahrbuch für Vor- und Frühgeschichte 20, 271–388.

Körber-Grohne, U. & Piening, U. (1979): Verkohlte Nutz- und Wildpflanzenreste aus Bondorf, Kreis Böblingen. Fundber. Baden-Württemberg 4,152-169.

Kreuz, A. (1991): Die ersten Bauern Mitteleuropas. Eine archäobotanische Untersuchung zu Umwelt und Landwirtschafl der ältesten Bandkeramik. Leiden, 305, 117.

Kroll, H. (1990): Melde von Feudvar, Vojvodina. Praehistorische Zeitschrift 65, 46–48. DOI: http://dx.doi.org/10.1515/pz-1990-0104

Kühn, F. (1981): Crops and weeds in Slapanice near Brno from Early Bronze Age to now. Z. Archäol. 15, 191-198.

Küster, H.-J. (1991): Mitteleuropa südlich der Donau, einschliesslich Alpenraum. In: Zeist, W. van, Wasylikowa, K. & Behre, K.-E. (eds.): Progress in Old World Palaeoethnobotany, Rotterdam, 179–188.

Medzrihradszky, Zs. & Járai-Komlódi, M. (1996): M.: Late Holocene vegetation history and the activity of man in the Tapolca Basin. Annls hist.-nat. Mus. nam. hung. 88, 21-29.

Pashkevich, G. A. (1983): Subfossile Reste von Kultur- und Wildpflanzen aus Fundplatzen in der Ukraine aus den letzten Jahrhunderten v. u. Z. und der ersten Hälfte des 1. Jahrtausends u. Z. Z Archäol 17, 47-57.

Piening, U. (1982): Botanische Untersuchungen an verkohlten Pflanzenresten aus Nordwürttemberg. Neolithikum bis Römische Zeit. Fundber Baden-Württemberg 7, 239-271.

Pósa, P., Emődi, A., Schellenberger, J., Hajdú, M., Mravcsik, Z. & Gyulai, F. (in print): Előzetes jelentés Miskolc-Hejő melletti szkíta kori kút növényi maradványainak feldolgozásáról. Gesta.

Schultze-Motel, J. (1988): Archäologische Kulturpflanzenreste aus der Georgischen SSR (Tell 1). Kulturpflanze 36, 421-435. DOI: http://dx.doi.org/10.1007/bf02034822

Stutz, C. H. (1972): On the origin of cultivated rye. American Journal of Botany 59/1, 59-70. DOI: http://dx.doi.org/10.2307/2441230

Sümegi, P., Heinrich-Tamáska , O., Törőcsik, T., Jakab, G., Pomázi, P., Majkut, P., Páll, G. D., Persaits, G. & Bodor, E. (2011): Reconstruction of the environmental history of Keszthely-Fenékpuszta. In: Heonrich-Tamáska, O. (ed.): Keszthely-Fenékpuszta im Kontext spätantiker Kontinuitätsforschung zwischen Noricum und Moesia. Castellum Pannonicum Pelsonense 2, 541-572. DOI: http://dx.doi.org/10.1017/s1047759413001001

Tempír, Z. (1966): Ergebnisse der paleoethnobotanischen Untersuchungen des Anbaus von landwirtschaflichen Kulturpflanzen auf dem Gebiet der CSSR. Ved. Pr. Cesk. Zemed. Muz. 1966, 27-144.

Tempír, Z. (1968): Archäologische Funde von landwirtschaflichen Pflanzen und Unkräutern in Böhmen und Mahren. Ved. Pr. Cesk. Zeroed. Muz. 1968, 15-88.

Tempír, Z. (1969): Archaeological finds of food plants and weeds in Slovakia. Agricultúra 8, 7-66.

 DOI: 10.18380/SZIE.COLUM.2014.1.2.25

Thomas, E. B. (1964): Römische Villen in Pannonien – Beiträge zur pannonischen Siedlungsgeschichte. Akadémiai Kiadó, Budapest. DOI: http://dx.doi.org/10.2307/297475

Wasylikowa, K., Cârciumaru, M., Hajnalová, E., Hartyányi, B. P., Pashkevich, A. & Yanushevich, Z. V. (1991): East-Central Europe. In: Zeist, W. van, Wasylikowa, K. & Behre K. E. (eds.): Progress in Old World Palaeoethnobotany. Balkema, Rotterdam/Brookfield, 207-239.

Werneck, H. (1969): Pflanzenreste aus der Stadt auf dem Magdalensberg bei Klagenfurt in Kärnten. Kärntner Museumsschr. 45.

Werneck, H. L. (1951): Ur- und frühgeschichtliche Roggenfunde in den Ostalpen und am Ostrande des Böhmerwaldes. Züchter 21, 107-108. DOI: http://dx.doi.org/10.1007/bf00709562

Werneck, H. L. (1954): Kulturpflanzen aus Lauriacum Lorch bei Enns. Forschungen in Lauriacum 2, 85–96.

Werneck, H. L. (1961): Ur- und frühgeschichtliche sowie mittelalterliche Kulturpflanzen und Hölzer aus den Ostalpen und dem südlichen Böhmerwald (Nachtrag 1949–1960). Archaeologia Austriaca 30, 68–117.

Willerding, U. & Wolf, G. (1990): Paläo-ethnobotanische Befunde aus einer Siedlung der jüngeren Vorrömischen Eisenzeit am Steinbühl bei Nörten-Hardenberg, Ldkr. Northeim. Nachrichten Niedersachsen Urgesch. 59, 111-140.

Yanushevich, S. V. (1976): The cultivated pants in the SW USSR according to palaeobotanical investigations (in Russian). Acad. NAUK Moldavian SSR, Kishinev.

Yanushevich, S. V. (1986): Cultivated plants north of the Black Sea. Palaeobotanical investigations (in Russian). Akad. NAUK Moldavian SSR, Bot. Garden, Kishinev, 90 p.

Zólyomi, B. (1971): 6000jährige Geschichte der Agricultur in der Umgebung des Balaton-Sees aufgrund von pollenstatistischen Untersuchungen der Seesedimenten. III^{ième} Congrès International des Musées d'Agriculture. Magyar Mezőgazdasági Múzeum Közleményei 1971, 194–195.

ARCHAEOBOTANICAL OVERVIEW OF RYE (*Secale cereale* L.) IN THE CARPATHIAN-BASIN II. FROM THE MIGRATION PERIOD UNTIL THE LATE MEDIEVAL AGE

Ferenc GYULAI

Szent István University, MKK Department of Nature Conservation and Landscape Ecology, H-2103 Gödöllő;
E-mail: Gyulai.Ferenc@kti.szie.hu

Abstract
It seems that rye is a twice domesticated cereal. Then, in the course of the Early Iron Age in Central or Eastern Europe, it is again transformed from weed to crop plant. We summarized the development of rye and his spread in the Carpathian Basin based on archaeobotanical data. The grains of the rye were found always in charcoal form. The rye appeared in Carpathian Basin during the Neolithic Age. Sporadic occurrences of rye were also found in Early Bronze Age Bell Beaker-Csepel Group and Early Iron age Scythian cultures. The prehistoric grains were small and longshaped. In the Prehistoric Ages rye should be exist as weed of hulled wheats. His cultivation started in the Roman Age. The grains found in roman sites are always big and reel shaped like recently. Only a few grains were found in the Migration Period. Among cereals of the conquering Hungarians rye is present. Rye became common product in the Late Medieval Ages. It was grown in a big quantity everywhere, but not independently, but mixed with the wheat.

Keywords: rye, archaeobotany, macroremains, Carpathian-Basin

Rye in Carpathian-Basin during the Early Medieval Ages

Contrary to the common bread wheat that was dominant in Pannonia but required an advanced level of agrotechniques, people in the Barbaricum continued to grow hulled wheat varieties such as einkorn and emmer. We believe that, rather than derived directly from eastern traditions, this reflects the cereal crop production heritage of earlier prehistoric peoples that once lived in the Great Hungarian Plain. Barley was found only at the sand pit of Szirmabesnyő-Sajópart. Here, rye was encountered as well (Hartyányi et al. 1967-68).

Further significant results of Sarmatian archaeobotany were obtained by Cs. Szalontai and K. Tóth in 1998 and 1999 at the Kiskundorozsma-Nagyszék site dated to the 3rd–4th centuries AD. Surprisingly, many grain species were encountered, however, only a few of them are of any significance. Their most important cereals were six-rowed barley and common millet, reflecting doubtlessly a continuation of their nomadic traditions. Of the naked grain common bread wheat and the characteristically cornered club wheat grains hardly any were found. Other grain types were also known, yet they played only a subordinate role: naked barley, two-rowed barley and rye. Many relict species from meadows and pastures suggest livestock grazing in the vicinity of the settlement. This meadow and pasture, having mainly typical habitat characteristics, could be effectively arid in some places, as shown by e.g. wild rye (Gyulai 2003).

In other Sarmatian deposits if not in large quantities, but rye also occurs in the following sites: Garadna (excavation by Á. Salamon 1974; Skoflek & Árendás 1971), Zalkod (excavation by Á. Salamon 1970; Skoflek & Árendás 1971), Gyomaendrőd (excavation by D. B. Jankovich 1987-‘90; Gyulai 2011), Budapest, Paskál park (excavation by A. Endrődi 2003), Mezőszemere-Kismari-Fenék (excavation by Cs. Ács 2002), Polgár-Kenderföld (excavation by P. Raczky 2001), Ebes-Zsong-völgy (excavation by J. Dani 2003), Szeged-Homokbánya (excavation by L. Bende and G. Lőrinczy 2004). The Sarmatians were characterized by the production of a mixture of grain and rye in it as well. In this site M0 East Pécel 02. (excavation by B. Maráz 2005-‘6)

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among the grains some germinated rye grains were available. In the site M0 East Budapest 06. Péceli Street (excavation by A. Korom 2005-‘6) near four hundred small and elongated rye grain were detected.

Characteristics of such ancient prehistoric types of rye are known from the sites: Szeged-Kiskundorozsma, Daruhalom-dűlő (excavation by G. Lőrinczy et al. 2005) and Hajdú-Bihar county excavations by K. Szilágyi et al. 2003-‘8 (Balmazújváros-Darucsorda, Debrecen-Józsa-Klastrompart, Debrecen-Józsa-Józsaplaza, Debrecen-Repülőtér, Ebes-Zsong-völgy, Berettyóújfalu-Nagy Bócs-dűlő). The latter site more them 400 rye grain were available. It is interesting that some wild rye was found as well, which is understandable because it is sandy steppe elements.

Unfortunately no ethnic identity is given to the 5–6th-century site in Sopron-Városháza street, yet species-rich crop grains and legumes were encountered here in great numbers (Hartyányi et al. 1967-68). Two type of rye were found equally among the half a liter of macroremains: “small, long and thin shaped” and “small and squat shaped”.

In 1986 excavations were carried out in Devín, near Bratislava, Slovakia, along the limes at the time, at a settlement dated to the 5th century AD, populated by Danube Germans or maybe Kvads. A carbonized grain layer, a completely intact bread and several pieces of bread were found among pot fragments in the demolished remains of a fireplace (Pieta 1988; Pieta & Plachá 1989). The composition of the cereals obviously stocked for kitchen use shows an advanced level of agriculture: 66% rye, 21% common bread wheat, 11% barley, 1.6% common millet.

The rye from the Early and Middle Avar Age still missing. But from the Late Avar Age a significant number of remains were known. The spread of rye in the Migration Period refers to Debrecen-Bordás-tanya site (excavation by K. Szilágyi et al. 2003) as well. L. Bende and G. Lőrinczy conducted an excavation 2003-ban in the Kistelek site in 2003. In addition to many rowed barley only rye was found. Interestingly, the wheat does not occur in the remains. Also some rye grains were found in Dunaszentgyörgy-Fadd Avar Age site (excavations by L. Szabó 2008).

Excavations in the planned cabbage deposit site of Felgyő-Kettőshalmi-dűlő led by G. Lőrinczy et al in the Avar Age samples can be found both type of rye: the big grains and small but elongated grains like in the prehistoric ages. This is the evidence of the presense of two different ecotypes or varieties. From the little grains his independent cultivation seems uncertain, but mixed with wheat is also conceivable. From the ratio of wheat and rye founded in the site Budapest, Csepel-Sewage (excavation by A. Horváth 2006) may indicate their mixed cultivation.

One of the most important botanical findings in Hungarian archaeobotanical research was uncovered from the late Migration Period Fonyód-Bélatelep site, the excavation of Béla Horváth in 1964 (Gyulai et al. 1992). Based on ^{14}C tests, the age of the settlement, made up of lake dwellings, can be dated from the second half of the 7th century to the end of the 9th century. According to evidence provided by these plant macrofossils, the inhabitants of the lake dwellings pursued extensive farming activities. The main crops of the inhabitants were barley, common bread wheat, club wheat, common millet, rye and common oat. Two thousand seeds of rye found here at Fonyód-Bélatelep are mostly of a larger type, yet the shape of the seeds puts them in the “squat” class, while a smaller proportion of the seeds are smaller and of a “narrow” type. The two types imply two varieties. The measurement data (length, width and height of grains) seem to indicate that there may have been several classes (ecotyp or variety) of the rye including wild rye recovered (Gyulai et al. 2014) (Figs. 1-3.).

Decades of excavations, led by Á. Ritoók and B. M. Szőke, of a parking lot exposing the 9th century site of Zalavár-Vársziget, initiated

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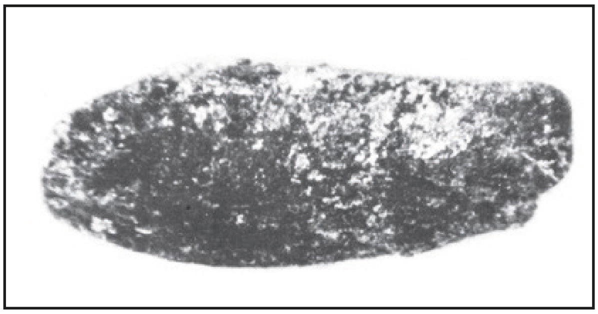
Figure 1. Rye (*Secale cereale*) short (wide) grains from Fonyód-Bélatelep. Late Migration Period lake dwelling settlement. Photograph by the author

Figure 3. Wild rye (*Secale sylvestre*) naked grain from the Fonyód-Bélatelep Late Migration Period settlement. Photograph by the author.



collection and flotation of soil samples for botanical remains in 1994 (Gyulai 1998). This was the first time that botanical macro remains, seeds and fruits, were recovered. According to historical data, the Carolingian court centre was populated by common people of mixed ethnic composition. Most seeds and fruit remains come from the fill of a "well", which was probably a planked storage pit. The carbonised diaspores were possibly deposited during the cleaning process after the burning of the settlement at the time. The botanical material is dominated by cultivated plants and their weeds. The most important crops were common millet, six-rowed barley and common bread wheat while. Rye was present only sporadically.

The near two hundred rye grains were found in presumably Late Avar Age level of Sopron Városháza Street were also different: "small,

Figure 2. Rye (*Secale cereale*) thin and large grains from Fonyód-Bélatelep. Late Migration Period lake dwelling settlement. Photograph by the author.

long and thin shaped" and "small and squat shaped" (Hartyányi et al. 1967-68).

Crop yields were not any higher in other parts of Europe. Valuable data have survived from Carolingian times. According to these, the yield was twice the amount sown in spelt, 1.6 times in rye, and 2.2 times in barley. Would it be possible that village people constantly lived at the verge of famine? According to the statement made by Füzes (1977), production yields of grains increased from the Neolithic to the middle 19th century not more than 15–20 percent in weight (acceleration percent), which is due to metric increase in grain size.

Rye in Carpathian-Basin during the Medieval Ages

The Hungarian word "rozs" is of Slavic origin occurring first in a document dated to 1292 as a place name (Molnár 1961). In the Hungarian Diploma Dictionary, a different date can be found: "Roswago" – 1478 (Szamota & Zolnai 1902-1906).

The botanical find from the age of the Hungarian conquest (beginning of the 10th century) comes from Lébény-Billedomb, the 1993 excavation of Miklós Takács. Several soil samples were collected from settlements of the conquering Hungarians. A great number of seeds and fruit remains of 30 different plant species were found (Gyulai 1997). Hulled wheat types, typical in prehistoric ages, were not grown at all, only the more advanced naked grain common wheat are found. In addition to sixrowed barley, albeit to a smaller extent, two-rowed barley and naked barley grains were also found. An important gruel plant was common millet, as unearthed carbonised common millet gruel pieces show. It has to be noted in connection with recovered rye grains that they can be sown separately or mixed with wheat (abajdoc, maslin).

About seven thousand rye grain were found in Edelény-Borsodi földvár (motte) settlement from the Conquest Period 10th century (excavation by M. Wolf 1987-'92). The rye was here the second most important cereal behind the wheat (A. Torma personal communication). The ratio of the common wheat and rye are 3:1. When exploring Edelény-Borsodi földvár in 1998, Mária Wolf found clay pots placed on their sides near the oven of a burnt house from the 10th century. On the side of one of the pots burnt food remains were discovered. They had homogenous structures mixed evenly. Neither grains, nor other kind of seeds or grists were distinguishable in them. They were made of evenly mixed, fine middling like cereal meals. The mixture thus prepared was not fermented but cooked right away. No traces of leavening of the dough, in other words lactic acid fermentation, were found. Microscopic examinations confirmed the observations. Indeed, a large number of flour particles were found in the aleuron layer of the grains. It was also possible to determine the material of the flour or rather the grist particles: they included common bread wheat epidermis and phytolith fragments, rye phytoliths (Gyulai 2014a).

Relatively more evidence has been recovered from the period (10th-11th centuries) after the conquest (Hartyányi et al. 1967-68; Hartyányi & Nováki 1973-74). In Kardoskút, during excavation of a 10th-13th century village, cereal grains were found among burnt straw under an oven. Numerically, the most important grain was common millet followed by bread wheat and rye. Adjacent to the Roman church at Esztergom-Kovácsi, 11th century graves provided botanical materials characterised by common bread wheat and rye, both having longer growing seasons. A carbonised grain layer was found during the excavation at the 10th-11th century castle of a count at Hont (Hartyányi 1981-83). The sample from this layer contained a small amount of rye and common bread wheat, together with a very high level of weed infestation (Fig. 4.). Also in the 10th-11th centuries level of Ebes-Zsong-völgy

Figure 4. Rye (*Secale cereale*) thin and large grains from Hont-Ispánsági vár, Hungarian, second half of the 10th century. After Hartyányi 1981–83

(10-11. century) rye was present. More them hundred rye grains were found in the above mentioned Kiskundorozsma-Nagyszék Early Arpad Period level. Extremely much nearly seven thousand rye were identified in the Győr-ECE Late Arpad Period site (excavation by Sz. Bíró 2004, 2006). The ratio of the rye and common wheat from a storage pit of former grain trader were 3:1. It is very likely that they have been grown together.

In the archaeobotanical finds of Early Árpádian Period houses and pits explored in Gyomaendrőd, Barley remained an important kind of grain (excavation by Dénes B. Jankovich, 1987-'90). Rye production was insignificant. Notwithstanding this, the production of cereals requiring a more advanced level agriculture was also started (Gyulai 2011).

When one compares the earliest finds of the period after the conquest, the conclusion is that common millet played an important role mainly in the Great Plain, while common bread wheat and rye did the same in Transdanubia.

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The finds from the Plain support the notion of limited nomadic patterns in the period after the conquest. The finds from Transdanubia and from the northern part of the country suggest a sedentary lifestyle and a more advanced level of agriculture. The Danube River, which is a historical as well as floristic boundary, also divided the country into two major areas of different crop production: the Great Plain, producing more archaic plants and Transdanubia, a more advanced region integrating the traditions of Roman agriculture.

Grain finds from later excavations in the Late Árpádian Period (12th-13th centuries) in the Great Plain start to show similarities with those found in Transdanubia both in terms of species composition and their relative importance (Hartyányi et al. 1973-'74). The alteration of sowing seeds, representing a quality change in crop production, was completed by this time. Growing high nutrient common bread wheat and rye became customary. Considerable amounts of carbonised common bread wheat grains and somewhat fewer rye grains were found in the Early Árpádian Period graveyard of Cegléd-Madarászhalom.

From the Árpád Period objects (pits, fireplaces and houses) of Solt-Tételhegy near by two thousand pieces of rye are available. The grains look characteristic spindle-shape. It is also conceivable that some of them or all of them can be grown together with wheat (Gyulai 2014b).

In 1995–1996, in the northern sector of the M0 highway under construction at Rákospalota-Újmajor site No 1, an Early-Late Árpádian Period village, the long forgotten former Sikátor, was explored under the leadership of Zoltán Bencze and Anna Gyuricza. The large majority of the carbonised diaspores unearthed from the waste pits and external or internal ovens dated to the 12th-13th centuries were cereals (Gyulai 1999). Corresponding to other sites from the Árpádian Period, six-rowed barley, common and club wheat, rye and certainly the inevitable gruel-plant, common millet, were also found here.

In 1996 at the Rákospalota-Újmajor site, most probably another segment of Sikátor village, was excavated by A. Gyuricza. This time, a well, dated to the 13th-14th centuries, rich in plant residues was also identified. According to the archaeobotanical finds recovered, the number of crops in cultivation in the Late Árpádian Period was further increased. The number of club wheat grains, like that of common millet grains, is low, both of them being typical for the Middle Ages in Hungary. There is somewhat more rye. The ratio between the grains of common bread wheat and rye is 3:1. This raises the possibility of their joint production (*Triticum mixtum*, abajdoc, abenas, maslin). Combine cultivation and harvesting of rye and wheat and joint preparation for bread was the medieval tradition and in the Great Plains until the mid-19th century remained.

During the excavation carried out at the outskirts of the city of Hajdúböszörmény-Téglagyár in 2011 (excavator: L. Szolnoki) the remains of a 12-13th century settlement part have been found. Linguistic and historical research have already presumed the Ismaelites's Árpád-era town in the northern part of the city. Most of the seeds came from wells and pits, the least amount came from houses. Most of the remains were rye and barley (Fig. 5.). Common wheat,

Figure 5. Rye (*Secale cereale*) grains from the Ismaelites's Árpád-era town Hajdúböszörmény-Téglagyár. Photograph by the author.

broomcorn millet and common oat were presented in much lower numbers. Comparing the identified plant remains and their composition to the other already known cultivated plant remains from the 12-13th century Árpád-era (Cegléd-Madarászhalom, Győr Gabona Square, Rákoskeresztúr-Újmajor, Kapuvár-Feketevár, Esztergom Kossuth Street, Vác Széchenyi Street, Lébény-Billedomb, Budapest-Csepel-Sewage, Debrecen Kölcsey Cultural Center, Ebes-Zsong-völgy, Solt-Tételhegy), we can find several peculiarities: in the city of Hajdúböszörmény fewer cereal species have been found, and their composition was different as well. Barley and rye was the most frequently found remain here not the common wheat (Gyulai et al. 2013).

In 1998, A. Endrődi and A. Horváth explored a Late Árpádian Period (12th-13th centuries) settlement segment in Budapest-Csepel Rákóczi Ferenc Street (ditches, open fireplaces/ovens, waste pits). Although the botanical material is poor, it indicates cultivation. Three quarters of the species are grain crops: six-rowed barley, rye and common bread wheat. The finds are dominated by six-rowed barley. A third of it is rye, and less common bread wheat.

Rye was found in Szigetszentmiklós-Waterworks as well, where A. Endrődi made a rescue excavation in 1999 before the construction of a MOL gas pipeline and explored some Árpádian Period structures. Grain remains coming from here correspond to the species composition found at other sites of 12th century Hungary and the data of written sources from the time: the main crops were six rowed barley, common bread wheat and rye. Remains of rye are known from the archaeological sites of surrounding of Debrecen: Balmazújváros-Darucsorda és Debrecen-Józsa-Józsapláza. The latter took place more than 800 charred rye grains were found. Also some rye were found in Balatonmagyaród, Alsókolon-dűlő, Gencsapáti-sziget, Cegléd sites.

The consolidated feudal order formed a uniform crop production system in Hungary. More or less the same plants (e.g. rye) were grown everywhere. To spread of rye was favored the cold and wet climate of the Medieval Little Ice Age (14th-19th centuries) (Rácz 1993). Also was necessary to carry out the lesser quality soils with the adaptive rye to meet the needs of still growing population.

This can be demonstrated by a number of archaeobotanical finds. Late medieval plantremains come usually from privies, wells, waste pits, i.e., places where the waste of households goes. Roads, cisterns and sewers, in particular, preserved seeds and fruit remains in good condition. Diaspores coming from here were conserved as a result of anaerobic conditions, although in certain instances surface corrosion can be significant. The seeds processed by I. Deininger, originating from Torna-Szádelő-sziget (today Slovakia) represent the heroic stage of archaeobotanical research (in: Lehoczky 1883). An approximately three centimetre thick cereal layer, consisting of mainly rye and to a smaller extent common wheat and barley, is dated to the Mongol invasion.

Important evidence for a uniform medieval crop production culture is seen in the late medieval botanical material of Muhi. Although the samples collected in 1995 at the Muhi medieval excavation site led by J. Laszlovszky and T. Pusztai. The cereals identified so far (common bread wheat, club wheat, rye, six-rowed barley, common millet) confirm the level of farming typical for the Middle Age (Gyulai 2010).

All cereals typical for the Hungarian Middle Ages were found in the samples taken from the medieval manor house excavation site in the forest beside Baj-Öregkovács-hegy (excavation by S. Petényi 1998): common bread wheat, six-rowed barley, rye, common millet.

In 1998 at Szarvasgede, at the excavation led by M. Takács and I. Paszternák, similar kinds of cereals were found. Archaeobotanical examinations indicated that intensive agricultural activities were carried out here in the middle 15th century. Along with common bread wheat, rye and sixrowed barley were also grown.

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In the late-medieval period (cca. 15th century) dated archaeological level of Solt-Tételhegy is the most majority of six-rowed barley, which is understandable, because in this era of barley was used as fodder plants. This is followed rye, common or sowing wheat and millet (Fig. 6.). The combined production of common wheat an rye is possible. Involved of his processing (threshing, cleaning) earspindle fragments are available as well (Gyulai 2014b).

Figure 6. Rye (*Secale cereale*) grains from late medieval site Solt-Tételhegy. Photograph by the author

In the course of the exploration and reconstruction of Buda castle over the past fifty years, archaeologists found a number of wells: Several earth and mud samples were taken for archaeobotanical examinations from two wells explored in the yard of the former Military Headquarters (Dísz Square No. 17) (excavations by Z. Bencze et al. 1999-2000) of Buda Castle in 1999 and Budapest, Szent György Square ex Teleki palace, well No.8. Kút (excavations by T. B. Nyékhelyi 1999-2000). Carbonised rye grains were found in all settlements, but not to much, because there kitchen trash. In the well of Debrecen Kölcsey Cultural Center site (excavation by Zs. Hajdú, et al. 2004) only a few rye was available, because the situation was the same (Gyulai 2010).

Led by András Horváth Pálóczi, archaeologist at the Agricultural Museum, several wells were explored at the late medieval Cuman settlement in 1984-87 at Lászlófalva-Szentkirály. It is not known whether six-rowed barley, a grain crop that occurred most frequently in the finds, was grown as fodder or was intended for human consumption. Common bread wheat and rye grain ratio is close to one to one. This raises the possibility of their mixed cropping (Pálóczi et al. 1996).

The rye was cultivated in the Medieval Europe sometimes separated but mostly mixed with wheat (*Triticum mixtum*, rye-wheat maslin, abajdoc in Hungary, suražica or napolica in Vojvodina) (Jones és Halstead 1995, Borojević 2005).

Mixed growing of these two cereals was typical in the Hungarian Middle Ages. As early as in the Árpádian Period, joint production of bread wheat and rye was widespread. “Abajdoc (abenác, abajdos)” means a mixed crop. It was also called “maslin”, “triticum mixtum”, or “cerealiam promiscuam”. King Ladislaus I provided for the taking of the tithe separately and not in the maslin: “In annona vero commixtum non accipiat, sed separatim”. Wheat and rye were not mixed subsequently but sown together. Allegedly, this was done for security purposes. This way, even if one of the species would not ripen, the other still might bear a yield. An interesting observation is that maslin was still grown at the beginning of the 19th century (Gaál 1978).

Not only archaeobotanical finds, but also written sources confirm the notion that the mixed cropping of common bread wheat and rye (called abajdoc, meslin, Triticum mixtum) was common as early as the Árpádian Period and continued through the Middle Ages until the modern times (Szamota & Zolnai 1902-1906).

Wheat and rye were not mixed subsequently, but sown and harvested, milled and used this way. It is also possible that abajdoc was grown for security purposes. This way, even if one of the species would not ripen, the other still might bear a yield. A further advantage is seen in the stalks being more resistant in maslin to being blown over. In our view, joint production of bread wheat and rye provided more advantages than simple harvest safety. Stalks of maslin are more resistant to being blown over. It is also possible that people realised at that time, also demonstrated in recent East-German crop production experiments, that mixed production of different cereal species results in more uniform stock, higher yields (stimulating effect), and more resistant to plants pests (host hiatus) (Á. Mesterházy personal communication).

The grain crop production boom was stopped by the Turks. The country was torn into three parts and the constant warfare did not favour

Figure 7. Rye (*Secale cereale*) grains from the 16th century settlement layer at Vác. Photograph by T. Kádas

crop production. The size of land left fallow increased and production yields fluctuated. A number of settlements were abandoned.

Turkish landlord taxes, collected in the part of the country under Turkish occupation, were as follows: wheat tithe, maslin ("mahlut" = mixed crop) tithe, must tithe, pasture benefit, wild cabbage tithe, barrel levy, and fruit tithe. The population – albeit suffering from the taxes – continued agricultural production. In the 16th century, tithe censuses mention lentil, pea, flax, buckwheat, cabbage, beetroot, onion and garlic, also fruits (apple, pear, grape) (Káldy-Nagy 1970).

Quite often common wheat and rye production was abandoned altogether in settlements dominated by the Turks, and only oat was grown, albeit in a limited manner. All this means spring crops, that is, less work and safer yields. This was obviously encouraged by the fact that autumn sowing cereals had a higher tax levied on them. The same conclusion could be drawn from examination of grain residues from the Turkish Period found in downtown Vác Széchenyi Street (Gyulai 1995) (Fig. 7.). A significant amount of seeds and fruit remains unearthed from excavations carried out in downtown Vác during the seventies and eighties represents well crop production in the Middle Ages. The archaeobotanical material

Figure 8. Rye (*Secale cereale*) grains from Nagyvázsony-Csepely (15th–16th centuries), Inventory of the Hungarian Agricultural Museum, Budapest

collected at excavations carried by Zs. Miklós between 1986 and 1992 originated from different centuries of the Middle Ages, thus opportunity to record changes in crop production spanning centuries at one special location. In the 13th century, albeit in small amounts, barley, common wheat and rye were still produced in nearly equal amounts. These species were accompanied by club wheat, six-rowed barley and common oat in the 15th–16th centuries. Regression of crop production stopped only after the Turks were driven out in the 18th century.

Charred rye seeds found in the 15th-16th century houses at Nagyvázsony-Csepely (excavation by J. Kovalovszky 1957-58) are also of two types, a longer squat and a shorter narrow type (Hartyányi et al. 1967-68) (Fig. 8.). They may

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Figure 9. Germinated rye (*Secale cereale*) grains from Dunaföldvár-Öregtorony, 17^{th} century, Inventory of the Hungarian Agricultural Museum, Budapest

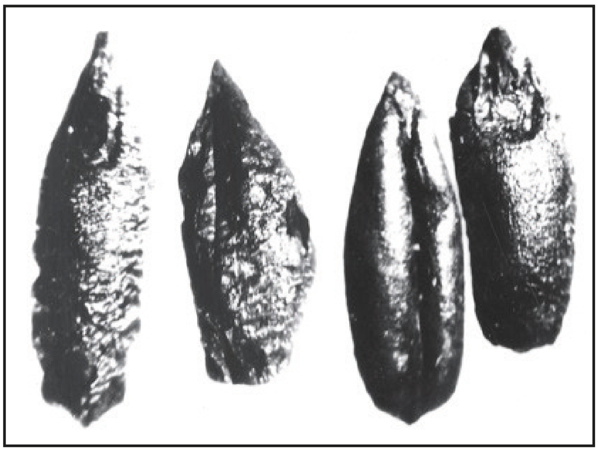

Figure 10. Rye (*Secale cereale*) grains from the gate of Hollókő castle, 17^{th} century, Inventory of the Hungarian Agricultural Museum, Budapest.

furnish evidence for the use of varieties in the Middle Ages. While in Gencsapáti-sziget not more them two dozen grain of rye were found until then in Sümeg-Sarvaly more than one liter charred rye. Devastation layers from the Turkish Period are relatively well researched from a botanical point of view. Plant remains of the Pogányszentpéter monastery, destroyed in the 16^{th} century (excavation by R. Müller 1967), included many common bread wheat and rye grains, in other word “maslin” (Füzes 1972). Between 1969 and 1974 I. Holl and N. Parádi led the excavation of a village razed during the Turkish era in the 16^{th} century in Sümeg-Sarvaly. Botanical finds from six houses devastated and burned during the Turkish Period and adjacent debris were processed by I. Skoflek (1984–85) and B. P. Hartyányi (in: Nováki 1984-85). Beside grains and seeds of carbonised common bread wheat, rye, common millet and weeds, fruit remains were also encountered. Prevalence of rye refers to Debrecen-Józsa Józsaplaza and Komádi-Gigánytó-dűlő sites (excavations by K. Szilágyi et al. 2003-‘8).

Half of the archaeobotanical material found at Dunaföldvár-Öregtorony site (ruins of a 17^{th} century house) consist of rye, somewhat less of common wheat and even less of common millet (Hartyányi & Patay 1970) (Fig. 9.). In the 16^{th}-17^{th} century layer of Hollókő-Castle, bread wheat and rye occurred in almost identical amounts (Hartyányi & Nováki 1973-74) (Fig. 10.). Sometimes rye surpassed the amount of wheat from the 16^{th}-17^{th} centuries, found at Kaposvár-Kaposszentjakab (excavation by Nagy, E. 1962), Pécs-Sebészeti Clinic (excavation by F. Fülep 1964), Szécsény-Plébániatemplom (excavation by K. F. Bodnár 1988-’93), Debrecen Kölcsey Cultural Center (excavation by Zs. Hajdú, et al. 2004).

Analysing the soilsamples from a 17^{th} century Cannon Foundry of II. György Rákóczi of Sárospatak Castle (excavation by I. Ringer 2006) took nearly one and a half thousand, spindle-shaped charred rye grains found. Behind the common wheat rye was the second important cereals. Joint cultivation is very likely (Gyulai et al. 2012).

Makkai (1968) has shown that in contrast to the slash and burn method practiced earlier on, which depleted soil quite quickly, the heavy plough used in the Árpádian Period conserved the fertility of the soil. “Crop yields were grown by the possibility to grow plants with a higher nutrient content. The typical grain of slash and burn farming, common millet, which prefers freshly cleared land, was pushed back gradually in favour of the more valuable but more demanding wheat, rye and barley”. In this period of the Middle Ages, a two-course rotation system was practiced. One part (campus) remained fallow, the other was ploughed three times using a plough draught by six oxen.

Mainly wheat, maslin (wheat and rye), barley and oat were produced. Twice as much autumn sowing grain was grown than spring sown. Sometimes only autumn sown grain crop was produced. Harvesting and threshing were done manually. We do not know the average yields of medieval cereals. Yet, one can draw some conclusions by assessing the evolution of ploughing methods in the Middle Ages combined with average yields of the 16th century as reconstructed from documented data. Accordingly, in the interval ranging over thirty years of duty registers, the following ratio can be applied to peasant farming: three times the amount sown in wheat, twice that of barley, five times as much in rye and three times as much in oats (Kirilly 1968).

References

Borojević, K. (2005): Nutrition and environment in medieval Serbia: charred cereal, weed and fruit remains from the fortress of Ras. Vegetation History and Archaeobotany 14, pp. 453–464. DOI: http://dx.doi.org/10.1007/s00334-005-0092-9

Füzes, M. (1972): Előzetes jelentés az 1967. évi pogányszentpéteri kolostor-ásatás XVI. század eleji gabonaleletéről (Präliminarer Bericht über die Getreidefunde der Klosterausgrabung in Pogányszentpéter im Jahre 1967). A nagykanizsai Thury György Múzeum Jublieumi Évkönyve 285–290. Nagykanizsa.

Füzes, M. (1977): A növénytermesztés kezdete hazánkban, különös tekintettel a fenékpusztai növényleletekre. Egyetemi doktori értekezés. Agrártudományi Egyetem Keszthely. Manuscript.

Gaál, L. (1978): A Magyar növénytermesztés múltja. Akadémiai Kiadó, Budapest.

Gyulai F. (1997): A honfoglaló magyarság ételeinek régészeti-növénytani forrásai. „Nyereg alatt puhtjuk..? avagy Vendéglátási és ételkészķtési szokások a honfoglaló magyaroknál és a rokon kultúrájú lovas népeknél." Kereskedelmi, Vendéglátó és Idegenforgalmi Főiskola Tudományos Közleményei I., Ómagyar kultúra 10. évf., Budapest, Separatum, 142 p., 113-134.

Gyulai F. (1998): A Kis-Balaton térségének archaeobotanikai kutatási eredményei. A Kis-Balaton térségének magasabbrendű növényzetével kapcsolatos kutatási eredmények. Magyar Hidrológia Társaság és a NYUVIZIG konferenciája, Keszthely, 1998. március 24., 17-24.Molnár 1961

Gyulai F. (1999): A Rákospalota-Újmajor 1. lelőhelyről származó növényleletek archaeobotanikai feldolgozása. (Archaeobotanical Analysis of the Botanical Remains Collected from the 1996. Excavations at the Motorway M0 site No. 1 Rákospalota-Újmajor). In: Bencze Z., Gyulai F., Sabján T. & Takács M.: Egy Árpád-kori veremház feltárása és rekonstrukciója. Monumenta Historica Budapestinensia 10, Budapest, 200 p., 73-91.

Gyulai F. (2003): Kiskundorozsma-Nagyszék szarmata kori település növénymaradványai. In: Szalontai Cs.: Úton útfélen. Múzeumi kutatások az M5 autópálya nyomvonalában. Móra Ferenc Múzeum, Szeged, 141-148.

Gyulai F. (2014a): Újabb eredmények a honfoglaló magyarság étkezési kultúrájának feltárásában: Edelény-Borsodi földvár ételmaradványainak vizsgálata. In: Révész l. & Wolf M.: (eds.): A honfoglalás kor kutatásának legújabb eredményei. Tanulmányok Kovács L. 70. Születésnapjára. Monográfiák a Szegedi Tudomnyegyetem Régészeti Tanszékéról 3. Szeged, 2013, 885 p., 715- 734.

Gyulai F. (2014b): Solt-Tételhegy régészeti lelőhely archaeobotanikai kutatása. Archaeologia Cumanica 3, 175-207.

Gyulai F., Pósa P. & Ringer I (2012): A kora újkor növényi sokféleségének maradványai Sárospatakon. Hogyan született az újkori falu? Régészeti adatok a mezőgazdaság- és a településtörténet kérdéseihez. Konferencia a Tudomány Hete alkalmából. Budapesti Történeti Múzeum, 2012. november 6., Összefoglalók, 4.

Gyulai, F. (1995): Natural environment and climate. Plant exploitation and agriculture. In: Bartosiewicz, L. (ed.) Animals in the Urban Landscape in the Wake of the Middle Ages. A case study from Vác, Hungary. Tempus reparandum/BAR, International Series 609, Oxford, 3-4., 93-110. DOI: http://dx.doi.org/10.1002/(sici)1099-1212(199709/10)7:5%3C566::aid-oa351%3E3.0.co;2-2

 DOI: 10.18380/SZIE.COLUM.2014.1.2.37

Gyulai, F. (2010): Archaeobotany in Hungary. Seed, Fruit, Food and Beverages Remains in the Carpathian Basin: an Archaeobotanical Investigation of Plant Cultivation and Ecology from the Neolithic until the Late Middle Ages. Archaeolingua, Budapest, 479 p.

Gyulai, F. (2011): The archaeobotanical study of plant remains from the Sarmatian Period and the Árpád Period recovered at the site of END0170. In: Vaday H. A., Jankovich B. D. & Kovács, L.: Archaeological Investigations in County Békés 1986–1992. Varia Archaeologica Hungarica 25, Budapest, 360 p., 359-404.

Gyulai, F., Hertelendi, E. & Szabó, I. (1992): Plant remains from the early medieval lakeshore settlement Fonyód-Bélatelep (Lake Balaton, Hungary) with especial emphasis on the history of fruit cultivation in Pannonia. Vegetation History and Archaeobotany 1, 177-184. DOI: http://dx.doi.org/10.1007/bf00191557

Gyulai, F., Kenéz, Á. & Pető, Á. (2014): Getreideökotypen oder Landsorten als archäobotanische Beweise für die prähistorische Sortennutzung. In: Heinrich-Tamáska, O. & Straub, P. (ed.): Mensch Siedlung und Landschaft im Wechsel der Jahrtausende am Balaton / People, Settlement and Landscape on Lake Balaton over the Millennia. Castellum Pannonicum Pelsonense Vol. 4. Verlag Marie Leindorf GmbH, Budapest–Leipzig–Keszthely–Rahden/Westf., 2013. 440 p., 429-436.

Gyulai, F., Szolnoki L., Daróczi-Szabó, L. & Emődi, A. (2013): Plant remains of Ismaeliti from Medieval Hungary. 16th Conference of the International Work Group for Palaeoethnobotany, Thessaloniki, 17th-22th June 2013. Abstracts book, 128-129.

Hartyányi, B. P. (1981-83): Kora Árpád-korból származó búza a Hont-i ispánsági várból (Aus der frühen Árpádenzeit stammender Weizen der Honter Gespannschaftsburg). Magyar Mezőgazdasági Múzeum Közleményei, 95–113.

Hartyányi, B. P. & Nováki, Gy. (1973-74): Növényi mag- és termésleletek Magyarországon az újkőkortól a XVIII. sz.-ig II. (Samen- und Fruchtfunde in Ungarn von der Jungsteinzeit bis zum XVIII. Jahrhundert II.). Magyar Mezőgazdasági Múzeum Közleményei, 23–73.

Hartyányi, B. P. & Patay, Á. (1970): A dunaföldvári öregtoronynál előkerült régészeti növények vizsgálata. Szekszárdi Balogh Ádám Múzeum Évkönyve, 209–222.

Hartyányi, B. P., Nováki, Gy. & Patay, Á. (1967–68): Növényi mag- és termésleletek Magyarországon az újkőkortól a XVIII. sz.-ig I. (Samen- und Fruchtfunde in Ungarn von der Jungsteinzeit bis zum XVIII. Jahrhundert I.). Magyar Mezőgazdasági Múzeum Közleményei, 5–85.

Jones, G. & Halstead, P. (1995): Maslins, Mixtures and Monocrops: On the interpretation of archaeobotanical crop samples of heterogeneous composition. Journal of Archaeological Science 22, pp. 103–114. DOI: http://dx.doi.org/10.1016/s0305-4403(95)80168-5

Káldy-Nagy Gy. (1970): Magyarországi török adóösszeírások. Akadémiai Kiadó, Budapest.

Kirilly, Zs. (1968): A jobbágyság gabonatermelésének vizsgálata a maghozam szempontjából. Agrártörténeti Szemle 10, 70–78.

Lehoczky, T. (1883): A szádelővölgyi sziklavölgy természetrajzi és régészeti tekintetben. Archaeologiai Értesítő 3, 140.

Makkai, L. (1968): Az idő sodrában. Budapest.

Nováki, Gy. (1984-85): Szántóföldek maradványai a XIV-XVI. századból a Sümeg-Sarvalyi erdőben (Überreste der Ackerfelder des 14.–16. Jahrhunderts aus dem Wald Sümeg-Sarvaly). Magyar Mezőgazdasági Múzeum Közleményei, 19–32.

Pálóczi Horváth A., Torma A. & Somhegyi T. (1996): Élet egy középkori faluban – Szentkirály. Magyar Mezőgazdasági Múzeum, Budapest, 67 p.

Pieta, K. (1988): Die Slowakei im 5. Jahrhunder. Germanen, Hunnen und Awaren. In: Bott, G. & Meier-Arendt, W. (eds.), Schätze der Völkerwanderungs zeit. 385–417. Germanisches Nationalmuseum, Nürnberg.

Pieta, K. & Plachá, V. (1989): Getreide- und Brotfunde aus der Völkerwanderungszeit in Devin. Slovenska Archeologia 37, 69–88.

Rácz, L. (1993): Éghajlati változások a középkori és kora újkori Európában. In: R. Várkonyi, Á. & Kósa, L. (eds.): Európa híres kertje. 67–83. Orpheusz, Budapest.

Skoflek, I. & Árendás, V. (1971): Botanische Untersuchung der aus den kaiserzeitlichen Siedlungen stammenden Lehmbewurfe (Methode und Ergebnisse). Mitteilungen des Archäologischen Instituts der Ungarischen Akademie der Wissenschaften 2, 119–129.

Szamota, I. & Zolnai, Gy. (1902-1906): Magyar Oklevél-szótár. Budapest.

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EFFECTS OF DIFFERENT BIOCHARS, COMPOST AND LIME TREATMENTS ON THE CHEMICAL PROPERTIES OF SANDY SOILS

Annamária HOLES [1] – Tamás SZEGI [1] – Márta FUCHS[1] – MiklósGULYÁS [1] – László ALEKSZA[2]

1. Department of Soil Science and Agrochemistry, Institute of Environmental Science, Faculty of Agricultural and Environmental Science, Szent István University, Holes.Annamaria@mkk.szie.hu, Szegi.Tamas@mkk.szie.hu, Fuchs.Marta@mkk.szie.hu, Gulyas.Miklos@mkk.szie.hu

2. Department of Water and Waste Management, Institute of Environmental Science, Faculty of Agricultural and Environmental Science, Szent István University, Aleksza.Laszlo@mkk.szie.hu

Abstract

Decrease in organic matter of the soil is one of the major threats to soils in Europe and other parts of the globe. Maintaining or increasing organic carbon is a great challenge in agricultural practices. Application of composts and other organic amendments is an important way of compensation of losses of organic carbon at the same time it is solving the placement and recycling of organic wastes and residues.

The favorable effect of these amendments on physical, chemical and biological properties has been proved by different studies, however the stability and the rate of the influence is an issue that can be improved.

The aim of the presented study was to investigate the effect of carbonates on the solubility of applied organic materials and selected soil parameters.

Different biochars, compost and carbonate were added to light textured soil. The pH and E4/E6 rates were studied under laboratory conditions.

Beside the increase of soil organic matter content, all studied parameters gave promising results. The decrease in E4/E6 rate suggests that the inorganic carbonates are stabilizing the fresh organic residues and prevent the leaching processes. Improved soil organic carbon stability is very important in light textured soils.

Further investigations are undergoing to determine the optimal rate of components and extend the kinds of material available for application.

Keywords: biochar, compost, sand, soil, biodegradable waste

Introduction

Composting and pyrolysis technologies can be solutions for biodegradable waste reduction, stabilization and environmentally friendly use and recycling of the by-products. (Christensen *et al.*, 1983; Joseph-Taylor, 2014). All of these techniques are advantageous for environmental and waste management (Hayland-Sarmah, 2014).

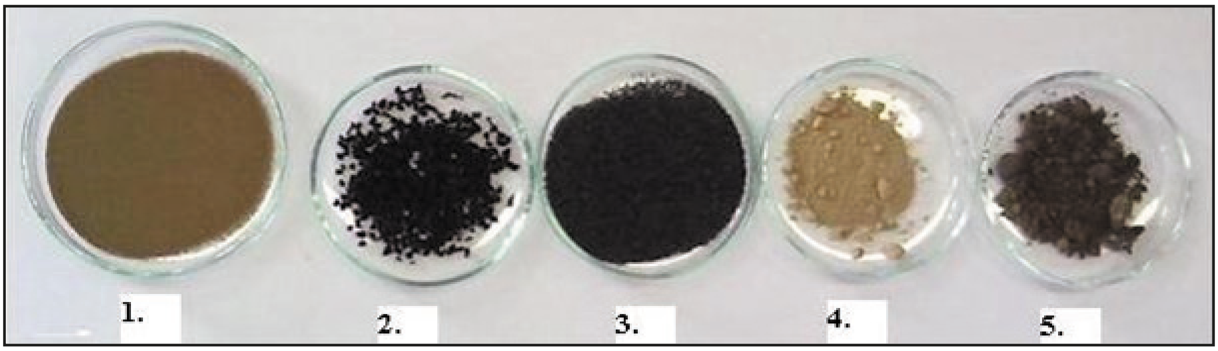
Favourable soil conditions can be achieved by applications of different soil amendments (Chen *et al.*, 2013). Applications of composts (Kádár, 2013) and biochars strongly increase the capacity of nutrient supply, soil organic matter content, soil buffering capacity and water holding capacity which stabilize and improve the soil structure, nutrient and water retention capacity (Van Zweiten *et al.*, 2010; Tammeorg *et al.*, 2014; Schmidt *et al.*, 2014).

The aim of this study was to investigate the effects of different composts and biochars treatments on sandy soils. The changes of chemical and physical parameters of the treated soil samples were investigated.

Materials

The chemical and physical properties of different composts can differ from one another depend largely on the source of raw material. The applied composts: COMPOST I. (compost produced from the green waste of park maintenances), COMPOST II. (compost produced from garden waste separately collected from households), COMPOST III. (compost exclusively produced from fallen leaves). The soil samples (Figure 1.) were collected in Szárítópuszta Experimental farm (Gödöllő, Hungary), from 0-20cm depth of an Arenosol. Two types of biochars (Figure 1.) were compared: plant origin biochar (POB) and animal origin biochar (AOB, bonechar) produced by pyrolysis. The samples were examined with added lime and without added lime. Table 1. shows the parameters of the sample mixtures. The experiments of the sixteen sample mixtures were replicated three times.

Figure 1. Investigated materials(1. - sand, 2. - animal origin biochar (AOB), 3. - plant origin biochar (POB), 4. - lime and 5. - green waste compost)



Methods

The following parameters were measured from the three types of composts (Table 2.): dissolved organic carbon (DOC) content [based on Tyurin method], soil organic matter content (SOM) [loss on ignition] and $CaCO_3$ content [calcimeter method]. The organic matter quality, pH, cation exchange capacity (CEC) and DOC of the investigated materials were measured (Table 1.). The organic matter quality was measured by E4/E6 metbod (Trubetskaya *et al.*, 2013), the CEC was measured by Mehlich method (Buzás, 1988) and the DOC was measured by Tyurin method (Buzás, 1988).

For the further biochar tests COMPOST I. was used, because it contained the highest proportion of DOC (Table 1.). Both biochar and bonechar were mixed with sandy soil in similar proportions (Table 3.) and replicated three times. The samples were incubated for four weeks in room temperature and moisturized with 150g distilled water (in 24th June to 22th July, 2013) after preparation. 1000g sandy soil was used as a control. The samples were examined with adding 0,5g lime and without adding lime (Table 1.). Every sample was air dried before measuring the parameters.

The samples of biochar-compost treatments (Table 1.) were tested for pH, and organic matter quality. The quality of the dissolved organic matter was measured by E4/E6 method (Trubetskaya *et al.*, 2013).

The results were analyzed by One Way Anova method using SPSS 16.0 Program Package [$P < 0,05$] (SPSS Inc. 2005).

Results and discussion

Table 2 shows the results of comparison experiments of different compost types.

The results show significant differences between COMPOST III. and the two other composts in SOM% (soil organic matter) and lime concentration. The significant differences are shown 'a' and 'b' in the indexes of the values in Table 2. There is no statistically significant difference between COMPOST I and COMPOST II in terms of SOM% and lime concentration. The DOC quantity solved in cold water shows opposite result: COMPOST I. is significantly different from the two other types of composts regardless if lime was added or not. There are no significant differences between the DOC quantity (solved in hot water) and the CEC results (Table 2.).

The soluble organic carbon sequestration

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Table 1. Treatments of samples

sample name	sand (g)	AOB [charcoal](g)	POB [biochar](g)	compost (g)	$CaCO_3$ (g)
sand	100				
compost				100	
compost + sand	80			20	
POB			100		
AOB		100			
1%AOB	495	5			
2.5%AOB	487.5	12.5			
5%AOB	475	25			
10%AOB	450	50			
1%POB	495		5		
2.5%POB	487.5		12.5		
5%POB	475		25		
10%POB	450		50		
1%AOB+comp	395	5		100	
2.5%AOB+comp	387.5	12.5		100	
5%AOB+comp	375	25		100	
10%AOB+comp	350	50		100	
1%POB+comp	395		5	100	
2.5%POB+comp	387.5		12.5	100	
5%POB+comp	375		25	100	
10%POB+comp	350		50	100	
1%AOB+$CaCO_3$	495	5			0.25
2.5%AOB+$CaCO_3$	487.5	12.5			0.25
5%AOB+$CaCO_3$	475	25			0.25
10%AOB+$CaCO_3$	450	50			0.25
1%POB+$CaCO_3$	495		5		0.25
2.5%POB+$CaCO_3$	487.5		12.5		0.25
5%POB+$CaCO_3$	475		25		0.25
10%POB+$CaCO_3$	450		50		0.25
1%AOB+comp+$CaCO_3$	395	5		100	0.25
2.5%AOB+comp+$CaCO_3$	387.5	12.5		100	0.25
5%AOB+comp+$CaCO_3$	375	25		100	0.25
10%AOB+comp+$CaCO_3$	350	50		100	0.25
1%POB+comp+$CaCO_3$	395		5	100	0.25
2.5%POB+comp+$CaCO_3$	387.5		12.5	100	0.25
5%POB+comp+$CaCO_3$	375		25	100	0.25
10%POB+comp+$CaCO_3$	350		50	100	0.25

capacity of sandy soils was improved with added lime. The quantity of soluble organic carbon is 1 mg/ml less as average with using the cold water method than in the samples treated with 0,5g lime (Table 2.).

The different degrees of decomposition and stability of the applied composts can be explained by the original 3-5% lime content (Table 2.). The different SOM content explains the differences in quantity of DOC in certain compost types during the hot water solution. It can be concluded that the DOC in COMPOST III. (compost exclusively produced from fallen leaves) is less than in the two other types of composts.

The cause of this probably is the high concentration of lignin of leaves. It is known that the decomposition of lignin is a very slow process (Fioretto *et al*, 2005).

COMPOST I. was choosen for further examinations: testing the biochars, sandy soil and lime mixtures, because it has the highest CEC value (Table 2.).

Table 2. Comparison of composts
1. compost produced from green waste of park maintenance; 2. composts produced from garden waste separately collected from households; 3. compost exclusively produced from fallen leaves; 4. DOC: dissolved organic carbon

	SOM %	Lime concentration %	CEC	The quantity DOC (mg/ml) average [solved in cold water]		The quantity DOC (mg/ml) average [solved in hot water]	
				no added lime	added 0,5g lime	no added lime	added 0,5g lime
COMPOST I.[1]	31.84_b	3.67_a	16.32_a	5.53_b	4.52_b	8.65_a	5.44_a
COMPOST II.[2]	33.43_b	3.53_a	13.08_a	4.19_a	3.18_a	8.33_a	6.40_a
COMPOST III.[3]	20.73_a	4.39_b	13.05_a	3.18_a	2.18_a	7.37_a	3.84_a

Significant differences in DOC were detectable in samples containig 10% biochar (100g POB+C, 100g AOB+C). The KCl pH results showed the same tendency as DOC, but the three samples containing lower concentrations of biochars had no significant differences (10g POB+C, 25g POB+C, 50g POB+C, 10g AOB+C, 25g AOB+C, 50g AOB+C). Significant differences were shown between control and the other samples by the pH. The reason of this is that biochar and bonechar increase the pH even at low concentrations.

The samples mixed with lime and without lime (Table 1.) were measured for E4/E6 ratio (Diagram 1.) and pH (Diagram 2.).

Table 3. Parameters of investigated materials

sample	E4/E6	pH (distilled water)	pH (KCl)	cation exchange capacity	DOC (mg/ml)
sand	7.15	6.5	5.74	7.04	5.52
compost (COMPOST I.)	7.42	7.6	7.18	72.18	13.46
sand (80%) + compost (20%)	7.9	7.64	7.3	19.14	7.63
AOB	0.35	9.56	9.02	3.54	6.97
POB	7.58	6.62	5.45	41.64	3.36

The results showed the high concentration (10%) of AOB (sample 4) decreased the E4/E6 rates (Diagram 1.) and increased the pH (Diagram 2.). This can be explained by the alkaline pH and the quite low E4/E6 ratio of AOB (Table 3.). The lower E4/E6 ratio (about 4-5) showed more stable dissolved organic materials (Aranda *et al*., 2011). The stable organic materials like humic acids had a positive effect on soil properties

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Diagram 1. E4/E6 rates (numbers of samples - horizontal axis - sample 1-4: mixed with AOB, samples 5-8: mixed with POB). The biochar concentrations: 1%, 2.5%, 5%, 10%.

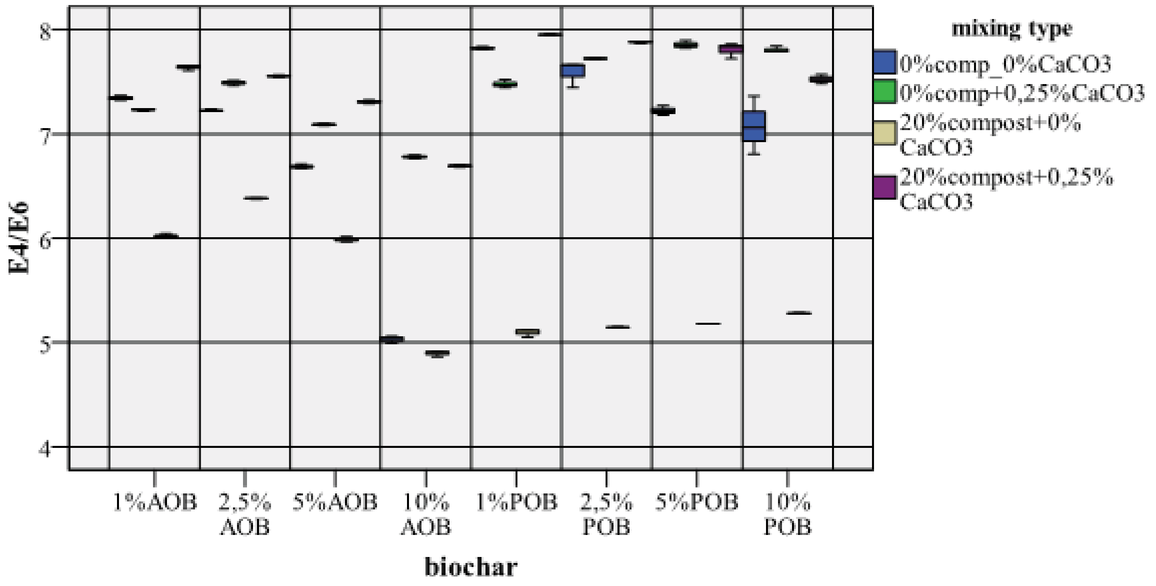
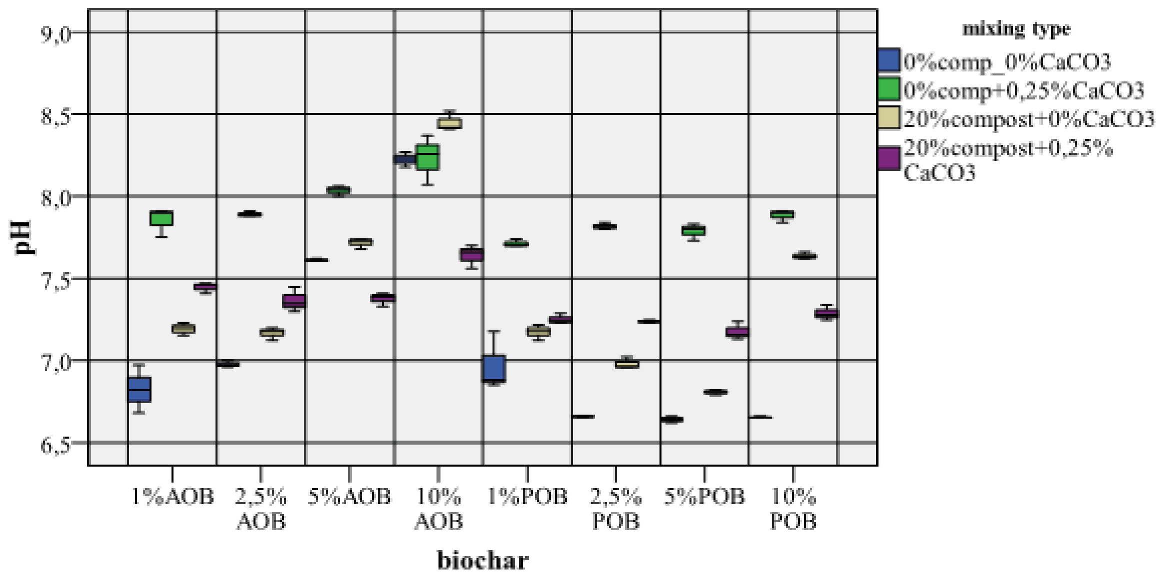


Diagram 2. pH results (numbers of samples - horizontal axis - sample 1-4: mixed with AOB, samples 5-8: mixed with POB). The biochar concentrations: 1%, 2.5%, 5%, 10%.



(Abdollahi *et al*., 2014). The animal origin biochar could be useful for the soil melioration.

The AOB increased the E4/E6 ratio and the POB did not cause a significant modification in the E4/E6 ratio (Diagram 1.). The AOB increased the pH because the pH of AOB is about 9-10 (Table 3.) but the added $CaCO_3$ (0,5m/m %) buffered this effect (Diagram 2.).

The $CaCO_3$ had a decreasing effect on the E4/E6 ratio. This can be explained by the generated Ca-humus complexes in the soil (Six *et al*., 2004).

The compost extremely increased the E4/E6 ratio, because it has high content of fresh organic matter compared to the soils.

Summary

The planned further experiments are measuring DOC, loss on ignition and CEC before and after pot experiments on the samples of Table 1. In the future the purpose of the experiments is to produce the compost-biochar-lime mixture with the most favourable impact to the soil fertility and crop yield. It could be useful in agricultural practice.

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References

Buzás I. (1988): Talaj- és agrokémiai vizsgálai módszerkönyv 2., Mezőgazdasági Kiadó, Budapest 106-116, 152-155, 179.

Christensen T. H. and Nielsen C. W. (1983): Leaching from land disposed municipal composts: 1. organic matter, Waste Management & Research 1, 83-94 DOI: http://dx.doi.org/10.1177/0734242x8300100107

Hayland C., cSarmah A. K. (2014): Advances and Innovations in Biochar Production and Utilization for Improving Environmental Quality, Bioenergy Research: Advances and Applications, Pages 435–446. DOI: http://dx.doi.org/10.1016/b978-0-444-59561-4.00025-5

Fioretto A., Di Nardo C., Papa S., Fuggi A. (2005): Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem, Soil Biology and Biochemistry, Vol 37, Issue 6, Pages: 1083-1091. DOI: http://dx.doi.org/10.1016/j.soilbio.2004.11.007

Golueke, C.G. (1972): Composting, A Study of the Process and its Principles. Rodale Press, Inc., Emmaus, Pennsylvania, USA, Page: 110.

Schmidt H-P. *et al*. (2014): Biochar and biochar-compost as soil amendments to a vineyard soil: Influences on plant growth, nutrient uptake, plant health and grape quality, Agriculture, Ecosystems & Environment, Environmental Benefits and Risks of Biochar Application to Soil, Volume 191, 15 June 2014, Pages 117–123. DOI: http://dx.doi.org/10.1016/j.agee.2014.04.001

Chen J. *et al*. (2013): Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China, Applied Soil Ecology, Volume 71, Pages 33–44. DOI: http://dx.doi.org/10.1016/j.apsoil.2013.05.003

Kádár I. *et al*. (2013): Szennyvizek, iszapok, komposztok, szevestrágyák a talajtermékenység szolgálatában, Magyar Tudományos Akadémia ATK Talajtani és Agrokémiai Intézet, 9421549 Akaprint Nyomda Kft., ISBN 978-963-89041-9-5, Budapest, Pages: 79, 102.

Lal R. (2004): Soil Carbon Sequestration Impacts on Global Clomate Change and Food Security, Science 304, 1623. DOI: http://dx.doi.org/10.1126/science.1097396

Abdollahi L. *et al*. (2014): The effects of organic matter application and intensive tillage and traffic on soil structure formation and stability, Soil and Tillage Research, Volume 136, Pages 28–37. DOI: http://dx.doi.org/10.1016/j.still.2013.09.011

Trubetskaya O. E. *et al*. (2013): Determination of hydrophobicity and optical properties of soil humic acids isolated by different methods, Journal of Geochemical Exploration 132, 84-89. DOI: http://dx.doi.org/10.1016/j.gexplo.2013.06.004

Tammeorg P. *et al*. (2014): Short-term effects of biochar on soil properties and wheat yield formation with meat bone meal and inorganic fertiliser on a boreal loamy sand, Agriculture, Ecosystems & Environment Environmental Benefits and Risks of Biochar Application to Soil, Volume 191, Pages 108–116. DOI: http://dx.doi.org/10.1016/j.agee.2014.01.007

Six J., Bossuyt H., Degryze S., Denef K., (2004): A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till Research 79, 7–31. DOI: http://dx.doi.org/10.1016/j.still.2004.03.008

Sorensen H. (1961): Decomposition of Lignin by Soil Bacteria and Complex Formation between Autoxidized Lignin and Organic Nitrogen Compounds, Denmark, Pages:21-22. DOI: http://dx.doi.org/10.1099/00221287-27-1-21

SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc. DOI: http://dx.doi.org/10.2307/2986184

Joseph S. –Taylor P. (2014): The production and application of biochar in soils, Advances in Biorefineries, Biomass and Waste Supply Chain Exploitation, Pages 525–555. DOI: http://dx.doi.org/10.1533/9780857097385.2.525

Aranda V. *et al*. (2011): Effect of soil type and management (organic vs. conventional) on soil organic matter quality in olive groves in a semi-arid environment in Sierra Mágina Natural Park (S Spain), Geoderma, Volume 164, Issue 1-2, Pages 54–63. DOI: http://dx.doi.org/10.1016/j.geoderma.2011.05.010

Van Zweiten L. *et al*. (2010): Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility, Plat Soil 327:235-246. DOI: http://dx.doi.org/10.1007/s11104-009-0050-x

STORAGE PROTEINS IN WHEAT (*Triticum aestivum* L.) AND THE ECOLOGICAL IMPACTS AFFECTING THEIR QUALITY AND QUANTITY, WITH A FOCUS ON NITROGEN SUPPLY

Csaba HORVÁTH

Szent István University, Crop Production Institute, Gödöllő
e-mail: hoce.csaba@gmail.com

Abstract
Wheat (*Triticum aestivum* L.) is the most widely used basic staple for mankind. Wheat is also one of the most important cereals in Hungary with a high economic value. The aim of wheat production is twofold; to provide quantity and quality. Milling and baking quality of wheat is mainly determined by the genetic basis, however it can be influenced by management techniques.

Storage proteins account for more than half of the total protein in mature cereal grains and have important impacts on their nutritional quality for humans and livestock and on their functional properties in food processing. Gluten proteins - gliadins and glutenins of wheat determine the quality of the grain for breadmaking and their amount and composition can be influenced by agronomic impacts leading to changes in dough properties and that of baking quality.

The present review is dealing with the impact of various agronomic and environmental factors on the performance of storage proteins and within them gluten proteins.

Keywords: review, wheat, proteins, nitrogen suply

Introduction

Wheat is one of the most important cereals used for bread making, a key source of food all over the world. The total global wheat output exceeded 670 million tonnes in 2012, according to FAOSTAT data (FAOSTAT 2012). Bread made from wheat is a staple foodstuff because it fills the stomach, it is easy to digest and it is not expensive (Pollhamerné 1973).

The success of wheat relies partly on its adaptability and high yield potential but it is also important that the dough made from its flour is suitable for making a wide variety of bakery products. Wheat contains essential amino acids, minerals and vitamins, along with useful secondary metabolites and dietary fibres (whole grain products are particularly rich in the latter two). It should also be noted however, that meals containing wheat products may be responsible for a variety of undesired side effects, such as intolerance (wheat sensitivity) or allergic symptoms (respiratory allergy and food allergy).

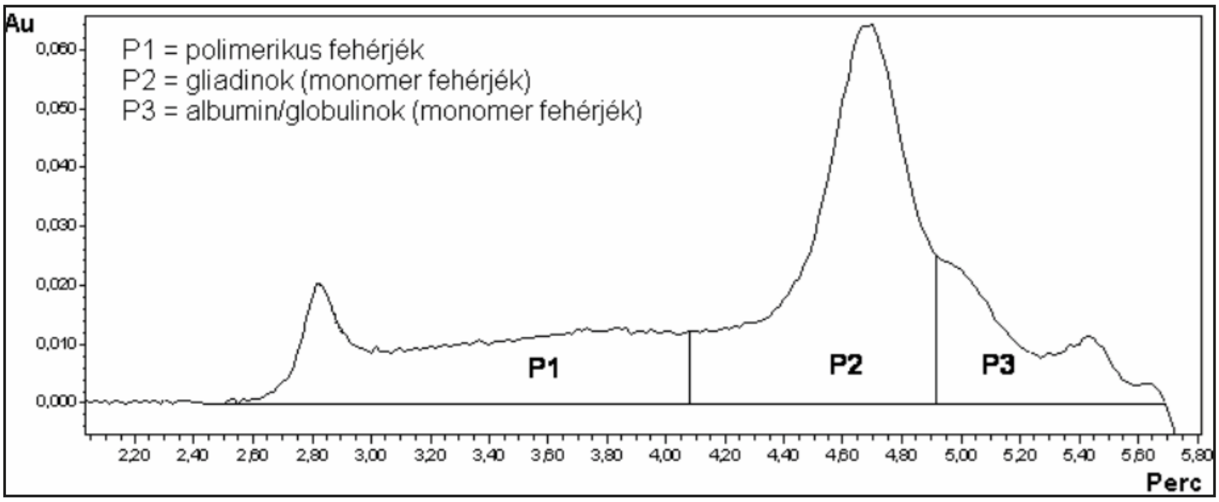
The topics of ongoing and prospective research projects include sustainable wheat production and quality using reduced amounts of chemicals, breeding of new wheat strains for end-users with special requirements, including the bio-fuel industry and public catering (Shewry 2009).

Plant breeders have, for quite some time now, been working on developing wheat varieties and strains meeting a wide range of different requirements on the part of users. The quality attributes of common – or bread – wheat are highly important for different users (Bedő et al. 1998). Different types of wheat of different protein contents are required for the production of biscuits, different types of bread or pasta. The possibilities of using a given wheat variety are determined primarily by two of its attributes: grain hardness and protein content: higher protein contents make it possible to make more valuable, higher quality products (Békés 2014).

Wheat's storage proteins

It has been known since 1728 when Jacopo Beccari carried out his studies that it is possible to produce more or less clean gluten – a sticky

Figure 1. Polymeric (glutenin-P1) and monomeric (gliadin-P2 and albumin, globulin-P3) proteins in the flour of the "Ukrainka" wheat variety (24. DPA) separated on the basis of its SE-HPLC chromatogram (Abonyi Tibor, 2010. p. 72.) (P1 = polymeric proteins, P2 = gliadins (monomeric proteins), P3 = albumin/globulins (monomeric proteins) **Minutes**)



matter of a peculiar consistency – by rinsing dough produced from wheat flour in water. This matter has a complex structure that is created by the interaction between elastic glutenin and plastic gliadin proteins. The gluten proteins are the most important storage proteins of a wheat grain, to be found exclusively in the starchy endosperm, the part of the grain that is ground into flour. During the ripening process the proteins combine with each other forming large polymers and when the flour is mixed with water while being worked into dough they build a continuous network of proteins. This network of protein molecules gives flexibility and viscosity to the dough, making it possible for instance to produce raised bakery products (Tosi et al. 2011). It is the storage proteins of wheat, called prolamins, making up what is commonly known as gluten, that make it possible to create such a wide variety of products from wheat flour. The relative proportions of the different types of proteins making up a given wheat flour's protein content however, are extremely important. Accordingly, the baking quality of wheat is determined by the quantity of the total protein content and the relative proportions of the different types of proteins to be found in it.

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 conventionally assigned to two different groups: the density and extensibility of the dough is determined by the monomeric gliadins, while its flexibility and strength is determined by polymeric gluteins. Within these groups the various proteins are categorised further according to their electrophoretic mobility: gliadins are sub-divided into type α-, γ- and ω proteins, depending on their mobility during electrophoresis, in a low pH environment.

Under chemical reduction glutenin decomposes into its subunits. The glutenin components are categorised into high molecular weight (HMW) and low molecular weight (LMW) groups based on the SDS-PAGE separation technique (Shewry et al., 2009).

Similarly to other wheat storage proteins its prolamins are also polymorphic, encoded by multigene families that are present in the form of homeologous alleles on three genomes (A, B and D). A high degree of variation of the gluten protein alleles is observed across different genotypes as well. Different wheat varieties can be separated from one another

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Figure 2. Fractioning of gliadin proteins according to charge (Békés 2014)

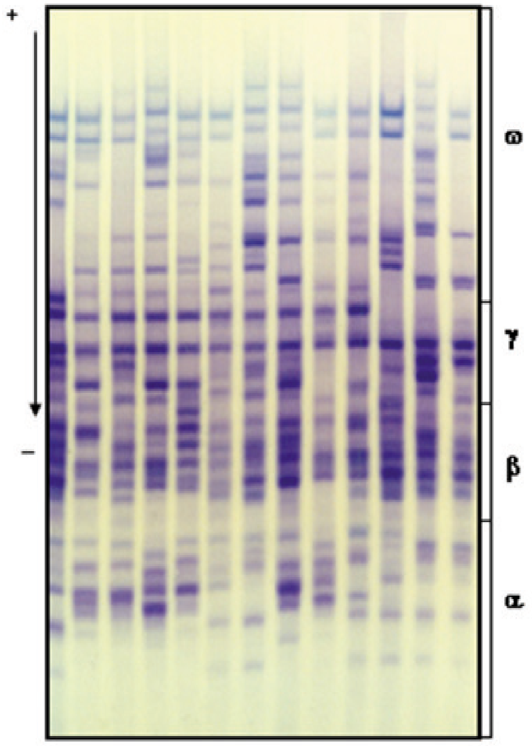
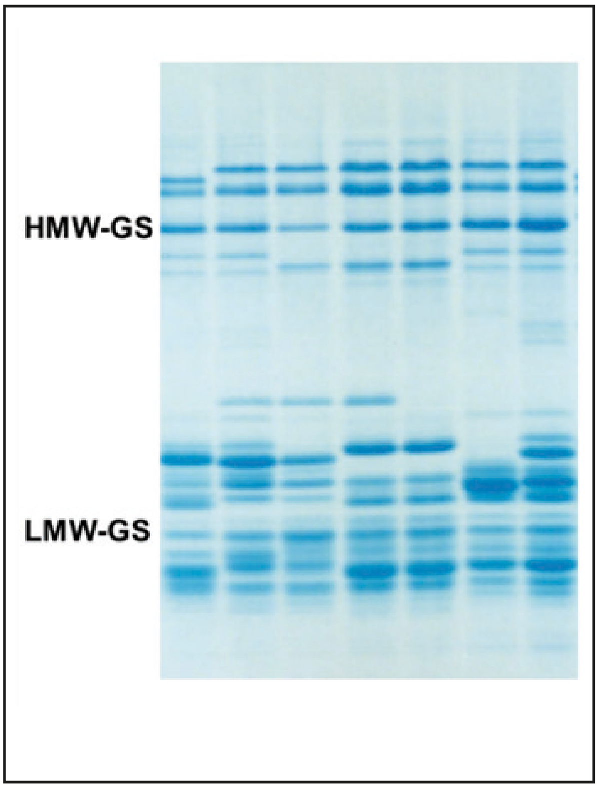


Figure 3. Separation of glutenin components by size (Békés 2014)



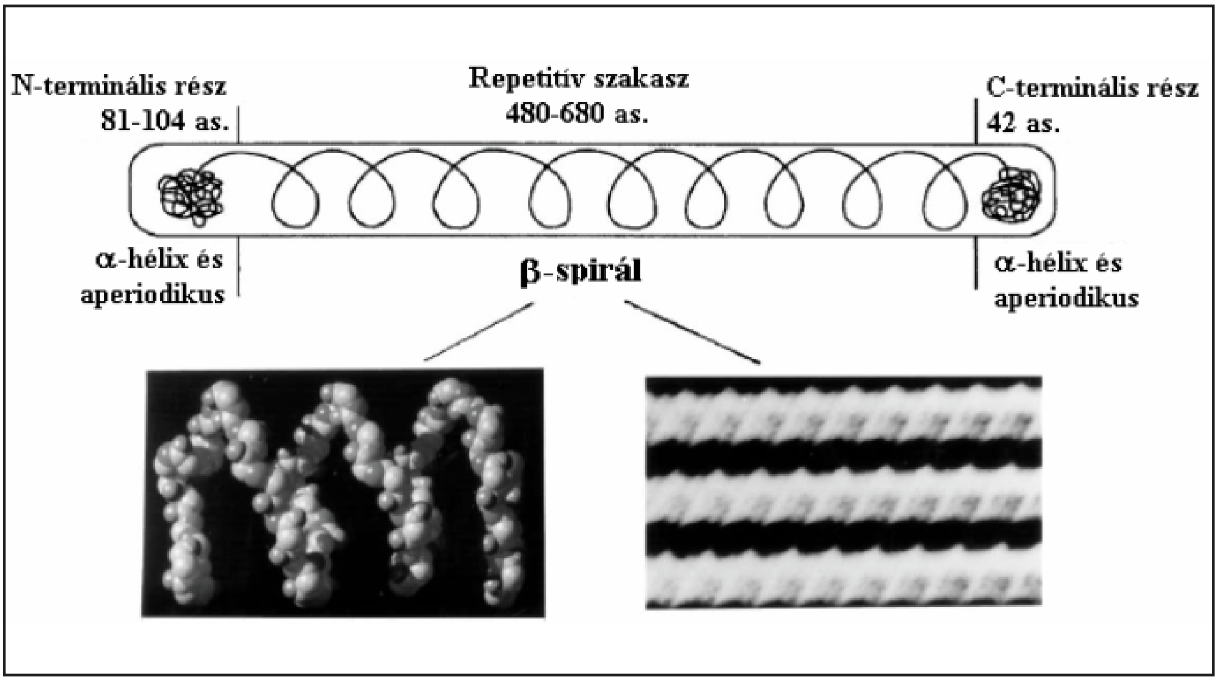
on the basis of the identification of the storage proteins, e.g. by the electrophoresis of gliadins (Bushuk et Zillman 1978).

The division into gliadins and gluteins - though it has proven to be a rather durable technique over time – sheds no light onto the molecular and evolutionary aspects of proteins. In this regard only three groups can be distinguished (Shewry et al., 1986): HMW proteins (comprising glutenins' HMW components), prolamins that are rich in sulphur (comprising α-, β- and γ-gliadins and glutenins' LMV components) and low-sulphur prolamins (comprising ω -gliadins and this type of proteins of the glutenin fraction – these are referred to as group D within the LMW category) (Masci et al. 1993, 1999).

Most ω-gliadins are encoded by the genes (called *Gli-A1*, *Gli-B1* and *Gli-D1*) on the *Gli-1 loci* on chromosomes A, B and D however, some other loci are also located on the same chromosome arm.

The structures of the proteins encoded by the genes *Gli-A1*, *Gli-D1* and *Gli-B1* are clearly different from each other. Although both groups of the proteins are created by repetitions of – for the most part – short peptide patterns, the patterns themselves are different from each other: in the case of the proteins encoded by *Gli-A1* and the *Gli-D1* genes a repetition of the PQQPFPQQ sequence, in the case of those encoded by the *Gli-B1* genes a repetition of the PFQ$_{2–4}$ sequence is observed (P=proline, Q=glutamine, F=phenylalanine). These differences in pattern appear, of course, in the amino-acid compositions of the resulting proteins as well: the ω-gliadins encoded by the genes *Gli-A1* and *Gli-D1* contain about 40 n/n% Glutamine and 30 n/n% Proline while the proteins encoded by the *Gli-B1* genes contain approx. 50 n/n% Glutamine and n/n% Proline. Moreover, these two different types of ω-gliadins are easily separated by electrophoresis at a low pH value. The *Gli-A1* and the *Gli-D1* proteins move much slower: these are called ω-1/2 gliadins, while the faster moving *Gli-B1* proteins are called ω-5 gliadins. The different ω-gliadins are distinguished from each other according to their N-terminal amino acid sequences, such as the SRLLSPQ sequence in ω-5 gliadins, the ARQLNPSNKELQ or KELQSPQQS sequence in ω-1 gliadins and the ARELNPSNK sequence in ω-2 gliadins (A=alanine , E=glutamic acid , L=leucine, S=serine , R=arginine, N=asparagine, K=lysine) (Shewry et al., 2009). The protein subunits making up gluten protein are bound together through disulphide bridges in the polymer. distribution of the special gluten proteins in the endosperm. The large molecule weight glutenin subunits (HMW - glutenin) and the γ gliadins are to be found primarily in the innermost layers of the endosperm, while the low molecule weight subunits of glutenin (LMW - glutenin), the ω and the α gliadins, are more typically to be found in the subaleuron layer. Immune localisation made it possible to demonstrate that the segregation of

Figure 4. The assumed structure of the HMW gluten subunit, according to spectroscopic and hydrodynamic examinations (Shewry et al., 2000)



The distribution and accumulation of proteins

Proteins are concentrated predominantly in the subaleuron layer of the ripe grain (Bradbury et al., 1956; Normand et al., 1965; Kent, 1966; Kent and Evers, 1969; Tosi et al., 2009, 2011; He et al., 2013). Accordingly, the endosperm is not a homogeneous tissue and its key components (proteins, starch and cell-wall polysaccharides) show a qualitative and a quantitative gradient as well. The gradient observed in the protein content and its composition is clearly evident and highly important because it plays a major role in the determination – by the gluten proteins – of the value of wheat for the milling industry. By means of Western blot analysis, using antibodies, it is possible to display the the gluten proteins occurs between and within the protein particles in the course of protein accumulation and this is preserved in the ripe wheat grain as well. Accordingly, a qualitative and a quantitative gradient develops among the gluten proteins of the endosperm during the development and maturing of the wheat grain. This may be caused perhaps by the origin of the subaleuronic cells which – unlike the other endosperm cells – develop from redifferentiated aleuronic cells but it may also possibly be a consequence of special controlling signals created by the matured tissue on the special domain of the gluten protein's gene promoter (Tosi et al. 2011).

The application of nitrogen fertilisers however, affects the expression patterns within the grain:

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Figure 5. LMW glutenin subunits (Békés 2014)

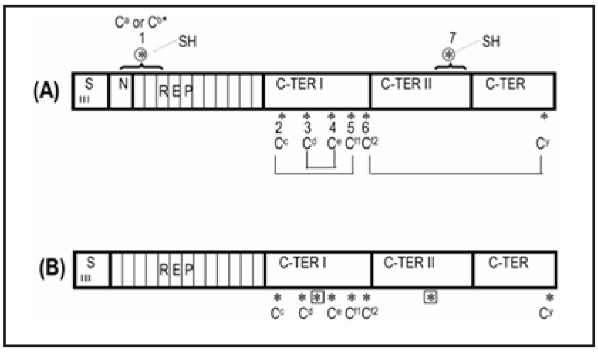
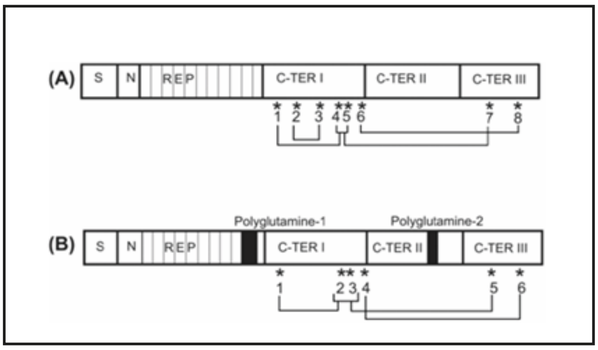


Figure 6. Gliadins (Békés 2014)



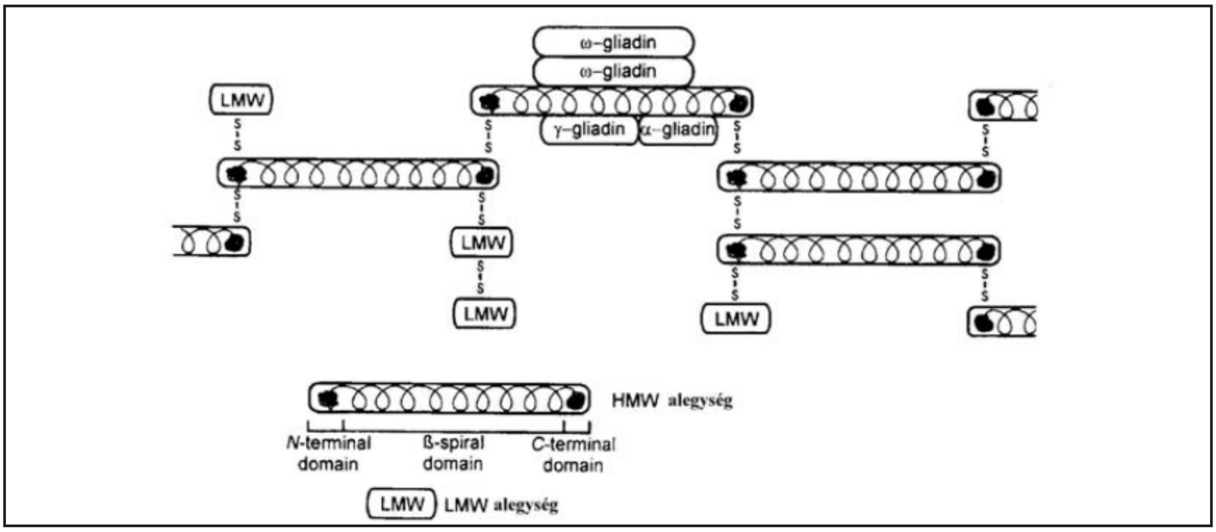
the ω gliadin genes got expressed mainly in the core of the endosperm rather than in the subaleuronic layer in the case of low N levels (100kgN-1), while in the case of high (350kgN-1) nitrogen levels they got expressed in the subaleuronic cells. (Wan et al., 2013b).

NIR spectroscopy may be an effective tool for monitoring the physiological processes of plants in terms of both qualitative and quantitative aspects also in regard to protein accumulation, the interaction between gliadins and glutenins and the development of the gluten network, while the spectrum also contains some additional hidden information that can be used for determining the degree of development of a particular wheat grain (Salgó and Gergely 2012).

The impacts of fertilisers/manure on plant development, yields and quality

Excellent raw materials is the first prerequisite for good bakery products. Of plant nutrients nitrogen has the most dramatic impact on both the quality and the amount of the yield. The application of a nitrogen fertiliser boosts protein content, the amount of wet gluten, vitrenousness and the thousand grain weight, indeed even the starch content of the grain (Pollhamerné 1973). 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 roper 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). Findings of long term experiments that have been continued in Hungary since 1967 at nine sites of different agro-ecological conditions, involving

Figure 7. A model illustrating the role of proteins making up gluten protein (Wieser et al. 2006)



long term N and P fertiliser experiments as well, show that increasing the amount of the P and the N fertiliser has a remarkable impact on the quantity, composition and quality of the yield, in comparison to the average of the data collected and evaluated during the first 20 years of the experiment (Ragasits et al., 2000). These impacts depend on the agro-ecological features of the area concerned. Even the application of a smaller dose of fertiliser triggers an increase in yield, while a higher dose generates a quality improvement as well.

In the case of winter wheat a harmonised supply of nutrients (NPK) is a dominant technique in crop production even in areas with favourable nutrient and water supply. Experiments carried out in 2001 showed an increase from the 3193 kg/ha average yield, without fertilisation, of the various wheat varieties involved in the trial, to 4 tonnes after the application of optimised quantities of fertilisers. Moreover, the application of fertilisers also affected, besides the quantity of the yield, the quality and the stability of the quality of the produce. The effects of the application of fertilisers are influenced by agro-ecological, biological and agro-technical elements (e.g. genotype, water supply, the preceding crop, the crop protection technology) (Pepó P. 2002, 2005, 2006, 2007). Nitrogen top dressing, even in doses as low as 40 kg/ha, but much more so in doses up to 80-120 kg/ha, triggers yield increases even under very dry conditions. Experiments have shown that increasing doses of nitrogen top dressing can enable outstanding quality improvements even under unfavourable ecological conditions (Szentpétery 2004). It is also confirmed by the results of long term fertiliser application experiments set up back in 1967, that the decrease in yields caused by aridification can be alleviated by the application of the right doses of fertilisers. (Láng et al 2007.)

As a general rule, yields, as well as grain protein contents, are also increased significantly by irrigation besides the application of fertilisers. These details are highlighted by the experiments carried out by Pushman and Bingham (1976): they applied fertilisers in amounts of 90 kg/ha, delivered to the experiment plots in the form of granules, as a result of which the yield increased by 12. 4 % and 6.1 %, while the grain protein content grew by 13.0 % and 33.7 % , without and with irrigation, respectively. The application of another dose of N fertiliser – liquid urea, in the form of top dressing during the anthesis phase – resulted in a 12.4 % and a 8.8 % increase in the amount of proteins with and without irrigation, respectively, along with a positive impact on the total amount of the yield as well. The significant difference in the yields and the protein contents of the produce of the different varieties resulted, in the case of each nitrogen treatment, in an inverse relationship between yield quantity and protein content. The amount of protein produced (N mass/unit of area) was similar in the case of every variety but the flour yield decreased as a consequence of the urea treatment. The differences between the varieties in terms of flour yield appeared to be stable and they were in no correlation with the thousand grain weight or with the bulk density. The bulk density may be a useful aid in the calculation of the flour yield on the basis of a sample taken from one variety but it would be probably misleading when it comes to comparing different varieties (Pushman and Bingham 1975). The test loaf volume increased as a consequence of the application of the granulated fertiliser. Urea top dressing had no such effect, despite the fact that it lead to increased flour protein content and decreased α-amylase activity showing that the application of nitrogen fertiliser after flowering is somewhat belated if the aim is to improve the product's value for the baking industry (Pushman and Bingham 1976).

Berecz and Ragasits (1990) examined the impacts of N fertiliser applied in the form of ammonium nitrate, in various doses (80-200 kg/ha), during different Feeks phases of flowering, in different distributions among the examined Feeks phases, on the dry matter accumulation in the winter wheat variety named Martonvásári-4 (*Triticim aestivum, L.*). In the

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initial growth phase neither the amount of nitrogen fertiliser applied, nor the timing of its application had any impact on dry matter accumulation. The plant's N accumulation positively correlated with the amount of N, with a 349.5 mg maximum amount per 10 plants when 200 kg/ha nitrogen was applied. Distributed application of the N fertiliser had no material impact on N accumulation. The grains' raw protein content increased along with the increase in the applied amount of nitrogen, but it was not affected by distributed allocation. The highest raw protein content was observed after the distributed application of 160 kg/he nitrogen fertiliser. The lysine and threonine content decreased as the raw protein content increased.

According to experiments performed by Győri (2006) the protein content determining the quality of wheat is radically increased by the application of a harmonised combination of nitrogen, phosphorous and potassium (NPK). This is also confirmed by the results of experiments carried out in 2001 and 2003, in the course of which the lower than 10 % protein content of the wheat produced on the control plot without fertilisers had been increased to 12 % or higher by the application of 60 kg/ha N+PK fertiliser. The wet gluten content of the produce on the control plots where no fertiliser was applied was below 25 %, while the application of 60 kg/ha N+PK resulted in a higher than 30 % wet gluten content in each of the experimental years. The application of the combined fertiliser in an 60 kg/ha dose improved the bakery value of the output in the case of each of the varieties involved in the experiments, however, the application of a higher doses does not result in further improvements in the case of every variety. In the course of their experiments Tanács et al. (2006) found that the application of higher doses of fertilisers usually lead to higher wet gluten contents. Reliable wet gluten content increase was produced by the application of 40+40 kg/ha N, 40 kg/ha P, 40 kg/ha K. The application of fertilisers in combination with fungicide treatments further increased the wet gluten contents. The baking value numbers were significantly different across varieties in both the annual evaluations and in the three-year averages in the wake of the treatments.

In experiments carried out by Szentpétery et al. (2005) increasing doses of nitrogen top dressing and their distributed application lead to quality improvements even in unfavourable ecological circumstances. In a series of experiments with the following doses: 40, 80, 120, 40+80 and 80 + 40 kg/ha the 120 kg/ha dose reduced the yield but in terms of total protein and gluten content the higher nutrient doses produced higher quality yields. Applying the amount of nitrogen used for top dressing in several rounds had a very positive impact on the crop. From the aspect of baking quality the growing amounts of fertiliser, particularly when applied in two rounds (one applied at a later stage) resulted in an increase in value. The best combination of treatments was the one (80+40 kg/ha) where a relatively large dose provided the wheat with the nutrient boost required for the first phase of its growth and the during the flowering phase another application of top dressing definitely helped the variant in coming closer to the maximum possible baking quality enabled by its genetic potential and the conditions of the given season. According to Fuertes-Mendizábal et al. (2010) the N-content of the wheat grain depends on the variant, the environmental impacts and the distribution of the applications of fertilisers. In comparing the response to increasing doses of nitrogen and to their application in different quantities at different times Soissons found that not only the increased amounts but also the distribution of their application over time had a positive impact on the quality of the wheat. Indeed, the distribution of the applications of a small total amount of nitrogen fertiliser over the growing season had an added positive impact in making the quality of the wheat more evenly balanced. The composition of the metabolic proteins remained unchanged regardless of the grains' nitrogen content, yet the amount of storage proteins increased along with the growth of the grains' nitrogen content. The flexibility, extensibility and strength of the dough improved dramatically, which was related to a slight increase in the glutenin fraction whereby it exceeded a certain threshold, indicating that a higher degree of glutenin polymerisation explains the improvement in quality. Both the raising of the doses of the N fertiliser and the distribution of the applications of the given amount over time, led to an increase in the amount of HMW-GS, enabling to the forming of an increased number of disulphide bridges, leading, in turn, to a higher degree of polymerisation which may ultimately drive the improvement in quality.

Raising the doses of the N fertiliser (from 0 to 288 kg/ha) resulted in an increase in the proportion of gliadin proteins and the extensibility of the dough (Godfrey et al., 2010). The flour ground from wheat produced on a field where 192 kg/ha N and 0 kg/ha S fertiliser had been applied was similar in quality to the flour made from wheat grown in a field where a total of 192 kg/ha N and 53 kg/ha S fertiliser had applied. The share of ω-gliadins however, increased and the strength of the dough was similar to that of the doughs made from wheat grown with smaller doses of N fertilisers. The N content of wheat grown on fields after the delivery of 35 t/ha farmyard manure equalled to that of wheat grown on fields where 144 kg/ha N fertiliser had been applied, indicating that most of the nitrogen present was not available for the wheat plants. The protein composition of the flour ground from wheat grown on this plot and the parameters of the dough produced from that flour were similar to those of flour and dough made from wheat of similar N content grown on a field enriched by conventional farmyard manure. Similar differences were observed in terms of the grains' N content, protein composition and functional attributes between wheat produced by organic farming and wheat grown by conventional techniques.

The protein content of grains is of relevance not only for the purposes of the baking industry but also from the aspect of crop production. Ayers et al. (1976) examined the relationships between grain size, total, fractional and individual protein compositions and the viability of the seedlings in winter wheat (*Triticum aestivum* L.) in response to the application of urea top dressing. They found that there was a close interrelationship between the viability of the seedling and the total protein content of the grain and of the endosperm. The viability of the seedling was greatly affected by the relative proportions of the salt solution-soluble and the salt solution insoluble fractions in the endosperm. The majority of the individual proteins separated by SDS gel electrophoresis - both the endosperm's proteins that are soluble in a thin salt solution and those that are not – positively correlated with the seedlings' viability. In the growth experiments while the weight and protein content of the grains of high protein contents decreased more rapidly than did those of the low protein grains, no such difference could be observed in the relative growth rates or the leaf surface area ratios. The ratio of the insoluble to the soluble proteins did not change along with changing grain sizes, but this ratio increased after the application of urea. The N content of the gliadin fraction grew in response to the application of N fertiliser, and upon the combined application of urea top dressing and herbicides in sub-toxic amounts the N content of the gluten fraction decreased.

Monitoring the biosynthesis of gluten forming polypeptides shows that small amounts of gliadin and glutenin monomers can be identified in the early stages of grain development but the bulk of these proteins is synthesised during later stages of development. Most experts think that the synthesis and accumulation of glutenin polymers begins later than that of monomers and this is confirmed by the conclusions drawn from experiments. It has been found that during the initial phase of protein synthesis the monomers are in a "free" state and it is not until later stages that polymeric glutenin can be identified. The HMW glutenin subunits are synthesised

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concurrently and the quantity is dominated by polypeptides encoded by chromosomes B and D. Although there may be significant differences between specific varieties in terms of total protein quantity, gliadin, glutenin and the individual gluten-forming polypeptides, the curve of the accumulation of the various protein components – in terms of protein mass/grain – is of a similar sigmoid shape (Abonyi et al. 2007). The experiment conducted by Liu et al. (2007) showed that the application of a N fertiliser ((0, 120, 240 and 360 kg/ha urea) resulted in a significant increase in the albumin and globulin content during the early stage of the grain-filling period but this impact gradually dampened later on. The different doses of N had no perceptible impacts on the albumin and globulin content by the ripening of the grains. The application of N fertiliser resulted in increased levels of both gliadin and glutenin. The latter increased by a somewhat higher percentage therefore the glutenin to gliadin ratio increased to some extent. The N treatment also raised the flour's wet gluten content whereby it extended the farinographic dough forming time, increasing the its stability and longevity.

Two-dimensional gel electrophoresis has shown that the application of fertilisers after flowering causes changes to the proteome that has a profound impact on the quality of the flour and on immunogenicity (Altenbach et al. 2011). The supply of mineral nutrients has a great impact during grain development on the protein content and composition of the flour ground from the grains which, in turn, affects the quality and the immunogenic potential of this economically highly valuable commodity. As a result of the complexity of the wheat flour proteome it is difficult to precisely identify the impacts of mineral supply on the composition of the protein. The use of tandem mass spectroscopy (MS/MS) has improved the identification of flour proteins and the comprehensive proteome maps of flour ground from an American wheat (Butte 86) are now available. All of these make it possible to document the changes taking place in the individual proteins of flour that are caused by the application of fertilisers. The Butte 86 wheat variety was treated with fertiliser after flowering, while the same variety was also grown on a control plot. Quantitative 2D gel electrophoresis was applied in order to determine the protein compositions of the resulting flours. The treatment was found to have caused significant changes to the proportions of 54 individual proteins. The ratios of most of the omega-gliadins, the HMW-GSs and the serpins (serine protease inhibitors) as well as those of some alpha-gliadins increased as a result of the treatment. By contrast, the ratios of alpha-amylase/protease inhibitors, farinins, purinins and puroindolines decreased. The ratios of a number of low molecule weight glutenin subunits (LMW-GS), globulins and enzymes also increased. The HMW-GS to LMW-GS ratio increased from 0.61 to 0.95, while the gliadin to glutenin ratio grew from 1.02 to 1.30 in response to the application of fertilisers. Since the protein content of the flour doubled (from 7 % to 14 %) in response to the application of fertiliser after flowering, the absolute quantities of most types of proteins increased. The data indicate that the flour proteins may change in response to the application of fertilisers after flowering in line with the amounts of sulphur containing amino acids (Cys and Met).

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. Using two varieties Kindred et al. (2008) found that an average of 10 kg of protein increase per tonne resulted in a 5.7 litre decrease in the alcohol output. The genotype had only a minor impact on the protein content. The two varieties responded to the application N in the same way, i.e. the interaction between the application of fertiliser and the wheat variety had no impact on either attribute being examined. Accordingly, in the case of the different grain protein levels the scientists found similar differences between the starch contents and consequently in the alcohol yield as well. An examination based on size exclusion chromatography revealed that upon each gram increase in the total grain protein the amount of gliadins grew by 0.56 g, i.e. gliadins made up the most important storage protein as regards mass itself. All of these findings indicate that plant improvement with the aim of reducing the grain's gliadin content reduces the total amount of grain protein and increases the alcohol yield. It has been proven statistically that the economically optimum amount of nitrogen is close to the amount that produces the maximum amount of alcohol.

The impacts of fertilisers on gene expression controlling

Gene expression in wheat is affected heavily by the application of farmyard manure or fertilisers (Lu et al. 2005). The greatest impact on yield and quality is produced by nitrogen, therefore the adequate and accurate planning of the application of N fertilisers is crucial for agriculture as well as for environmental protection. Nonetheless, we still know fairly little about the impacts of different doses and forms of nitrogen on gene expression in the case of field-grown cereals. Samples originating from the Rothamsted Broadbalk winter wheat experiment and from three other experimental fields were examined using the EST (expressed sequence tag) based wheat microarray technique, finding that the various genes responded to nitrogen delivered in the form of farmyard manure or fertiliser with surprisingly different expression levels. A number of genes showing different gene expression levels are known to participate in N metabolism and in the synthesis of storage proteins. Others play hitherto unknown roles which may be a suitable subject for future research. Typical gene expression may be used for distinguishing between organic and conventionally grown wheats.

The controlling of one of the new ω gliadin gene families is largely influenced by the N supply in the course of grain development (WAN et al. 2013a). A total of 6 different wheat varieties were grown at Rothamsted with different (100, 200 and 350 kg/ha) N treatments in 2009 and 2010. The gene expression of the developing wheat grains were described using the Affymetrix wheat GeneChip® on the 21st day after flowering. Of the 105 transcripts 4 – whose transcription was significantly overridden by N – were identified as γ-3 hordein. The identification of the expressed sequences prove that their amino acid sequences were different from those of the earlier described (typical) γ-gliadins, representing a new family of γ gliadins. The transcripts were examined during the ripening of the wheat grain using the technique called Real time reverse transcriptase PCR method on the 14th, 21st, 28th and 35th day after flowering and they found this transcription to be the most abundant on the 21st day which is when it responded most markedly to N treatment. As many as four new γ-globulin genes were isolated from the wheat variety Hereward and from the close relative species *Aegilops tuschii* and *Triticum monococcum,* with the PCR technique, while three such genes were identified from the wheat (Chinese Spring variety) genome sequence database. The amino acid sequence associated with the seven genes so identified revealed that they displayed a mere 44.4-46.0 % correspondence to the typical γ-gliadins but there was a 61.8-68.3 % match with the γ-3 hordein sequences of the wild barley species *Hordeum chilense*. The new γ-gliadin gene was localised in the first chromosome group (1, 1B, 1D)

Other factors' impacts on the quality and quantity of the yield

According to Van Lili et al. (1995) the baking industry is supplied with flour of rather widely varying quality, as a consequence of environmental impacts affecting both yield quantities and technological quality, having a negative impact on the market value of wheat. The baking quality of flour may be affected by a variety of genetic and environmental factors. Yields and protein quantities (concentrations) are affected by environmental impacts while dough quality is determined predominantly by wheat's inherited qualities.

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According to Dupont and Altenbach (2003) when the grains are filling up environmental factors have a major impact on both yield and flour quality. Environmental variables (temperature, water and nutrient supply) affect the duration and ratios of the growth and development of wheat, the accumulation of protein and the deposition of starch in unique ways, through various mechanisms. The environmental impacts are added to gene expression's internal chronological pattern in the course of the development of wheat. Comparisons of genetic and proteomic research to experiments carried out under controlled environmental conditions may reveal the complex pattern of gene expression during the development and maturing of the grain, identifying the key controlling processes that are affected by environmental impacts and demonstrating the molecular foundations of the impacts of environmental factors on flour quality and composition.

According to Borojevic and Williams (1982) choosing the best variety for the given environmental conditions is a crucial prerequisite for maximising the yield. This is said because it is the genotype that most dominantly determines the number of grain per ear ratio, the thousand grain weight, resistance to diseases and endurance, just like the yield level. Experiments conducted over a decade showed how the interaction between genotype and environment affect the parameters that determine the plant's nutrient absorption and source capacity and their impact on the yield. In relation to leaf area index (LAI) and leaf area durability (LAD) the "year effect" is more important than the genotype or the year or the interaction between the two. Environmental factors had a greater impact on source capacity than on the factors relating to the utilisation of nutrients. The direct impacts of other variables differed across varieties: some had positive, others had negative direct impacts on yield. Of the climatic impacts only higher numbers of sunny hours in May and June showed a positive correlation with yield, for instance during the period of the formation of the reproductive cells, fertilisation and grain filling.

The impacts of the different years on the quality of the produce manifested in different ways in the case of wet gluten content and valiographic value. Experiments showed that while in the case of the examined varieties the wet gluten content could be kept at a high level – subject to the year effect – in the premium quality category, the valiographic value number was much more exposed to the year effect, ranging in a very wide interval of 39 (C1) to 80 (A2) even in the case of suitable application of fertilisers or manure. Experiments proved that in years of drought the process of grain fill-up was disrupted, the required gluten composition could not develop and no favourable gluten skeleton could grow (Pepó P. 2004).

The application of N fertilisers combined with farmyard manure increases gluten content and the farinographic value, and even the Zeleny number grows substantially. When N fertilisers are applied in low doses no general improvements are observed despite the favourable impacts of farmyard manure. These results were found in an experiment carried out on Ramann brown forest soil of average K supply, low phosphorous level and medium N content (Kismanyoky and Ragasits 2003). The experiment conducted at Keszthely examined how the application of farmyard manure and N fertiliser affects wheat yields and quality, applying 0-200 kg/ha N fertiliser and 100 kg/ha phosphorous (P2O5) and 100 kg/ha potassium (K2O), farmyard manure, straw manure and green manure, along with a control plot without manure or fertiliser. The application of N fertiliser had a profound impact on yield (the control 1.98 t/ha yield was tripled by the application of 200 kg/ha N fertiliser). The treatments had a marked impact on the quality of wheat as well. Grain weight, protein content, gliadin composition and content are affected in different ways by the temperature and the nitrogen supply. Higher temperatures and more ample nitrogen supplies raise the ratios of proteins and gliadins in the flour, while at the same time higher temperature has an adverse impact on the quality of proteins or gliadins, while these are favourably influenced by ample nitrogen supply. Both factors increase the ratios of ω gliadins within the total gliadin content, while the ratio of α- and β-gliadins increases as the temperature rises or the supply of nitrogen decreases. The proportion of γ-gliadins is reduced by higher temperatures and it is increased by higher nitrogen supplies. Unlike the total amounts of proteins or gliadins accumulated in the grain, the different impacts of temperature and nitrogen supply on the ratio of gliadin within the proteins contained in the flour and on the relative proportions of the different gliadins can be explained. The two approaches are found to supplement one another at the level of flour and grain, the first can be used for describing the raw material, while the other can be used in understanding and modelling the variants of grain composition. (Daniel and Triboi 2000)

Gliadin content and composition at harvest time play a key role in determining the attributes and utility of wheat flour. An increase in temperature after flowering results in increased daily gliadin accumulation and in reduced periods of gliadin accumulation during the day. This impact is more marked in the case of the α- and the β-gliadins, than in that of the ω-gliadins. The application of nitrogen fertilisers increases the rate and duration of accumulation within a day. The N level before flowering affects the impact of N supply at flowering. The N supply has a relatively greater impact on the accumulation of ω-gliadin than on that of α-, β- and γ-gliadins. The final composition of gliadins is affected by the ratio of accumulation and the duration of synthesis, which, in turn, is determined by the timing of the beginning and the end of the period of synthesis. The dynamic allocation across the various gliadin fractions – which may be described from the aspect of thermal treatment – is suitable for modelling the gliadin content and composition of wheat (Daniel és Triboï 2001).

The impacts of the application of nitrogen fertilisers, the temperature after flowering, and dry spells are reflected by winter wheat's (*Triticum aestivum* L.) dry matter accumulation kinetics, the total N quantity and the relative proportions of the protein components (albumins, globulins, amphiphile compounds, gliadins and glutenins). Temperature or water supply effects applied during the post-anthesis period have no significant impact on the kinetic of the accumulation of the protein fractions, while the application of nitrogen fertilisers have a profound impact on the duration of the accumulation of storage proteins and their relative proportions. The accumulation of albumin-globulin proteins takes place during the early phase of grain development. The rate of the accumulation of this fraction significantly decreases after flowering (at about 250°Cd), when the intensive accumulation of storage proteins (albumins and globulins) begins. Simple allometric relationships exist under different environmental conditions between the total N per grain and the quantity of each protein fraction. The process of N distribution is not affected by post-flowering temperature or drought, or by the timing of the application of N fertiliser or its dose. Changes in the composition of protein fractions are affected primarily by the total N accumulated during the grain-filling phase (Triboï et al. (2003).

The impacts of mineral nutrients and temperature on the accumulation and composition of wheat grain proteins and on its baking quality were studied under controlled circumstances (Dupont et al., 2006). At controlled daytime and overnight temperature of 24°C and 17°C respectively (24/17°C), the continuous supply of nitrogen, phosphorous and potassium fertiliser (N:P:K=20:20:20) in a drip irrigation system after flowering increased the rate of protein accumulation, doubled the ratio of flour protein and slightly increased the grain weight. By contrast, post-flowering NPK treatment had nearly no impact at all on the rate or duration of protein accumulation or on the ratio of flour proteins when the nutrients were supplied in parallel with a high temperature treatment (37/28°C). The 37/28°C treatment shortened

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the period of grain fill-up, along with the period of dry matter accumulation and reduced the grain weight by 50 %. In the case of the 37/28°C temperature regime the rate and duration of grain protein accumulation and the quantity of total protein per grain was similar – with or without NPK supply – to the above characteristics of the wheat yield produced by wheat raised under a 24/17 ºC temperature regime but without NPK treatment after flowering. The transcription and the protein profile studies confirmed that the 37/28 ºC treatment shortens the development period without disruption of the coordinated synthesis of gliadins and glutens, although some specific impacts of the supply of NPK fertiliser and the temperature on the relative amounts of certain gliadins and gluteins could be observed. The transcription levels of the ω-gliadins, the α-gliadins and some high molecule weight glutenin subunits (HMW-GS) dropped during the 24/17 ºC temperature treatment without the application of NPK after the flowering phase, while the transcription level of the low molecule glutenin subunits (LMW-GS) and the γ-gliadins changed only modestly. The two-dimensional gel electrophoresis examination showed that the relative quantities of a number of ω-gliadins, α-gliadins and HMW-GS were lower without than with NPK treatment after flowering, whereas the relative quantities of the majority of the LMW-GSs were lower after NPK treatment. The impact of the temperature on the relative quantities was usually less dramatic than that of the application of NPK. The relative quantities of some α-gliadins and the HMW-GS were higher at 37/28 ºC with and without NPK treatment after flowering than at 24/17 ºC without NPK, while at 37/28 ºC the relative quantities of the majority of the LMW-GSs decreased. The test loaf volume was in correlation with the ratio of flour protein, regardless of temperature treatment, but the highest kneading tolerance indices were measured in the case of the flours ground from wheat grown at 24/17 ºC with the application of NPK treatment as well.

Transgenic strains to improve wheat quality

Some experiment findings show that it is possible to create transgenic wheat strains whose grains make higher baking quality flour. The transgenic wheat strain identified as B73-6-1 developed and described by British scientists includes the original HMW-glutenin gene (*1Dx5*) in 10-15 extra copies, increasing the amount of the protein encoded in the gene to about four times the original quantity. The technological and rheological attributes of this transgenic wheat strain were studied at Martonvásár between 2000 and 2002, comparing them to the non-transgenic control. The results show that the transgenic and the associated functional attributes are reliably passed down through a number of generations. No differences could be identified between the yields of the transgenic and the original genotypes, but in terms of grain hardness and grain size there were genotype related differences. The transgenic strain grew harder but smaller grains. The transgenic B73-6-1 plants tended to have higher protein contents than the control (L88-6) but the difference was not statistically significant. By contrast, the amount of the *1Dx5* HMW glutenin subunit and the *Dx/Dy*, the HMW/LMW and the glutenin/gliadin ratios increased by a significant 400 %. At the same time, the wet gluten ratio and the SDS sedimentation index decreased. The attributes of the flour changed owing to the changes in the protein matrix structure, caused by the altered x:y ratio of the HMW-GS. The attributes that characterising the stability and strength of the dough showed that the dough made with the flour ground from B73-6-1 was stronger but it was less extensible. The flour produced from this transgenic strain may be suitable for improving lower quality flours (Rakszegi et al. 2005). A bread cereal (Cadenza) (*Triticum aestivum*) was transformed by the albumin gene encoding the 35 kDa AmA1 grain protein with a high level of essential amino acids, contained in a cockscomb species (*Amaranthus hypochondriacus*). The

Southern-blot analysis of the T1 line proved the integration of the alien gene while the Rt.-PCR and Western-blot analyses of the samples confirmed the transcription and translation of the same transgenes. The impact of the extra albumin protein on the attributes of the flour – ground from grains propagated from the T2 line – were identified by examining the total protein content, the essential amino acid content and the polymer/monomer and HMW/LMW ratios. The findings showed that not only the essential amino acid content may be increased but some parameters relating to the quality of the flour can also be improved by the expression of the AmA1 protein. (Tamás et al. 2009.)

Environmental aspects

Studies performed by Ragasits et al. (1996) have proved that different forms of nitrogen (ammonium nitrate, AN, urea (Formurin/FO) and paraffin coated urea (Paramid/PA) have an impact on nitrogen leaching and nitrogen absorption but they have no effect on yield or baking quality in the case of winter wheat, therefore their use may be justified more by environmental than by direct economic considerations. One of the most favourable attributes of slow-release N fertilisers is that they provide a steady nitrogen supply, while reducing the risk of N leaching. The application of FO and PA in the late autumn resulted in 38 % and 15 % lower N-minimum percent levels in the top 90 cm soil layer, respectively, than did the application of AN. The N-minimum level was not excessively high in this layer in the control plots. The differences between the nitrogen supply levels were reflected by wheat's nitrogen uptake as well. After AN or PA treatment the rate of nitrogen uptake was similar in both the stem growth and the flowering phases but when FO was applied, the N uptake slowly decreased during the life of the wheat plants. Nonetheless, the yields were not influenced by the form of N supply. The baking quality was not affected more favourably by the slow-release N fertilisers than by the application of AN. An amount of 160 kg/ha of N was required for reaching the maximum wet gluten content, Zeleny number and valorigraphic value. Neither FO, nor PA treatments resulted in significant differences in the above parameters in comparison to the AN treatment.

The application of N fertilisers is a costly technique for the farmer and at the same time the leaching of N, the consumption of fossil fuels during the production and delivery of the fertilisers as well as the N_2O emission from the soil during denitrification may also entail environmental impacts. The development of N-efficient cultures will provide farmers with economic benefits and helps reduce the environmental loads entailed by the application of excessive amounts of N fertilisers. It is concluded that (i) increased root length density (RLD), (ii) the stalk's high nitrogen absorbing capacity perhaps in combination with a high maximum N uptake ratio, (iii) the low N concentration of the leaf blade, (iv) more efficient N remobilisation capability from stalk to grain after flowering but reduced efficiency of N remobilisation from leaf blade to grain, both preferably together with delayed senescence and (v) the grain's lower N concentration may be particularly crucial in the efforts at attaining high nitrogen use efficiency (NUE) in the case of wheat varieties grown for livestock feed, and (vi) in the case of bread wheat varieties the high NUE may be accompanied by the highly efficient uptake and assimilation of N, with high efficiency in N remobilisation after flowering and/or special grain protein compositions (Foulkes et al., 2009).

Health implications

The processing industry and food allergy research are avidly interested in wheat ω-gliadins. The ω-5 sub-group is particularly important, owing to its role in the development wheat-dependent exercise-induced anaphylaxis (WDEIA) (Morita et al., 2003; Matsuo et al., 2005). This allergic response occurs when wheat was digested by the sensitive individual before physical exertion and the symptoms may be extremely acute – in some cases fatal (Palosuo et al., 2001; Morita et al., 2003). Similarly to the other gluten

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proteins these also show genetic polymorphism. (Metakovsky, 1991; Denery-Papini et al.,2007). Accurate identification of the number of the wheat ω-gliadin proteins and genes is still to be worked on. Sabelli and Shewry (1991) applied the method of Southern blot analysis and found that bread wheat contains up to 15-18 ω-gliadin genes. A number of scientists have identified various ω-gliadin N-terminal by sequencing or electrophoresis or reverse phase HPLC (Kasarda et al., 1983; Masci et al., 1993, 1999; Dupont et al., 2000). Dupont et al. (2011) identified 7 ω-gliadins by 2-D gel electrophoresis and tandem mass spectrometry but they did not specify whether those were monomeric or polymeric. Wan et al. (2013b) identified two patterns of ω-gliadins in six wheat varieties, including the monomeric "gliadin" proteins and the subunits contained in the polymeric "glutenin" fraction. They concluded that the two groups of the six wheat varieties they studied contained at least seven and five ω-gliadin proteins, respectively. The polymeric type of the ω-5 gliadin (ω-5b) was found in three varieties (Hereward, Istabraq, Malacca), the polymeric form of the ω-2 gliadin (ω-2b) was identified in each of the six varieties. This indicates that the polymeric forms of the ω gliadins are widely present in modern wheat varieties and shows that continued studying of their roles in glutenin's polymeric structure and in the baking quality of the dough will be fully justifiable.

Moss et al., (1981); Wieser and Seilmeier (1998); Godfrey et al. (2010); Altenbach et al. (2011) proved that and the application of higher quantities of N fertilisers leads to an increase in the ratio of ω-gliadins. Raising the N dose within a plot drove the proportion of ω-5 gliadins also upwards. The increase in the proportion of ω-5 gliadins as a result of the application of more ample doses of N fertilisers is probably related to the fact that the N content of these proteins is higher than those of ω-1/2 gliadins.

The role of ω-gliadins in determining the baking value has not yet been clarified. The adding of purified protein to the flour may have either a positive (Khatar et al., 2002a, b) or a negative (Uthayakumaran et al., 2001; Fido et al., 1997) impact on baking quality, though these studies were limited to the monomeric fractions. The marked growth of the polymeric ω-5b gliadins may lead to an increase in the overall glutenin polymer content but it is not likely that they would result in improved quality because the polymeric ω-5 gliadins (that is, their free cysteine groups) may form disulphide bridges between chains therefore they may act as chain terminators reducing the lengths of the polymers (Gianibelli et al., 2002).

The increased accumulation of ω-gliadins and other gluten proteins in the subaleurone layer of the starch-containing endosperm is enabled by the influx of a large quantity of amino acids into these cells as a consequence of the ample N supply. The impact of the level of N supply on the spatial distribution and composition of the ω-gliadins in the wheat grain's endosperm shows that the composition of ω-gliadins in wheat and in foodstuffs can be controlled by plant improvement, agronomical solutions or industrial methods in order to optimise functional.

References

Abonyi, T., Király, I., Tömösközi, S., Baticz, O., Guóth, A., Gergely, S., ... & Lásztity, R. (2007). Synthesis of gluten-forming polypeptides. 1. Biosynthesis of gliadins and glutenin subunits. Journal of agricultural and food chemistry, 55(9), 3655-3660. DOI: http://dx.doi.org/10.1021/jf063143z

Altenbach, S. B., Tanaka, C. K., Hurkman, W. J., Whitehand, L. C., Vensel, W. H., & Dupont, F. M. (2011). Differential effects of a post-anthesis fertilizer regimen on the wheat flour proteome determined by quantitative 2-DE. Proteome science, 9(1), 46. DOI: http://dx.doi.org/10.1186/1477-5956-9-46

Ayers, G. S., Wert, V. F., & Ries, S. K. (1976). The relationship of protein fractions and individual proteins to seedling vigour in wheat. Annals of Botany, 40(3), 563-570.

Bedő, Z., Láng, L., Vida, G., Juhász, A., & Karsai, I. (1997). A minőségi tulajdonságok felértékelődése a búzanemesítésben. (Increased role of quality attributes in wheat improvement) Agro-21 Füzetek, 23, 19-30.

Békés Ferenc (2014). A búza ‘sütőipari minőség’- fogalom alakulása a kezdetektől napjainkig – a gabonavegyész szemével (Evolution of the concept of wheat “baking quality” from the beginning up till now – through the eyes of a cereal chemist). Inaugural Lecture, Hungarian Academy of Sciences

Berecz, K., & Ragasits, I. (1990). Effect of nitrogen fertilization on the dry matter, nitrogen accumulation and amino acid content of wheat. Pol’nohospodarstvo, 36(6), 489-499.

Borojevic, S., & Williams, W. A. (1982). Genotype Environment Interactions for Leaf Area Parameters and Yield Components and Their Effects on Wheat Yields. Crop Science, 22(5), 1020-1025. DOI: http://dx.doi.org/10.2135/cropsci1982.0011183x002200050030x

Bradbury, D., Cull, I. M., & MacMasters, M. M. (1956). Structure of the mature wheat kernel. I. Gross anatomy and relationships of parts. Cereal chemistry, 33(6), 329-342.

Bushuk, W., & Zillman, R. R. (1978). Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. Canadian journal of plant science, 58(2), 505-515. DOI: http://dx.doi.org/10.4141/cjps78-076

Daniel, C., & Triboi, E. (2000). Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: effects on gliadin content and composition. Journal of Cereal Science, 32(1), 45-56. DOI: http://dx.doi.org/10.1006/jcrs.2000.0313

Daniel, C., & Triboï, E. (2001). Effects of temperature and nitrogen nutrition on the accumulation of gliadins analysed by RP-HPLC. Functional Plant Biology, 28(12), 1197-1205.

Denery-Papini, S., Lauriére, M., Branlard, G., Morisset, M., Pecquet, C., Choudat, D., ... & Moneret-Vautrin, D. A. (2007). Influence of the allelic variants encoded at the Gli-B1 locus, responsible for a major allergen of wheat, on IgE reactivity for patients suffering from food allergy to wheat. Journal of agricultural and food chemistry, 55(3), 799-805. DOI: http://dx.doi.org/10.1021/jf062749k

Dupont, F. M., & Altenbach, S. B. (2003). Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. Journal of cereal science, 38(2), 133-146. DOI: http://dx.doi.org/10.1016/s0733-5210(03)00030-4

Dupont, F. M., Hurkman, W. J., Vensel, W. H., Tanaka, C., Kothari, K. M., Chung, O. K., & Altenbach, S. B. (2006). Protein accumulation and composition in wheat grains: effects of mineral nutrients and high temperature. European Journal of Agronomy, 25(2), 96-107. DOI: http://dx.doi.org/10.1016/j.eja.2006.04.003

DuPont, F. M., Vensel, W. H., Chan, R., & Kasarda, D. D. (2000). Characterization of the 1B-type ω-gliadins from Triticum aestivum cultivar Butte. Cereal Chemistry, 77(5), 607-614. DOI: http://dx.doi.org/10.1094/cchem.2000.77.5.607

Dupont, F. M., Vensel, W. H., Tanaka, C. K., Hurkman, W. J., & Altenbach, S. B. (2011). Deciphering the complexities of the wheat flour proteome using quantitative two-dimensional electrophoresis, three proteases and tandem mass spectrometry. Proteome Sci, 9(10). DOI: http://dx.doi.org/10.1186/1477-5956-9-10

FAOSTAT: http://www.fao.org/home/en/ (2014.05.28.)

Fido, R. J., Bekes, F., Gras, P. W., & Tatham, A. S. (1997). Effects of α-, β-, γ-and ω-gliadins on the dough mixing properties of wheat flour. Journal of Cereal Science, 26(3), 271-277. DOI: http://dx.doi.org/10.1186/1477-5956-9-10

Fiona M. Pushman and J. Bingham (1976). The effects of a granular nitrogen fertilizer and a foliar spray of urea on the yield and bread-making quality of ten winter wheats. The Journal of Agricultural Science, 87, pp 281-292. DOI:10.1017/S0021859600027556.

 DOI: 10.18380/SZIE.COLUM.2014.1.2.57

Foulkes, M. J., Hawkesford, M. J., Barraclough, P. B., Holdsworth, M. J., Kerr, S., Kightley, S., & Shewry, P. R. (2009). Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. Field Crops Research, 114(3), 329-342. DOI: http://dx.doi.org/10.1016/j.fcr.2009.09.005

Fuertes-Mendizábal, T., Aizpurua, A., González-Moro, M. B., & Estavillo, J. M. (2010). Improving wheat breadmaking quality by splitting the N fertilizer rate. European journal of agronomy, 33(1), 52-61. DOI: http://dx.doi.org/10.1016/j.eja.2010.03.001

Gianibelli, M. C., Masci, S., Larroque, O. R., Lafiandra, D., & MacRitchie, F. (2002). Biochemical Characterisation of a Novel Polymeric Protein Subunit from Bread Wheat (Triticum aestivum L.). Journal of Cereal Science, 35(3), 265-276. DOI: http://dx.doi.org/10.1006/jcrs.2001.0401

Godfrey, D., Hawkesford, M. J., Powers, S. J., Millar, S., & Shewry, P. R. (2010). Effects of crop nutrition on wheat grain composition and end use quality. Journal of agricultural and food chemistry, 58(5), 3012-3021. DOI: http://dx.doi.org/10.1021/jf9040645

Győri, Z (2006) A trágyázás hatása az őszi búza minőségére (Impacts of fertiliser application on winter wheat quality). Agrofórum, 9, 14-16.

Gyori, Z (2008): Complex evaluation of the quality of winter wheat varieties. Cereal Research Communications. 36. 2. 1907-1910p.

He, J., Penson, S., Powers, S. J., Hawes, C., Shewry, P. R., & Tosi, P. (2013). Spatial Patterns of Gluten Protein and Polymer Distribution in Wheat Grain. Journal of agricultural and food chemistry, 61(26), 6207-6215. DOI: http://dx.doi.org/10.1021/jf401623d

Hurkman, W. J., Tanaka, C. K., Vensel, W. H., Thilmony, R., & Altenbach, S. B. (2013). Comparative proteomic analysis of the effect of temperature and fertilizer on gliadin and glutenin accumulation in the developing endosperm and flour from Triticum aestivum L. cv. Butte 86. Proteome science, 11(1), 8. DOI: http://dx.doi.org/10.1186/1477-5956-11-8

Jolánkai M (1985): Differences in fertilizer response due to winter wheat varieties. Agrokémia és Talajtan 34:(Supplement) pp. 57-60.

Kasarda, D. D., Autran, J. C., Lew, E. J. L., Nimmo, C. C., & Shewry, P. R. (1983). N-terminal amino acid sequences of ω-gliadins and ω-secalins: implications for the evolution of prolamin genes. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 747(1), 138-150. DOI: http://dx.doi.org/10.1016/0167-4838(83)90132-2

Kent, N. L. (1966). Subaleurone endosperm cells of high protein content. Cereal Chem, 43(5), 585-601.

Kent, N. L., & Evers, A. D. (1969). Variation in protein composition within endosperm of hard wheat. Cereal Chemistry, 46(3), 293.

Khatkar, B. S., Fido, R. J., Tatham, A. S., & Schofield, J. D. (2002). Functional properties of wheat gliadins. I. Effects on mixing characteristics and bread making quality. Journal of Cereal Science, 35(3), 299-306. DOI: http://dx.doi.org/10.1006/jcrs.2001.0429

Khatkar, B. S., Fido, R. J., Tatham, A. S., & Schofield, J. D. (2002). Functional properties of wheat gliadins. II. Effects on dynamic rheological properties of wheat gluten. Journal of Cereal Science, 35(3), 307-313. DOI: http://dx.doi.org/10.1006/jcrs.2001.0430

Kindred, D. R., Verhoeven, T. M., Weightman, R. M., Swanston, J. S., Agu, R. C., Brosnan, J. M., & Sylvester-Bradley, R. (2008). Effects of variety and fertiliser nitrogen on alcohol yield, grain yield, starch and protein content, and protein composition of winter wheat. Journal of Cereal Science, 48(1), 46-57. DOI: http://dx.doi.org/10.1016/j.jcs.2007.07.010

Kismanyoky, T., & Ragasits, I. (2003). Effects of organic and inorganic fertilization on wheat quality. Acta Agronomica Hungarica, 51(1), 47-52. DOI: http://dx.doi.org/10.1556/aagr.51.2003.1.6

Láng, I., Csete, L., & Jolánkai, M. (2007). A globális klímaváltozás: hazai hatások és válaszok Szaktudás Kiadó Ház, Budapest.("Global climate changes, Hungarian impacts and responses". VAHAVA Report. Published in Hungarian.) DOI: http://dx.doi.org/10.1556/agrokem.56.2007.1.16

Liu, X., Li, Q. C., Wang, Z. L., He, M. R., & Yin, Y. P. (2007). Effects of nitrogen rates on grain protein components and processing quality of wheat. Plant Nutrition and Fertilizer Science, 13(1), 70-76.

Lu, C., Hawkesford, M. J., Barraclough, P. B., Poulton, P. R., Wilson, I. D., Barker, G. L., & Edwards, K. J. (2005). Markedly different gene expression in wheat grown with organic or inorganic fertilizer. Proceedings of the Royal Society B: Biological Sciences, 272(1575), 1901-1908. DOI: http://dx.doi.org/10.1098/rspb.2005.3161

Masci, S., Egorov, T. A., Ronchi, C., Kuzmicky, D. D., Kasarda, D. D., & Lafiandra, D. (1999). Evidence for the presence of only one cysteine residue in the D-type low molecular weight subunits of wheat glutenin. Journal of Cereal Science, 29(1), 17-25. DOI: http://dx.doi.org/10.1006/jcrs.1998.0224

Masci, S., Egorov, T. A., Ronchi, C., Kuzmicky, D. D., Kasarda, D. D., & Lafiandra, D. (1999). Evidence for the presence of only one cysteine residue in the D-type low molecular weight subunits of wheat glutenin. Journal of Cereal Science, 29(1), 17-25.

Masci, S., Lafiandra, D., Porceddu, E., Lew, E. J. L., Tao, H. P., & Kasarda, D. D. (1993). D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. Cereal chemistry, 70.

Masci, S., Lafiandra, D., Porceddu, E., Lew, E. J. L., Tao, H. P., & Kasarda, D. D. (1993). D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. Cereal chemistry, 70.

Matsuo, H., Morimoto, K., Akaki, T., Kaneko, S., Kusatake, K., Kuroda, T. & Morita, E. (2005). Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis. Clinical & Experimental Allergy, 35(4), 461-466. DOI: http://dx.doi.org/10.1111/j.1365-2222.2005.02213.x

Metakovsky, E. V. (1991). Gliadin allele identification in common wheat. II. Catalogue of gliadin alleles in common wheat. J Genet Breed, 45, 325-344.

Morita, E., Matsuo, H., Mihara, S., Morimoto, K., Savage, A. W. J., & Tatham, A. S. (2003). Fast ω-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. Journal of dermatological science, 33(2), 99-104. DOI: http://dx.doi.org/10.1016/s0923-1811(03)00156-7

Moss, H. J., Randall, P. J., & Wrigley, C. W. (1983). Alteration to grain, flour and dough quality in three wheat types with variation in soil sulfur supply. Journal of cereal science, 1(4), 255-264. DOI: http://dx.doi.org/10.1016/s0733-5210(83)80013-7

Moss, H. J., Wrigley, C. W., MacRichie, R., & Randall, P. J. (1981). Sulfur and nitrogen fertilizer effects on wheat. II. Influence on grain quality. Crop and Pasture Science, 32(2), 213-226. DOI: http://dx.doi.org/10.1071/ar9810213

Normand, F. L., Hogan, J. T., & Deobald, H. J. (1965). Protein Content of Successive Peripheral Layers Milled from Wheat, Barley, Grain Sorghum, and Glutinous Rice by Tangential Abrasion. American Association of Cereal Chemists, 42(4), 359-367.

Palosuo, K., Varjonen, E., Kekki, O. M., Klemola, T., Kalkkinen, N., Alenius, H., & Reunala, T. (2001). Wheat ω-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. Journal of Allergy and Clinical Immunology, 108(4), 634-638. DOI: http://dx.doi.org/10.1067/mai.2001.118602

Pepó P. (2002): Őszibúza-fajták trágyareakciója eltérő évjáratokban (Wheat varieties' response to fertilisers in different years). Növénytermelés (Crop production). 51. 2. 189-198.p.

Pepó P. (2004): Az évjárat hatása az őszi búza termésmennyiségére tartamkísérletben (Impact of year effect on winter wheat yields in long term experiment). Növénytermelés (Crop production). 53. 4. 339-350.p.

Pepó P. (2005): A tápanyagellátás és néhány agrotechnikai elem interaktív hatása az őszi búza termésmennyiségére és minőségére. - Korszakváltás a hazai mezőgazdaságban: a modern növénytermesztés alapjai (Interactive impacts of nutrient supply and certain agro-technical elements on winter wheat yield and quality – Epoch change in Hungary's agriculture: the fundamentals of modern crop production). - Debrecen : Debreceni

 DOI: 10.18380/SZIE.COLUM.2014.1.2.57

Egyetem Agrártudományi Centrum, 192-199.p.

Pepó P. (2006): A tápanyagellátás és néhány agrotechnikai elem kölcsönhatása az őszi búza termésmennyiségére és minőségére (Interaction of nutrient supply and certain agro-technical elements on winter wheat yield and quality). Gyakorlati Agrofórum. Extra 14. 10-12.p. b:12.p.

Pepó, P. (2007). The role of fertilization and genotype in sustainable winter wheat (Triticum aestivum L.) production. Cereal Research Communications, 35(2), 917-920. DOI: http://dx.doi.org/10.1556/crc.35.2007.2.188

Pollhamer, E.-né. 1973. A búza minősége a különböző agrotechnikai kísérletekben (Wheat quality in various agro-technical experiments).(Martonvásár 1963-1971). Akadémiai Kiadó, Budapest, 199.

Pushman, F. M., & Bingham, J. (1975). Components of test weight of ten varieties of winter wheat grown with two rates of nitrogen fertilizer application. The Journal of Agricultural Science, 85(03), 559-563. DOI: http://dx.doi.org/10.1017/s0021859600062444

Pushman, F. M., & Bingham, J. (1976). The effects of a granular nitrogen fertilizer and a foliar spray of urea on the yield and bread-making quality of ten winter wheats. The Journal of Agricultural Science, 87(02), 281-292. DOI: http://dx.doi.org/10.1017/s0021859600027556

Ragasits, I., Balázs, J., & Berecz, K. (1996). Effect of slow-release N-fertilizers on yield and baking quality of winter wheat. In Fertilizers and Environment (pp. 237-240). Springer Netherlands. DOI: http://dx.doi.org/10.1007/978-94-009-1586-2_39

Ragasits, I., Debreczeni, K., & Berecz, K. (2000). Effect of long-term fertilisation on grain yield, yield components and quality parameters of winter wheat. Acta Agronomica Hungarica, 48(2), 149-154. DOI: http://dx.doi.org/10.1556/aagr.48.2000.2.5

Rakszegi, M., Bekes, F., Lang, L., Tamas, L., Shewry, P. R., & Bedő, Z. (2005). Technological quality of transgenic wheat expressing an increased amount of a HMW glutenin subunit. Journal of cereal science, 42(1), 15-23. DOI: http://dx.doi.org/10.1016/j.jcs.2005.02.006

Sabelli, P. A., & Shewry, P. R. (1991). Characterization and organization of gene families at the Gli-1 loci of bread and durum wheats by restriction fragment analysis. Theoretical and Applied Genetics, 83(2), 209-216. DOI: http://dx.doi.org/10.1007/bf00226253

Salgó, A., & Gergely, S. (2012). Analysis of wheat grain development using NIR spectroscopy. Journal of Cereal Science, 56(1), 31-38. DOI: http://dx.doi.org/10.1016/j.jcs.2012.04.011

Shewry, P. R. (2009). Wheat. Journal of experimental Botany, 60(6), 1537-1553.

Shewry, P. R., D'Ovidio, R., Lafiandra, D., Jenkins, J. A., Mills, E. N. C., Békés, F., & Khan, K. (2009). Wheat grain proteins. Wheat: chemistry and technology, (Ed. 4), 223-298. DOI: http://dx.doi.org/10.1093/jxb/erp058

Shewry, P. R., Tatham, A. S., Forde, J., Kreis, M., & Miflin, B. J. (1986). The classification and nomenclature of wheat gluten proteins: a reassessment. Journal of Cereal Science, 4(2), 97-106. DOI: http://dx.doi.org/10.1094/9781891127557.008

Szentpétery Zs, Jolánkai M, Szöllősi G (2005): Nitrogén-fejtrágyázás hatása a búza termésmennyiségére és minőségére. - Korszakváltás a hazai mezőgazdaságban: a modern növénytermesztés alapjai (Impacts of nitrogen top dressing on wheat yield and quality – Epoch change in Hungary's agriculture: the fundamentals of modern crop production). - Debrecen : Debreceni Egyetem Agrártudományi Centrum, 37-42.p.

Szentpétery Zs. (2004): A nitrogén-fejtrágyázás hatása a búza termésmennyiségére és minőségére nagygombosi kísérletekben (Impacts of nitrogen top dressing on wheat yield and quality in the experiments at Nagygombos). Növénytermelés (Crop production). 53. 6. 547-558.p.

Tamás, C., Kisgyörgy, B. N., Rakszegi, M., Wilkinson, M. D., Yang, M. S., Láng, L., & Bedő, Z. (2009). Transgenic approach to improve wheat (Triticum aestivum L.) nutritional quality. Plant cell reports, 28(7), 1085-1094.

DOI: http://dx.doi.org/10.1007/s00299-009-0716-0

Tanács L, Véha A., Petróczi I.M. (2006): Műtrágyával és fungiciddel kezelt aestivum búzák nedeves glutentartalom, valorigráfos és alveográfos vizsgálatai, az évjáratok függvényében (Wet gluten content, valiographic and alveographic tests of aestivum wheat varieties after fertiliser and fungicide treatment, depending on year effect). – Növénytermelés (Crop production). Tom. 55. No. 5-6. 335 - 355.

Tosi, P., Gritsch, C. S., He, J., & Shewry, P. R. (2011). Distribution of gluten proteins in bread wheat (Triticum aestivum) grain. Annals of botany, 108(1), 23-35. DOI: http://dx.doi.org/10.1093/aob/mcr098

Tosi, P., Parker, M., Gritsch, C. S., Carzaniga, R., Martin, B., & Shewry, P. R. (2009). Trafficking of storage proteins in developing grain of wheat. Journal of experimental botany, 60(3), 979-991. DOI: http://dx.doi.org/10.1093/jxb/ern346

Triboï, E., Martre, P., & Triboï-Blondel, A. M. (2003). Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. Journal of Experimental Botany, 54(388), 1731-1742. DOI: http://dx.doi.org/10.1093/jxb/erg183

Uthayakumaran, S., Tomoskozi, S., & Tatham, A. S. (2001). Effects of supplementing monomeric plant proteins on the functional properties of wheat dough. Cereal Chemistry, 77, 737-743.

Van Lili, D., Purchase, J. L., Smith, M. F., Agenbag, G. A., & De Villiers, O. T. (1995). Multivariate assessment of environmental effects on hard red winter wheat. I. Principal-components analysis of yield and bread-making characteristics. South African Journal of Plant and Soil, 12(4), 158-163. DOI: http://dx.doi.org/10.1080/02571862.1995.10634357

Wan, Y., Gritsch, C. S., Hawkesford, M. J., & Shewry, P. R. (2013b). Effects of nitrogen nutrition on the synthesis and deposition of the ω-gliadins of wheat. Annals of botany, mct291. DOI: http://dx.doi.org/10.1093/aob/mct291

Wan, Y., Shewry, P. R., & Hawkesford, M. J. (2013a). A novel family of γ-gliadin genes are highly regulated by nitrogen supply in developing wheat grain. Journal of experimental botany, 64(1), 161-168. DOI: http://dx.doi.org/10.1093/jxb/ers318

Wieser, H., & Seilmeier, W. (1998). The influence of nitrogen fertilisation on quantities and proportions of different protein types in wheat flour. Journal of the Science of Food and Agriculture, 76(1), 49-55. DOI: http://dx.doi.org/10.1002/(sici)1097-0010(199801)76:1%3C49::aid-jsfa950%3E3.0.co;2-2

Wieser, H., Seilmeier, W., & Belitz, H. D. (1994). Quantitative determination of gliadin subgroups from different wheat cultivars. Journal of Cereal Science, 19(2), 149-155.
DOI http://dx.doi.org/10.1006/jcrs.1994.1020

 DOI: 10.18380/SZIE.COLUM.2014.1.2.57

THE INFLUENCE OF CULTIVATION METHOD ON THE SOIL'S ORGANIC CARBON CONTENT CALCULATIONS (HUNGARIAN SOC REFERENCE VALUES *VS.* IPCC DEFAULTS)

Attila KOVÁCS – Bálint HORVÁTH – Csaba FOGARASSY

Szent Isván University, H-2100, Gödöllő, Páter Károly utca 1.
e-mail: kovacs.attila@gtk.szie.hu

Abstract

In the majority of the IPCC (Intergovernmental Panel on Climate Change) studies the third most significant segment among the activities of the agricultural enterprises is the GHG emission from the soil. It is important to examine how this value could be reduced. Our research aims to find the modeling system which is able to provide us with accurate data regarding the difference of the several cultivation methods and easy to apply on the basis of the facts of practical. IPCC methodology has been applied in the calculation specified with the results of certain Hungarian studies. As an early outcome we have come to see that in comparison with the regular methodology our results showed more precise values. Where the IPCC demonstrates 1-2 t CO_{2e} we were able to witness 8-10 t CO_{2e} on soils with better humus content. Therefore we concluded that accurate values of the changes can only be calculated with the involvement of the local conditions.

Keywords: IPCC, Soil organic carbon (SOC), GHG calculation, anthropogenic activities, CO2 emission/saving

Introduction

The IPCC's research back from 2007 highlights the fact for us how much human activity contributes the growth of GHG emissions. We are able to witness every day the extreme weather changes and how frequent they have become. Nowadays we cannot talk about it, only the trap of heuristic thinking (Bazerman, Moore 2008), people from the world of science are more eager than ever to protest against the tendency of the negative effects. That is what makes it so important to be aware of how we can be more efficient towards the unfavorable environmental changes caused by human activity in the different economic systems. Unfortunately money is still a crucial decision factor in the world of business so we must find those systems that have interest and willing to contribute the activities which generate short-term disadvantage regarding their competitiveness (Fogarassy, 2012). What we should keep in mind that these are the answers for the opposing ones that have no vision for the future and any solution for our long-term problems. This study does not include economic examinations but aims to draw attention to the differences of certain methods which could be the ground for the ideal model.

Literature background

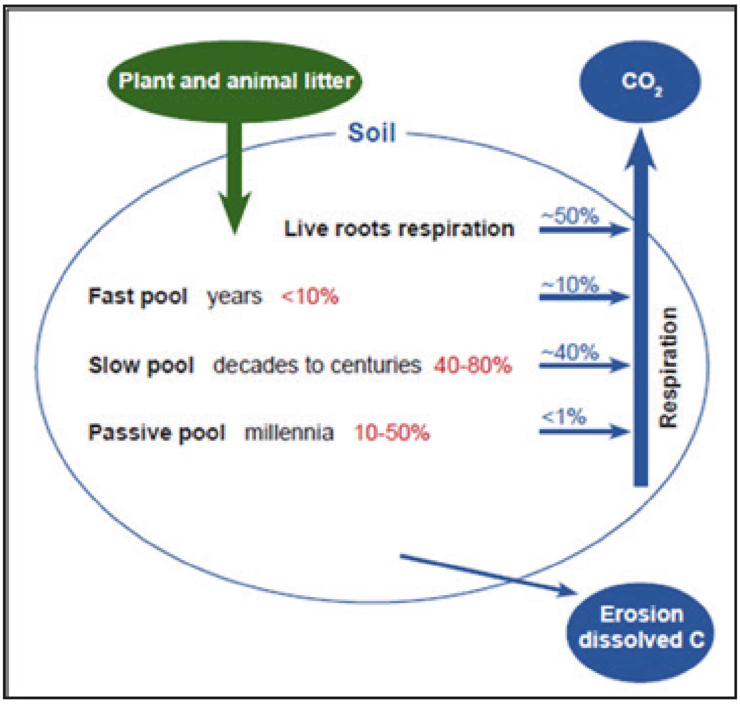
The CO_2 emissions and savings of the soil

The influence of the cultivation method on the soil's carbon content is mostly defined by the effect of the current process. (Paustian et al., 1997; Bruce et al., 1999; Ogle et al., 2005). The main aspects that determine the carbon assets of the soil regarding plant production are the followings: tillage, nutrient management (fertilizers and organic amendments), rotation and the intensity of the production, irrigation, various plant production systems and pasture, hay rotation sequences. Furthermore the drainage and the tillage of natural areas also decrease the carbon content of the soil (Armentano, Menges, 1986).

The soil's carbon turnover is mostly the impact of decomposition of the inorganic limestones. In this field stability and the lacking cycle of organic origin are recognized as general patterns so whenever it comes to the measurement of CO_2 emissions, most of the studies neglect them.

So in the rest of this study they will be ignored as well because the authors of this article find those cycles more important which are related to human activity. The CO_2 cycles of the soil are demonstrated on the first figure (Fig. 1.). The organic material of the fast pool is the easiest to increase but it also degrades really quick (for instance the carbon into the atmosphere). The slow pools are more important regarding binding the CO_2 but it is not that easy to implement them.

Figure 1. The CO_2 cycles of the soil



Source: Bureau of Rural Sciences, Dairy Australia, 2009.

The soil's carbon-absorbing capacity is based on a process which indicates carbon-dioxide binding plant material within the soil (mostly from dead plants and animal waste). The soil is a mixture organic compounds that are in the several phases of the decomposition. The organic carbon within the soil can be distributed into different „pools" according to it's nature of decomposition (like it is pictured on the first figure).

The following pools are:

- Fast pool: the added vegetable, animal and microorganism residues in the current year which decompose easily
- Slow pool: a stronger organic material, the humus. This pool is more or less stable, as long as it is not bothered by any human activities.
- Passive pool: the „oldest" phase. It resists to the further demolition and it is placed in the end of the decomposition process like becoming coal.

The amount of coal in the soil depends on several aspects and processes:

- The weather and the fertility of the soil: the fertile soils and wet zones (or highly irrigated), and the mixture of these two highly contributes the plant production so it is capable to get more organic material into the soil. The proportion of this organic material depends on the throughput, respiration of the living organisms which is also influenced by the temperature (higher temperature, more intense respiration) and the water content of the soil. So the weather and the soil determines the upper limit of the carbon sequestration.
- The system of agricultural production: usually more coal gets installed in the case of pastures than during the cultivation of cash crops.

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- Management: at plowed soils or at differently cultivated areas which were protected by microbial activities the carbon-dioxide emission increases. Those natural processes that stimulates the protective plant layer (for instance the stubble or the creation of th pasture) increase the soil's organic matter storage and eventually it's carbon sequestratuin but this modification is really slow.

So it can be concluded that the possibility of the human intervention is only possible in the case of production systems or management approaches because the climate or soil attributes cannot be changed.

Matherials and Methods

How to calculate the changes of carbon within the biomass

The calculation method examines the changes of land use and forestry in the case of land use and management mode modifications. Regarding the CO_2 emissions and savings we should put our focus on the following four important aspects:

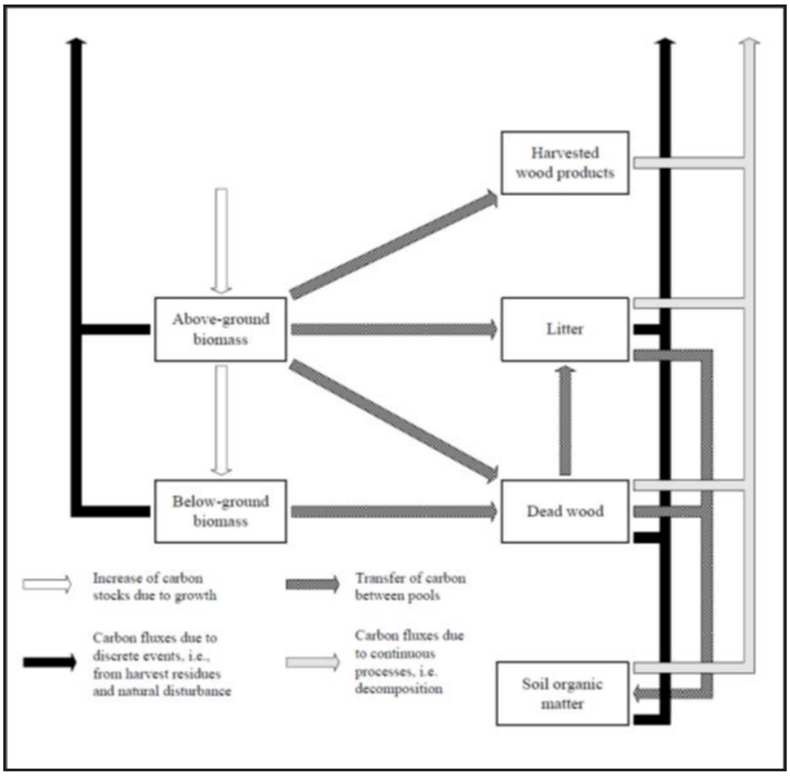
- changes within the woody biomass feedstock,
- cultivation changes,
- fields withdrawn from cultivation,
- the CO_2 emissions and savings of the soil

Basically the term „CO_2 savings" means a transformation from the atmosphere into a storage, meanwhile „CO_2 emissions" equals the opposite, when the atmosphere becomes polluted by the material of a storage. Not all of these transformations result in emissions or savings. Even though processing from a storage opportunity into another is considered as a reduction, for the receiving storage it still counts as a growth so it is not necessarily an emission.

Simplifications in the methodology

- The change of the *below-ground biomass stocks* equals 0.

Figure 2. The changes of carbon stocks between the different carbon storage systems in the perspective of the biomass turnover



Source: IPCC 2006 Volume 4.; Chapter 2., p. 8.

Table 1. Hungarian SOC values that differs from the numbers of the regular IPCC methodology (dimensionless)

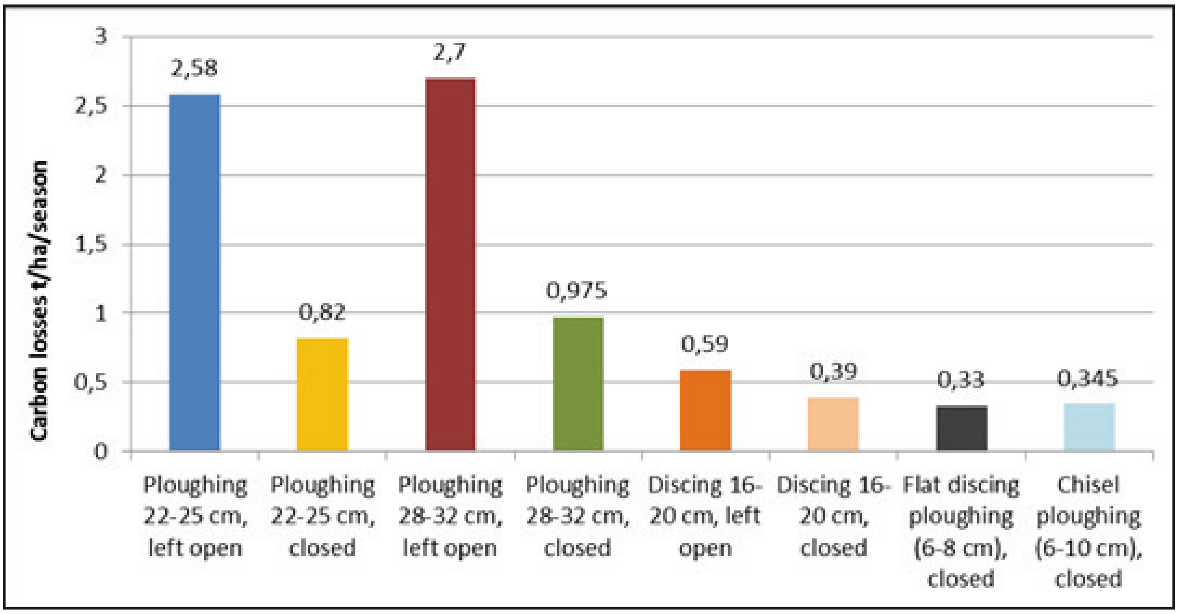
Fmg	Ploughing 28-32 cm	Ploughing 22-25 cm	Discing 16-20 cm	Flat discing ploughing 6-8 cm	Chisel ploughing 6-10 cm	Cultivation 30cm
Ploughing 28-32 cm	1.13	1.14	1.17	1.18	1.17	1.13
Ploughing 22-25 cm	1.12	1.13	1.16	1.17	1.17	1.13
Discing 16-20 cm	0.97	0.98	1.01	1.02	1.02	0.98
Flat discing ploughing 6-8 cm	0.97	0.98	1.01	1.02	1.02	0.98
Chisel ploughing 6-10 cm	0.97	0.98	1.01	1.02	1.02	0.98
Cultivation 30cm	0.93	0.94	0.97	0.98	0.97	0.93

Source: Authors' factor for F_{MG} (dimensionless) after the research of Birkás (2008)

- In this case the crop residue is usually classified as the part of the *plant-derived organic residues* category.
- The plant-derived organic residue aims to the 0 value in the case of the non-woody plants.

In the first place this method is based on the notion that in a long term perspective the annual plants produce no change according to their carbon balance. It concentrates more on the changes of the production intensity during the direct and indirect emissions of the soil. So it tries to measure the significance of the several cultivation forms in the CO_2 and non-CO_2 emissions. The schematic illustration (Fig. 2.) about the changes of the carbon stocks between the different carbon storage systems in the perspective of the biomass turnover.

Figure 3. Carbon losses during different soil preparation processes



Source: Birkás, 2008.

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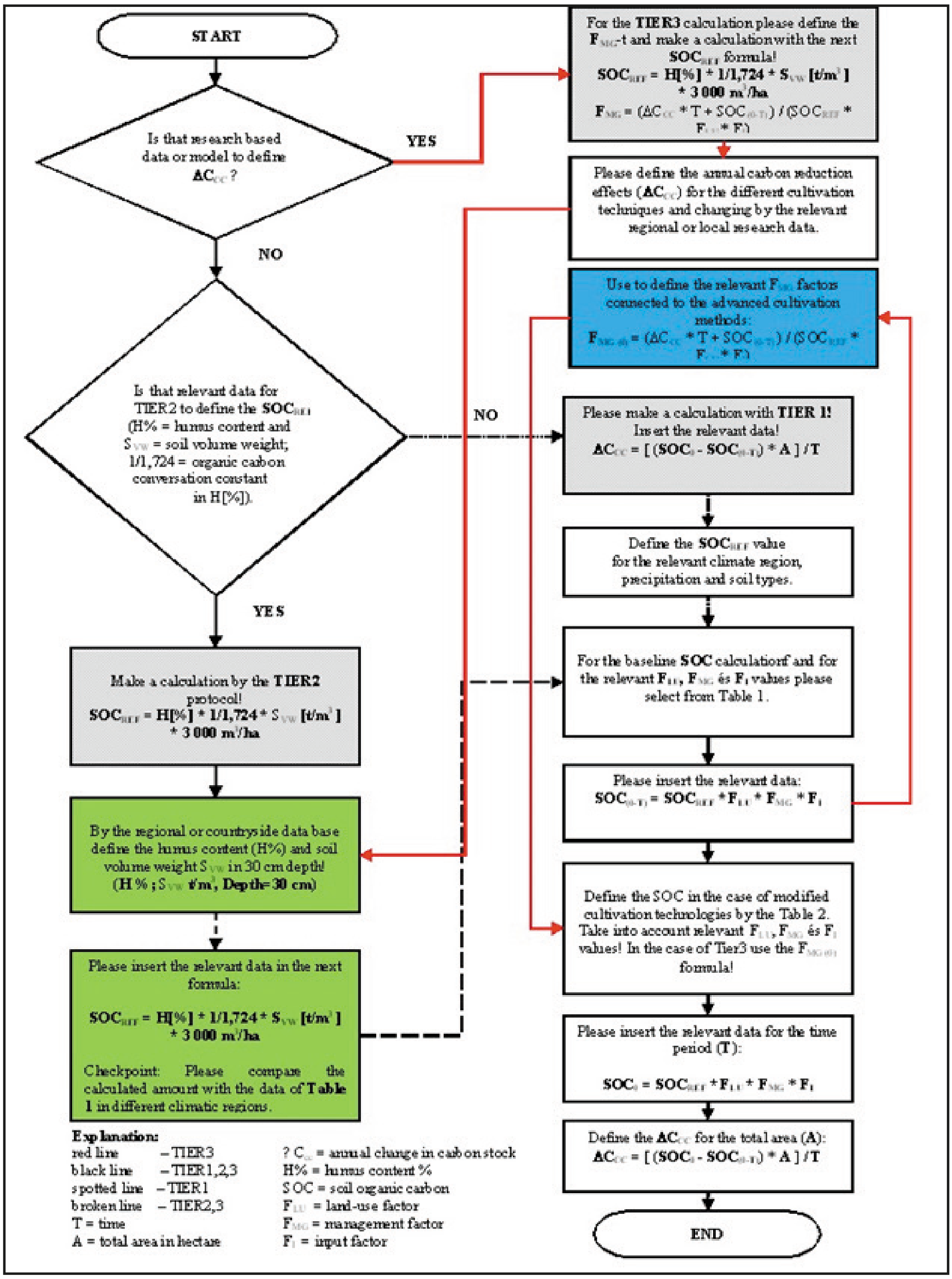
Figure 4. TIER Decision Tree



Source: Authors's graph after the Decision Tree of the IPCC

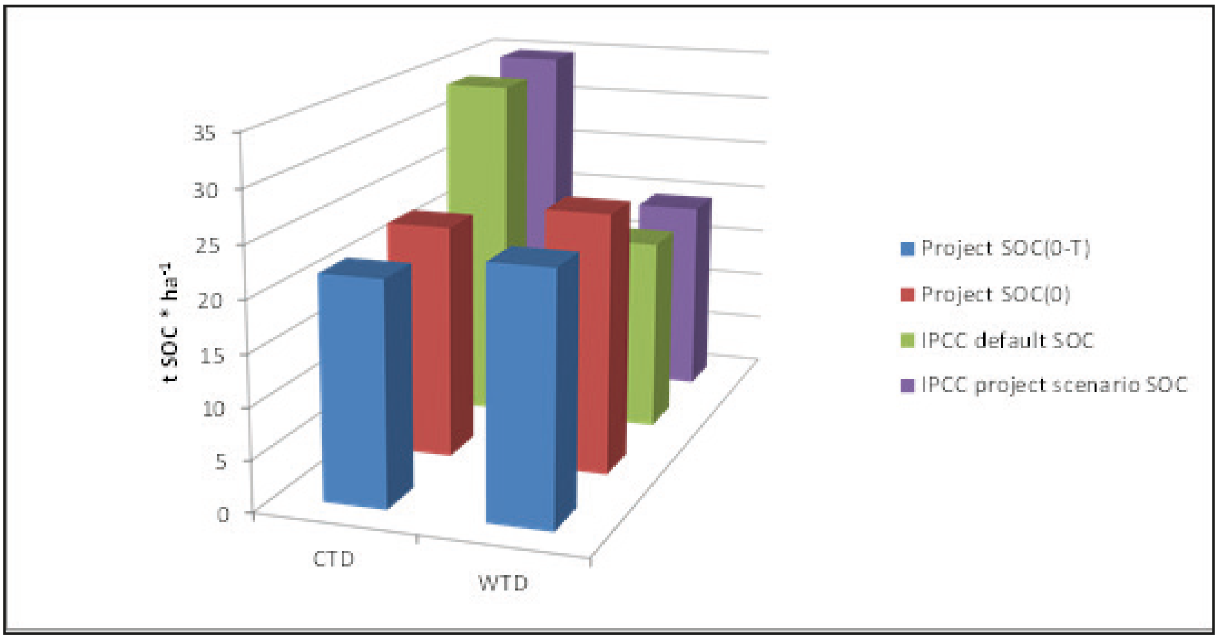
The calculation of the savings originated from the cultivation method

The examination results of BIRKÁS (Fig. 3.) about the soil loss caused by the different soil preparation processes. It shows that on one hectare of soil we would be able to reach almost a 2 t carbon saving which is 7,33 t CO_2/ha within the CO_2 balance (counting with 44/12 conversion factor). Her research points out the fact that the carbon saving happens only with the condition of not harvesting the crop residues. In case we gather the crop residues it causes a long term effect which is going to decrease the organic material puffering capacity of the soil. Eventually it also leads to crop yield losses within the next 2-3 years.

Besides the cultivation method we should associate a huge importance with the soil type as well. As the literature also highlights this point the real differences between countries will occur at their basic attribute: the land itself. Even though we included some calculations regarding the landuse and the management, we cannot forget about the quality of the soil. For this aspect we are going to use the help of the Debrecen University that already had a research about the several types of Hungarian soils and their humus content. They distribute the soils into „Cold" (CTD) and „Warm" (WTD) categories based on the country's temperature zones and from each group we picked out the six most important types (note: the different country level categories from Zsembeli at al. (2011) are not exacly suitable for the IPCC subcategories but these are not the most relevant factors in the SOC_{REF} calculations). What we learned from that study is the difference between the a basic IPCC carbon stock numbers and the real amount calculated in the mentioned soil types (Zsembeli et al., 2011). We made an own interpretation of the IPCC Tier decision tree (Fig. 4.) which is adapted to the Hungarian conditions. The two green boxes mark the place of the calculations of the Debrecen University where we could use their research outcomes. The blue box is the part of this study where we were able to paste the previous data gathered by Birkás (2008) during her research at Experimental Farm of Józsefmajor. (It includes the results of her research which has been taking place at Experimental Farm of Józsefmajor from the year of 2002; Birkás, 2009).

The mentioned calculation (Table 1.) which takes place in the blue box presents the Hungarian SOC values that differs from the numbers of the regular IPCC methodology. We distributed the different carbon contents by the several cultivation methods and illustrated how they would change during shifting into another process.

Figure 5. The changes of the carbon content in „Arenosols (humus)" soil type according to IPCC and measured SOC data (SOC_{REF} Zsembeli et al., 2011)



Source: Authors' graph

The regular method of our calculations

In all the cases we modified the IPCC standards for Debrecen's calculated SOC_{REF} values during the examination of the three main factors: land use (F_{LU}), cultivation method (F_{MG}), the number and the amount of the certain inputs (F_I). The first step was to analyze the research data of

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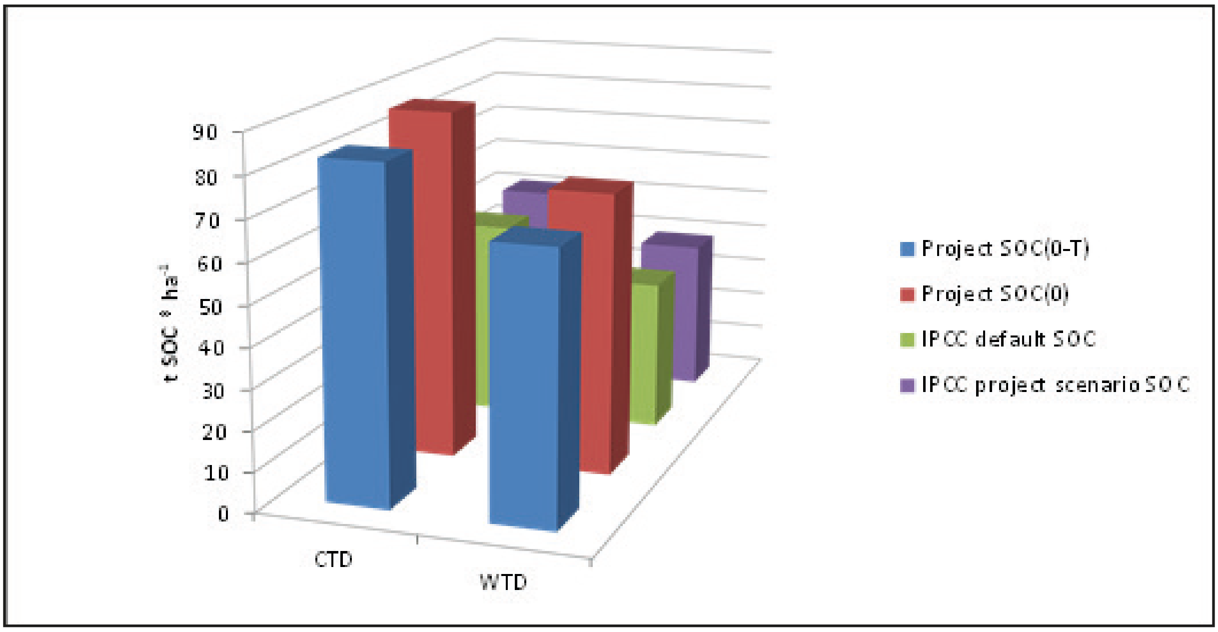
Figure 6. The changes of the carbon content in „Gleyosols" soil type according to IPCC and measured SOC data (SOC_{REF} Zsembeli et al., 2011)



Source: Autors's graph

the IPCC and the University of Debrecen than to translate their „CTD" and „WTD" (soils from cold and warm temperature zones) categories. After we have been able to measure the changes of the carbon content in the soil we calculated the difference between the IPCC and local results. Unfortunately we cannot illustrate our entire research outcomes for all of the soil types, cultivation methods and inputs so we selected only the most important and illustrious ones. From the previous studies of the IPCC we picked the two main categories (HAC - high activity clay- and SANDY) and in relation with the Hungarian background from both groups we involved the six most relevant soil types from each temperature zones. Eventually our results are going to present the carbon and CO_2 content changes of several soils using international and local calculations. Therefore the readers of this report will be able to see the difference between the methods.

Results and conclusions

On figure 5 and figure 6 we can see that the IPCC carbon content standards highly differ from the numbers of our research. In the case of the sandy soils the IPCC SOC_{REF} values are more favorable, meanwhile the HAC soils show better data in the local measures. After seeing the results of this table it is obvius that using our own SOC_{REF} database is more efficient because they turned to be the most accurate. But this difference also points out that the effect of the antrophogenic activity also diverges from the IPCC data because the landuse, the cultivation method and the inputs can also change in certain countries. For the characterization of this problem the technological-map might serve as the best solution which does tend to illustrate the application of the several method in a geographical perspective.

As another result of our research (Table 2. and Table 3.) we can see the changes of cultivation methods from medium left open into all the other examined closed processes that leave the organic materials entirely on the field. This outcome might be even more significant than the previous one because it shows the differences in comparison with the IPCC calculations in every single category. It is obvious to see that with our own method we are able to reach higher CO_{2e} savings than with the IPCC standards.

In the end of our research it can be concluded that even though the IPCC GHG calculation

Table 2. The changes of the CO_2 content in the certain soil types after a cultivation method modification from „Ploughing 28-32" into others (Cold temperature)

$\Delta t\ CO_{2e} * ha^{-1}$						
Temperature zone:	**CTD**					
IPCC classification:	**HAC**				**SANDY**	
Cultivation method/ soil type	Cambisols. Luvisols (Clay)	Cambisols. Luvisols (Ramann type)	Cambisols. Luvisols (Chernozem)	Gleyosols	Arenosols	Arenosols (Humus)
Cultivation 30cm	3.32	3.16	4.63	7.41	0.73	1.64
Chisel ploughing 6-10 cm	4.52	4.32	6.22	9.83	1.10	2.27
Flat discing ploughing 6-8 cm	4.56	4.35	6.26	9.90	1.11	2.29
Discing 16-20 cm	4.42	4.22	6.09	9.63	1.07	2.22
Ploughing 22-25 cm	3.46	3.30	4.82	7.70	0.77	1.71
Ploughing 28-32 cm	3.12	2.97	4.36	7.00	0.67	1.53
Average:	**3.90**	**3.72**	**5.40**	**8.58**	**0.91**	**1.94**

Source: Compiled by the authors (based on Kovacs-Bottlik, 2009)

provides a safe ground as a framework we still cannot rely on it's standards entirely. It might include the main intervention possibilities through a special model which makes sure that the certain data will not be multiplied but every model is just as good as the values they use during the function. Therefore our study pointed out the fact that we cannot implement any GHG reduction project based only on the IPCC system because we also need to be aware of the local conditions. It means everytime we would like to use this calculation method we must modify it first according to the attributes of the examined local soil.

Table 3. The changes of the CO_2 content in the certain soil types after a cultivation method modification from „Ploughing 28-32" into others (Warm temperature)

$\Delta t\ CO_{2e} * ha^{-1}$						
Temperature zone:	**WTD**					
IPCC classification:	**HAC**				**SANDY**	
Cultivation method/soil type	Cambisols, Luvisols (Clay)	Chernozems	Fluvisols	Gleyosols	Aernosols	Arenosols (Humus)
Cultivation 30cm	2.91	6.46	4.93	5.99	1.48	2.09
Chisel ploughing 6-10 cm	3.95	8.55	6.56	7.94	2.01	2.80
Flat discing ploughing 6-8 cm	3.98	8.61	6.61	7.99	2.02	2.82
Discing 16-20 cm	3.86	8.38	6.43	7.78	1.96	2.74
Ploughing 22-25 cm	3.04	6.72	5.13	6.23	1.54	2.18
Ploughing 28-32 cm	2.74	6.12	4.66	5.67	1.39	1.97
Average:	**3.41**	**7.47**	**5.72**	**6.93**	**1.73**	**2.43**

Source: Compiled by the authors

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References

Armentano, T. V. Menges, E. S. (1986): Patterns of Change nt he Carbon Balance of Organic Soil-Wetlands of the Temperate Zone. *Journal of Ecology*. Vol. 74, No. 3, pp. 755-774 DOI: http://dx.doi.org/10.2307/2260396

Bazerman, M. H. Moore, D.A. (2008): Judgement in Managerial Decision Making. *Wiley.* 7th edition. 2008. pp. 15-20

Birkás M. (2008): Scientific measurement methods in soil tillage experiments. Gödöllő.

Birkás M., Stingli A., Bottlik L., Takács T. (2008): A klímakár csökkentő talajművelés magyarországi lehetőségei. Vajdasági Magyar Tudományos Társaság „A Magyar Tudomány Napja Délvidéken", Újvidék, 2008. nov. 8. Kiadvány (szerk. Szalma József), Atlantis, Újvidék. pp. 14-23

Birkás M. (2009): Land use bases for soil quality improvement and maintenance. OTKA T049.049 research project final report.
http://nyilvanos.otka-palyazat.hu/index.php?menuid=930&num=49049

Bruce, K. D. Holt, R. J: Strike, P. (1999): Conservation of transposon structures in soil bacteria *FEMS Microbioloby Ecology* 30: pp. 25-37 DOI: http://dx.doi.org/10.1111/j.1574-6941.1999.tb00632.x

Bureau of rural sciences report (2009): Science for decision makers series - Soil Carbon Management and Carbon Trading, 2009 pp. 1-5

IPCC 2006 Guidelines for National Greenhouse Gas Inventories**.** Volume 4, Agriculture, Forestry and other Land Use; Chapter 2-11.

Fogarassy, c. (2012) Karbongazdaság (Low-carbon economy). L'Harmattan, Budapest, 2012 pp. 23-25

Kovács, a., Bottlik, l. (2009) Reducing the Detrimental Effects of the Climate Change. Research Report. Szent István University, Józsefmajor Farm of Experiments and Model Farm Hatvan – Józsefmajor, 2009 pp. 7-11

Ogle, S. M. Breidt, F. J. Paustian, K. (2005): Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. *Biogeochemistry,* 72, pp. 87-121 DOI: http://dx.doi.org/10.1007/s10533-004-0360-2

Paustian, K. Andren, O. Janzen, H. H. Lal, R. Smith, P. Tian, G. Tiessen, H. Van Noorwijk, M. Womer, P. L. (1997a): Agricultural Soils as a sink to mitigate CO2 emissions. Soil Use and Mgt. 13:230-244. pp DOI: http://dx.doi.org/10.1111/j.1475-2743.1997.tb00594.x

Zsembeli, J. Czimbalmos, R. Kovács, Gy. Somlyai, L. (2011): Calculation of Organic Carbon Stocks of the Soil Types of Hungary on the Base of Their Humus Content. University of Debrecen, Centre for Agricultural and Applied Economic Sciencesm Research Institutes and Study Farm, Karcag Research Institute. pp. 3

NEW RESULTS OF NUTRIENT UTILIZATION AND RESPONSE OF MAIZE *(Zea mays* L.*)* HYBRIDS

Péter PEPÓ – Lajos Gábor KARANCSI

Institute of Crop Scoience of Agricultural and Food Sciences of Environmental Management, Centre of Agricultural Sciences, University of Debrecen, 138. Böszörményi út, Debrecen H-4032, Hungary
e-mail: pepopeter@agr.unideb.hu

Abstract
In a long-term experiment, the fertilizer response of 10 maize hybrids of different genotypes was studied on chernozem soil in a favourable year. The yields of the hybrids varied between 9500 and 18600 kg ha^{-1} depending upon the fertilizer treatment and the genotype. The excellent nutrient and water management of the chernozem soil was proved by the high yield (9500 – 14600 kg ha^{-1}) obtained in the control. Even in such a good soil, the maize hybrids demanded fertilization and responded to it with a high yield increasement (3200 – 6500 kg ha^{-1}). From among the tested hybrids, 8 hybrids gave the highest yield in the N_{120}+PK treatment, while 2 hybrids reached their maximum yield in the N_{150}+PK fertilizer treatment (13500 – 18600 kg ha^{-1} depending upon the hybrid). The water utilization of maize was improved as a result of the optimum fertilization. The water-use efficiency (WUE) was 30.35 kg mm^{-1}, while it increased to 42.22 kg mm^{-1} at the optimum fertilizer dosage (N_{120}+PK). The natural nutrient utilization ability (yield in the control treatment) and the yield obtained at the optimum fertilizer dosage (N_{120}+PK and N_{150}+PK) were used in a special coordinate system.

Keywords: maize, yield, fertilization, water utilization, nutrient efficiency

Introduction

The most critical factors determining maize yield are the water and the nitrogen supply (Moser et al., 2006). Nagy (1996) finds positive correlations between irrigation and fertilization as well as plant population and fertilization. Fertilization, irrigation, soil cultivation and plant population increased the yield by 48 %, 28 %, 18 % and 6 %, respectively. Maize is a plant that demands high amounts of nutrients and also requires and gives good responses professional fertilizer application (Pakurár et al., 2004). The cropyear and different agrotechnical factors (fertilization, crop rotation, irrigation etc) could modify the yields of different maize genotypes (Pepó et al., 2006; Berényi et al., 2007; Karancsi and Pepó, 2012). Széles et al. (2013) proved that the yield was influenced not only by the precipitation of the crop year, but also by the amount of precipitation and temperature during the winter. Results of Kuscu et al. (2013) proved that the better water supply resulted higher yields because the main limitation factor was water deficit. Huang et al. (2010) stated that imbalanced fertilization did not increase the yield of maize in the long run, furthermore, it resulted in soil acidification. Maize requires a balanced NPK fertilization and nitrogen has a determining role from among the macroelements (Kovačevic et al. 2006). Uribelarrea et al. (2007) found that the applied hybrid and the N supply have a great role in the N accumulation and in the efficacy of N uptake in maize. According to their results, the grain yield of maize increased gradually with increasing fertilizer doses up to the N_{160} fertilization level. On chernozem soils with medium-good NPK supply, the dosages above 120 kg ha^{-1} N active ingredient did not increase yields efficiently, furthermore, they even reduced it without irrigation (Zhou et al, 2011). According to Azeez (2009), the dosage of 90 kg/ha N significantly increased the maize yield. As opposed to that, Berenguer et al. (2009) stated that the highest yields were obtained at 96, 153 and 159 kg ha^{-1} N in 2003, 2004 and 2005, respectively. Idikut and Kara (2011) stated that the effect of nitrogen doses was significant for the tasseling period, 1000 grain yield of different maize varieties. Similar results were obtain in silage maize by Budakli Carpici et al. (2010). According to their results the dry matter yield

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responded linearly to nitrogen rates with the highest dry matter yield at 300 – 400 kg N ha^{-1}.

Mateials and methods

The experiment was carried out at the experimental farm of the University of Debrecen Centre for Agricultural Sciences, Institute of Crop Sciences at Látókép. The site is located in Eastern-Hungary, 15 km from Debrecen in the Hajdúság loess region and its soil is calcareous chernozem soil (N 47°33', E 21°27'). The experimental soil is of good culture-state, medium-hard loam. Its humus content is medium, 2.8 %, its pH value is almost neutral, pH_{Kcl}=6.4. The soil has good water management characteristics. The long-term experiment was set up in 1983. The hybrids studied in the experiment were P9578 (FAO 320), DKC 4014 (FAO 320), NK Lucius (FAO 330), P9175 (FAO 330), DKC 4025 (FAO 340), PR37M81 (FAO 360), DKC 4490 (FAO 370), PR37N01 (FAO 380), P9494 (FAO 390) and SY Afinity (FAO 470). The hybrids were sown with a seed number of 72.000 plants/ha. Six fertilization treatments were applied (Table 1). Nitrogen was applied in the form of 34% ammonium-nitrate (50% in the spring), the nitrogen 50% and the phosphorus and potassium fertilizers were applied in full dosage (100%) in the autumn as a 10:15:18 special complex fertilizer. The forecrop was winter wheat. The major meteorological data are presented in Table 2.

Results

From among the field crops in Hungary, maize has the widest biological bases. Currently, the number of state registered hybrids is almost four hundred (390 hybrids on the variety list of 2013). There are great differences among the maize hybrids of different genetic background. The differences are manifested not only in the yield potential and yield stability of the given hybrid (in its abiotic and biotic adaptation ability), but also in the responses of the hybrids to the different agrotechnical inputs. The responses to the agrotechnical inputs have a great influence on the biological, agronomical and economic efficiency of maize production technology. From among the agrotechnical responses, one of the most important ones is the fertilizer response of maize hybrids, on the one hand, because fertilizer represent a very high cost, on the other hand, because overfertilization is not only ineffective from the economic point of view, but it can also indicate severe environmental problems.

In the vegetation period of 2013, the fertilizer response of maize hybrids with different genetic

Table 1. Fertilizer doses of long-term experiment (Debrecen, chernozem soil)

Treatment	N	P_2O_5	K_2O
		kg ha^{-1}	
Control	0	0	0
1	30	22.5	26.5
2	60	45	53
3	90	67.5	79.5
4	120	90	106
5	150	112.5	132.5

Table 2. Some important meteorological data (Debrecen)

Precipitation (mm)	Oct-Feb.	March.	Apr.	May.	June	July	Aug.	Total
2012/2013	196.4	136.3	48.0	68.7	30.8	15.6	32.2	528.0
30 years average	186.7	33.5	42.4	58.8	79.5	65.7	60.7	527.3
Temperature (°C)	Oct-Feb.	March.	Apr.	May.	June	July	Aug.	Average
2012/2013	3.7	2.9	12.0	16.6	19.6	21.2	21.5	13.9
30 years average	2.4	5.0	10.7	15.8	18.7	20.3	19.6	13.2

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Table 3. The effect of fertilization on the yield of maize hybrids (kg ha^{-1}) (Debrecen, chernozem soil, 2013)

Hybrids	Control	N_{30}+PK	N_{60}+PK	N_{90}+PK	N_{120}+PK	N_{150}+PK
P9578	11428	15710	15869	16105	16838	16475
DKC 4014	9774	11846	12349	12437	13622	13011
NK LUCIUS	11237	14392	15112	15017	16572	15553
P9175	11226	14880	15851	16311	16713	17736
DKC 4025	9530	11011	12982	12299	13514	12943
PR37M81	10630	14123	14611	14757	14838	16754
DKC 4490	11148	12741	13790	14364	14789	14414
PR37N01	14250	15641	15965	16519	17476	17127
P9494	11293	14388	15092	16263	17132	15206
SY AFINITY	14550	16570	16643	16736	18619	17718
$LSD_{5\%}$ (Hybrid)			1230			
$LSD_{5\%}$ (Nutrient level)			408			

backgrounds was studied on chernozem soil in a long-term experiment started 30 years ago. The weather of 2013 was favourable for the vegetative and generative development and yield formation of maize. As a joint effect of the favourable crop rotation (wheat – sunflower - wheat – maize), careful agrotechnique and favourable weather, the tested maize hybrids could realize a significant ratio of their yield potential. The yields of the hybrids ranged from 9500 to 18600 kg ha^{-1} depending upon the hybrid and the fertilizer treatment (Table 3). The favourable physical, chemical and biological characteristics of the chernozem soil and its excellent water and nutrient management are well indicated by the high yields of the hybrids obtained in the control (non-fertilized) treatment. The yields of the hybrids varied between 9500 and 14600 kg ha^{-1} in the control treatment. This means a difference of 5100 kg ha^{-1} between the tested genotypes in 2013, which illustrates that there are huge differences between maize hybrids in their natural nutrient utilization ability. In 2013, the hybrids DKC 4025, DKC 4014, PR37M81 gave a relatively moderate yield in the control treatment (9500 – 10600 kg ha^{-1}). These hybrids had different FAO numbers, which indicates that the natural nutrient utilization ability of hybrids is primarily determined by the genotype. The hybrids PR37N01 and SY Afinity gave outstandingly high yields (14200 – 14500 kg ha^{-1}) in the control treatment in 2013, these hybrids also differed in their vegetation season-length.

In the season of 2013, the maximum yields of the hybrids varied within a very favourable range between 13500 and 18600 kg ha^{-1} (Table 3). The yield maximum of the hybrids DKC 4025, DKC 4014 and DKC 4490 was relatively lower than the average (between 13500 and 14800 kg ha^{-1}). Outstandingly high yields were obtained in the case of the hybrids SY Afinity, P9175, PR37N01, and P9494 (between 17100 and 18600 kg ha^{-1}).

The evaluation of the nutrient utilization of the tested maize hybrids is included in Table 4. As

Table 4. Parameters of maize hybrids nutrient utilization (Debrecen, chernozem soil, 2013)

	Average of 10 hybrids	The minimum and maximum yield of hybrids
Control yield (kg ha^{-1})	11507	9530 – 14550
Maximum yield (kg ha^{-1})	16305	13514 – 18619
The yield surplus of fertilization (kg ha^{-1})	4798	3226 – 6510
Optimum N +PK (kg ha^{-1})	126 N+ PK	120 N+PK - 150 N+PK

Table 5. Study of nutrient efficiency of different maize genotypes (Average of ten hybrids) (Debrecen, chernozem soil, 2013)

	Control	N_{30}+PK	N_{60}+PK	N_{90}+PK	N_{120}+PK	N_{150}+PK
Average yield (kg ha^{-1})	11507	14130	14826	15081	16011	15694
Absolute yield surplus of fertilization (kg ha^{-1})	-	2623	696	255	930	-317
Relative yield surplus of fertilization (kg 1 kg NPK^{-1})	-	33.20	8.81	3.23	11.77	-4.01
WUE (kg mm^{-1}) (Rainfall March – Sept.)	30.25	37.26	39.10	39.77	42.22	41.39

an average of the tested 10 hybrids, the control and the maximum yields were 11507 kg ha^{-1} and 16305 kg ha^{-1}, respectively. The minimum and maximum values of the hybrids in control treatment and maximum yields represent well the natural nutrient utilization ability and realized yield potential of the different genotypes. As an average of the 10 tested hybrids, the yield increasement due to fertilization was 4798 kg ha^{-1}. The differences in the fertilizer response of the hybrids were proved by the minimum and maximum values of the yield increasement due to fertilization (3226 and 6510 kg ha^{-1}). The different fertilizer request was also proved by the optimum N + PK fertilizer dosage. In 2013, the N_{120}+PK fertilizer dosage was the optimal (the maximum yield was obtained at this dosage) for most of the hybrids, however, the highest yield was obtained at the dosage of N_{150}+PK in the case of two hybrids (P9175 and PR37M81). As for the latter, the importance of the genotype should be highlighted, that is the hybrids with different vegetation season length can be characterized by the same optimum fertilizer dosage. It should also be emphasized that the newest (P9175) and the old (PR37M81) genotypes also had the same optimum fertilizer dosage. Based on our experimental results, however, a trend could be observed that the more modern maize genotypes gave higher yields at the same or lower N_{opt} +PK dosage in general, that is the fertilizer utilization ability of the hybrids was improved as a result of breeding.

When analysing the efficacy of fertilization and nutrient supply as an average of the ten tested hybrids (Table 5), it was found that the absolute yield increasement due to fertilization was the highest between the control and the N_{30}+PK treatment (2623 kg ha^{-1}). In the fertilization treatments of higher dosage, the fertilization resulted in a more modest yield increment (696, 255 and 930 kg ha^{-1} as an average of the hybrids), moreover, a small yield reduction (-317 kg ha^{-1}) was observed in the N_{150}+PK treatment. The relative yield increasement due to fertilization was also calculated as an average of the hybrids (Table 5), this index represents the maize yield increasement due to 1 kg NPK fertilizer. As regards the relative yield increment due to fertilization, the most favourable value (33.20 kg 1 kg NPK^{-1}) was also obtained between the control and the N_{30}+PK treatment, with increasing dosages, these valued were reduced (8.81, 3.23 and 11.77 kg 1 kg NPK^{-1}) and then became negative (-4.01 kg 1 kg NPK^{-1}).

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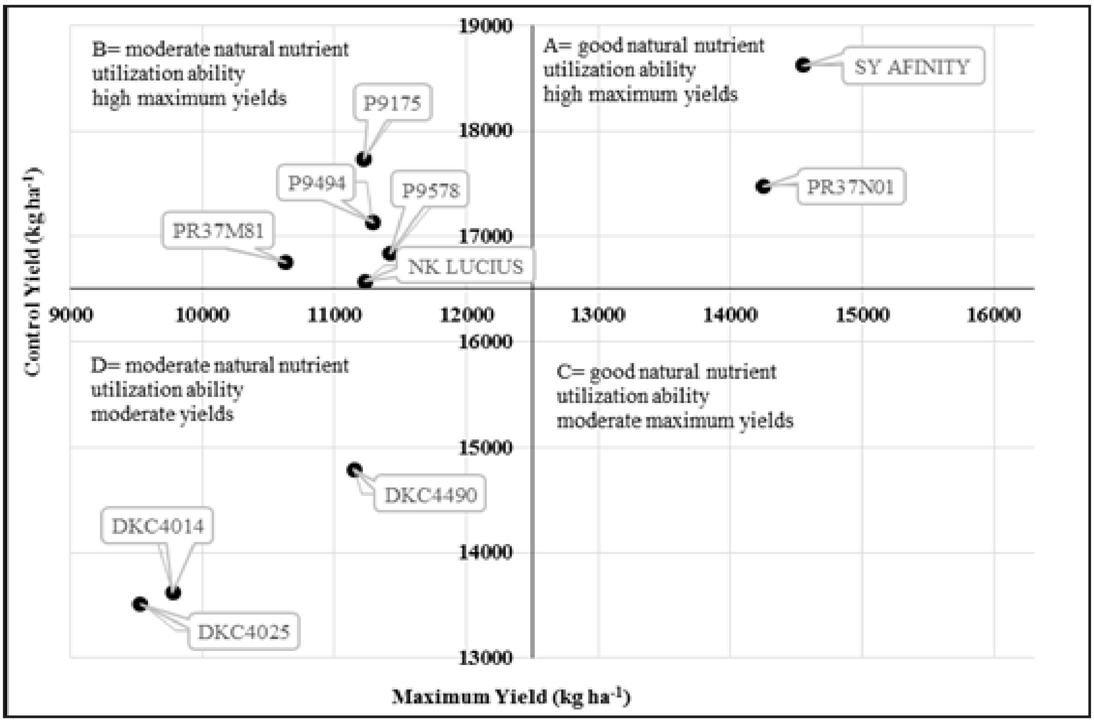
Figure 1. Complex evaluation of the nutrient response of maize hybrids (Debrecen, 2013)



Results of our long-term experiments proved that there is a strong correlation between the nutrient and water supply of maize. A more favourable nutrient supply improved the water utilization of the maize hybrids (Table 5). As an average of the ten tested hybrids, the amount of yield per 1 mm precipitation from March 2013 until September 2013 was determined in the different fertilizer treatments. Our experimental results proved that a more favourable nutrient supply resulted in the improvement of water utilization by maize hybrids that is they could produce more yield from 1 mm precipitation. While the water use efficiency (WUE) was 30.25 kg mm^{-1} in the control treatment as an average of the tested hybrids, it increased to 42.22 kg mm^{-1} in the N_{120}+PK fertilizer treatment.

For the complex evaluation of the fertilizer response of the tested maize hybrids such a graphic method was applied (Figure 1) which is suitable for the joint evaluation of

- the natural nutrient utilization ability (yield in the control treatment)
- the maximum yield due to fertilization (yield in the N_{opt} +PK treatment).

For this evaluation, such a coordinate system was used in which the maximum yields and the control yields were presented in the ordinate (vertical) axis and the abscissa (horizontal) axis, respectively. In this way, such a coordinate system was created in which the hybrids with a different fertilizer response could be illustrated in the different quarters. Based on this, the tested maize hybrids could be classified into the following four fertilizer response groups:

A = hybrids which have a good natural nutrient utilization ability and give high maximum yields as a result of fertilization (SY Afinity, PR 37N01).

B = hybrids which have a moderate natural nutrient utilization ability and give high maximum yields as a result of fertilization (P 9175, P 9494, PR 37M81, P 9578, NK Lucius).

C = hybrids which have a good natural nutrient utilization ability and give moderate maximum yields as a result of fertilization (-).

D = hybrids which have a moderate natural nutrient utilization ability and give moderate yields as a result of fertilization (DKC 4014, DKC 4025, DKC 4490).

Those hybrids are the best for the production, which can utilize well both the natural nutrient stock of the soil and the applied fertilizers (group A). Those hybrids can also be favourable, which can significantly increase their relatively lower control yield as a result of fertilization (group B) (Figure 1).

Discussion

The results of our long-term experiment on chernozem soil in 2013 proved that fertilization is a very important agrotechnical element of maize production technology. Maize has extremely high productivity. The year and the weather have a significant yield-determining effect in maize production. In the experiment, the yields of the ten tested maize hybrids varied between 9500 and 18600 kg ha^{-1} depending upon the fertilizer treatment. Very favourable yields were obtained also in the control, non-fertilized treatment (9500 – 14600 kg ha^{-1}), which proved the excellent qualities and the good water and nutrient management of the chernozem soil. Fertilization had a yield-increasing effect even in spite of these high control yields. The maximum yield of the maize hybrids varied between 13500 and 18600 kg ha^{-1} in 2013. The yield-increasing effect of fertilization was 4798 kg ha^{-1} as an average of the hybrids, ranging from 3226 to 6510 kg ha^{-1} depending upon the genotype. As an average of the hybrids, the optimum fertilizer dosage was the N_{126}+PK. Berényi et al. (2007) proved that the maize had very good fertilizer response on chernozem soil.

The absolute yield increment due to fertilization was the highest between the control and the N_{30}+PK treatment (2623 kg ha^{-1} as an average of the hybrids), then it decreased with increasing fertilizer dosages. A similar statement can be made for the relative yield-increment due to fertilization (the relative yield-increment between the control and the N_{30}+PK treatment was 33.20 -kg 1 kg NPK^{-1} (Kovacevic et al., 2006, Karancsi and Pepó, 2012).

Our experimental results proved that the water utilization of the maize hybrids can be improved with a proper nutrient supply and optimum fertilization. As an average of the hybrids, the WUE was 30.35 kg mm^{-1} in the control treatment, while it increased to 42.22 kg mm^{-1} in the optimum fertilization treatment.

Based on their fertilizer response, maize hybrids could be classified into different groups. Pepó (2006) proved the significance of hybrid-specific fertilization and the different nutrient utilization of maize hybrids based on their experimental results. For this classification, the nutrient utilization of the hybrids (yield in the control treatment), and the maximum yield due to fertilization (yield in the N_{opt}+PK treatment) were used. Based on that, the tested hybrids can be classified into four different groups. As regards the nutrient utilization, those hybrids are the most valuable, which can significantly increase their good control yield as a result of fertilization. Our research results can successfully contribute to a better knowledge of maize genotypes, to the exploration of their traits and to a rational, environment-friendly application of fertilizers adapted to the given hybrid.

References

Azeez J. O. (2009): Effect of nitrogen application and weed interference on performance of some tropical maize genotypes in Nigeria. Pedosphere. 19:5. 654-662.

Berenguer P., Santiveri F., Boixadera J., Loveras J. (2009): Nitrogen fertilisation of irrigated maize under Mediterranean conditions. European Journal of Agronomy. 30:3. 163-171.

Berényi S., Vad A., Dóka L., Pepó P. (2007): Effects of fertilization and crop years on maize (Zea mays L.) yields in different crop rotations. Cereal Research Communications. 35: 2. 241-244.

Budakli Carpici E., Celik N., Bayram G. (2010): Yield and Quality of forage maize as influenced by plant density and nitrogen rate. Turkish Journal of Field Crops. 15:2. 128-132.

 DOI: 10.18380/SZIE.COLUM.2014.1.2.87

Huang, S., Zhang, W., Yu, X., Huang Q. (2010): Effect of long-term fertilization on corn productivity and its sustainability in an Ultisol of southern China. Agriculture, Ecosystems and Environment. 138. 44-50. DOI: http://dx.doi.org/10.1016/j.agee.2010.03.015

Idikut L., Kara S. N. (2011): The effect of previous plants and nitrogen rates on second crop corn. Turkish Journal of Field Crops. 16:2. 239-244.

Karancsi L. G., Pepó P. (2012): Study of the effect of fertilization of maize (Zea mays L.) in crop years with different water-supply. Növénytermelés. 61: Suppl. 89-92.

Kovačevic V., Rastija M., Rastija D., Josipović M., Šeput M. (2006): Response of maize to fertilization with KCl on gleysol of Sava Valley Area. Cereal Research Communications. 34:2-3. 1129. DOI: http://dx.doi.org/10.1556/crc.34.2006.2-3.252

Kuscu H., Karasu A., Oz M., Demir A. O., Turgut I. (2013): Effect of irrigation amounts applied with drip irrigation on maize evapotranspiration, yield, water use efficiency, and net return in a sub–humid climate. Turkish Journal of Field Crops.18:1. 13-19.

Moser S. B., Feil B., Jampatong S., Stamp P. (2006): Effect of preanthesis drought, nitrogen fertilizer rate, and variety on grain yield, yield components, and harvest index of tropical maize. Agricultural Water Management. 81:1-2. 41-58. DOI: http://dx.doi.org/10.1016/j.agwat.2005.04.005

Nagy J. (1996): Effects of tillage, fertilization, plant density and irrigation on maize (Zea mays L.) yields. Acta-Agronomica-Hungarica. 196. 44:4. 347-354.

Pakurar M., Nagy J., Jagendorf S. (2004): Fertilisation and irrigation effects on maize (Zea mays L.) grain production. Cereal Research Communications 32:1. 151-158.

Pepó P., Vad. A., Nagy J. (2006): Effect of some agrotechnical elements on the yield of maize on chernozem soil. Cereal Research Communications. 34:1. 621-624. DOI: http://dx.doi.org/10.1556/crc.34.2006.1.155

Széles A., Ragán P., Nagy J. (2013): The effect of natural water supply and fertilization on maize (Zea mays L.) yield in the case of different crop year. Növénytermelés. 62:Suppl. 119-122.

Uribelarrea M., Crafts-Brandner S. J., Below F. E. (2009): Physiological N response of field-grown maize hybrids (Zea mays L.) with divergent yield potential and grain protein concentration. Plant Soil. 316. 151–160. DOI: http://dx.doi.org/10.1007/s11104-008-9767-1

Zhou J. B., Wang C. Y., Zhang H., Gong F., Zheng X. F., Gate W., Li S. X. (2011): Effect of water saving management practices and nitrogen fertilizer rate on crop yield and water use efficiency in a winter wheat-summer maize cropping system. Field Crop Reasearch. 122:2. 157-163. DOI: http://dx.doi.org/10.1016/j.fcr.2011.03.009

PLANT DENSITY IMPACT ON GRAIN YIELD OF MAIZE (*Zea mays* L.) HYBRIDS ON CHERNOZEM SOIL OF THE EASTERN HUNGARY

Péter PEPÓ[1] – Eszter MURÁNYI[2]

Institute of Crop Science of Agricultural and Food Sciences of Environmental Management, Centre of Agricultural Sciences, University of Debrecen, 138. Böszörményi út, Debrecen H-4032, Hungary
e-mail: 1. pepopeter@agr.unideb.hu; 2. emuranyi@gmail.com

Abstract

The plant density response of 12 maize hybrids was tested in a favourable year (2013) under optimum agrotechnical conditions on chernozem soil by applying plant densities of 50, 70 and 90 thousand plants ha^{-1}. Due to the favourable ecological and agrotechnical conditions yields varied between 11400 and 16300 kg ha^{-1} depending upon the hybrid and the plant density. From among the hybrids, 5 and 7 produced the maximum yield at 70 and 90 thousand plants ha^{-1}, respectively however the yield increment was not significant between 70 thousand plants ha^{-1} and 90 thousand plants ha^{-1}. No relationship was found between the season length and the optimum plant density of hybrids. Those hybrids were the most favourable, which gave a stable high yield at different plant densities that is their optimum plant density range was wide (P 9175, P 9494, SY Afinity). Hybrids with a small optimum plant density range (P 9578, DKC 4490, PR 37N01) gave the highest yield at a specific plant density (70 thousand plants ha^{-1} or 90 thousand plants ha^{-1}). A special complex coordinate system was developed for evaluation for the plant density response of maize genotypes specific ecological and agrotechnical conditions can be evaluated jointly.

Keywords: maize, plant density, hybrids, grain yields

Introduction

In Hungary, the number of registered hybrids has been the highest in maize for decades among the field crops. The number of maize hybrids has been around 400 for several years. This large hybrid portfolio provides the farmers with a great selection potential on the one hand, however, it also hinders the judgement of the objective traits of the hybrids. From among the agrotechnical elements applied in maize production technology several have a direct or indirect effect on the yield and the agronomical and economic efficiency of production.

According to Tokatlidis and Koutrubas (2004), the optimum plant density resulting in maximum yield has increased due to the great plant density tolerance of the new maize hybrids, but the potential yield per plant has not changed. Modern hybrids are highly dependent upon the plant density, because they can produce the maximum yield only in a small range due to the high number of plants. According to Sárvári (2005), plant density has a decisive effect on yield. With increasing plant density, the yield per plant decreases, but the yield per unit area increases until the optimal number of plants ha^{-1} is reached. Vad et al. (2007) found that, the optimum plant density is an important factor in sustainable maize production. Besides the genotypes and agrotechnical elements (fertilization etc.) the ecological factors (water supply, rainfall quantity and its distribution, physical and chemical properties of soil etc.) strongly modify the optimum plant density of maize. In the study of Pepó et al. (2006), increasing population density results in small amounts of additional yields (0.2-1.6 t ha^{-1}). In the study of Dawadi and Sah (2012), the highest yield (11.19 t ha^{-1}) was obtained at a plant density of as compared to the plant density of 55555 plants ha^{-1}. There was no significant difference between the yields of 66666 plants ha^{-1} and 83333 plants ha^{-1} (10.54 t ha^{-1}). Gozübenli et al. (2004) and Lashkari et al. (2011) found that plant density has a significant effect on yield. The yield increased up to the plant density of 90000 plants ha^{-1} (10973 kg ha^{-1}), above that value it was reduced. Widdicombe and Thelen (2002) also obtained the highest yield at the plant density of 90000 plants ha^{-1}. Hoshang (2012) also found that there is a significant difference between the

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yields at different plant densities and that the yield increases with increasing plant density. In the study of Mohseni (2013), the increase of plant density from 60000 plants ha^{-1} (9.09 t ha^{-1}) to 80000 plants ha^{-1} (11.14 t ha^{-1}) resulted in a yield increment. Roekel and Coulter (2011) stated that there is a close relationship between maize yield and plant density. The tested hybrid reached its maximum yield at the plant density of 81700 plants ha^{-1}.

Materials and methods

The plant density response of maize hybrids was studied in a small-plot field experiment at the experimental site of the University of Debrecen Centre for Agricultural Sciences at Látókép, which is located in Eastern Hungary in the loess region of Hajdúság (47° 33' N, 21° 27' E). The experiment was set up in four repetitions. The parcel size was 15.2 m^2. Three different plant densities (50, 70, 90 thousand ha^{-1}) were applied. In the season of 2013, an experiment was set up in which the plant density response of 12 maize hybrids of different genotypes and different vegetation period on a calcareous chernozem soil. The fertilizer doses applied in favour of the site-specific fertilization were N 108 kg ha^{-1}, P 0 kg ha^{-1} and K 0 kg ha^{-1}.

The hybrids were from the FAO 290-470 range. Most of the hybrids were developed in Hungary and taking into consideration on the local conditions. The examined hybrids were grown across the country. For example, the sowing area of NK Lucius was more than 40,000 hectares in 2013. In the last three years PR 37N01 were grown in the largest area in Hungary.

When evaluating the weather of 2013 and its effect on maize yields, the early spring weather should also be taken into consideration in addition to the weather during the season. The extremely high amount of precipitation (163.3 mm) in March 2013 greatly contributed to the fill-up of the water stock of the chernozem soil. The weather of May-June provided favourable conditions for the vegetative development of maize hybrids. The favourable fore crop (winter wheat), the applied proper agrotechnique and the water stock of the chernozem soil could reduce the unfavourable effects of the dry and hot weather of July (precipitation: 15.6 mm) and August (precipitation: 32.2 mm) (Table 1).

For the statistical analysis of the experiment, we used bi-factorial variance analysis in SPSS 13 for Windows.

Results

The significant vegetative mass provided for the physiological conditions of the extremely high yields obtained in 2013 (Table 2). The yields of maize hybrids varied between 11400 and 16300 kg ha^{-1} in 2013 depending upon the

Table 1. The meteorological data in the maize vegetation period (Debrecen, 2013 and 30-year average 1961-1990)

Months	March	April	May	June	July	August	September	Total/ Average
Precipitation (mm) 30-year average (1961-1990)	33.5	42.4	58.8	79.5	65.7	60.7	38.0	379.2
Precipitation (mm)	136.3	48.0	68.7	30.8	15.6	32.2	47.6	378.6
Difference (mm)	102.8	5.6	9.9	-48.7	-50.1	-28.5	9.6	0.6
Temperature (°C) 30-year average	5.0	10.7	15.8	18.7	20.3	19.6	15.8	15.1
Monthly average temperature (°C)	2.9	12.0	16.6	19.6	21.2	21.5	14.0	15.4
Difference (°C)	-2.1	1.3	0.8	0.9	0.9	1.9	-1.8	0.3

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Table 2. Effect of plant density on maize hybrids yield (Debrecen, chernozem soil, 2013)

Hybrids (the factor A)	Grain yield (kg ha^{-1}) Plant density (thousand plants ha^{-1}) (the factor B)			Average
	50000 plants ha^{-1}	70000 plants ha^{-1}	90000 plants ha^{-1}	
Sarolta (290)	11878	11997	11826	11900
P 9578 (320)	12463	15046	13826	13778
DKC 4014 (320)	12247	13770	12832	12950
DKC 4025 (330)	11908	12637	13748	12764
P 9175 (330)	15859	15948	15219	15675
NK Lucius (330)	13377	12935	13751	13354
PR 37M81 (360)	12991	12483	13182	12885
PR 37N01 (380)	13765	15421	16296	15161
DKC 4490 (380)	11882	12802	13214	12633
P 9494 (390)	15116	15619	14300	15012
Kenéz (410)	11388	11862	12387	11879
SY Afinity (470)	14682	15372	15946	15333
Average	13130	13824	13877	13610
LSD5% (A)		459 kg ha^{-1}		
LSD5% (B)		687 kg ha^{-1}		
LSD5% (A*B)		1190 kg ha^{-1}		

hybrid and the plant density. The higher plant density demand and plant density response of modern maize hybrids were proved by our experimental results. In our study, the lowest yield was obtained at the plant density of 50 thousand plants ha^{-1}. In 2013, the optimum plant densities of hybrids were 70 thousand plants ha^{-1} and 90 thousand plants ha^{-1}. Maximum yield was achieved at a plant density of 70 thousand plants ha^{-1} for the hybrids Sarolta, P9578, DKC 4014, P 9175 and P 9494 and at 90 thousand plants ha^{-1} in the case of DKC 4025, NK Lucius, PR 37M81, PR 37N01, DKC 4490 and SY Afinity. However, the results also proved that there was no significant yield increment in most hybrids when the plant density was increased from 70 thousand plants ha^{-1} to 90 thousand plants ha^{-1}.

Based on our experimental results, the effect of plant density on the yield of maize hybrids was evaluated as an average of the hybrids and also for the minimum and maximum values (Table 3). As an average of the hybrids, no difference was found in the yield between the different plant densities. The yields of the hybrids were 13130 kg ha^{-1} at 50 thousand plants ha^{-1}, 13824 kg ha^{-1} at 70 thousand plants ha^{-1} and 13877 kg ha^{-1} at 90 thousand plants ha^{-1}. It is very important to evaluate the minimum and maximum values at the different plant densities and the difference between the two values (Table 3). There were no significant differences in the yield for any of the plant densities neither in the minimum (11388 kg ha^{-1}, 11682 kg ha^{-1} 11826 kg ha^{-1}), nor in the maximum (15859 kg ha^{-1}, 15948 kg ha^{-1}, 16296

Table 3. Parameters of the plant density response of maize hybrids (Debrecen, chernozem soil, 2013)

	Plant density		
	50 thousand ha^{-1}	70 thousand ha^{-1}	90 thousand ha^{-1}
a) Yield average of hybrids (kg ha^{-1})	13130	13824	13877
b) Minimum yield (kg ha^{-1})	11388	11862	11826
c) Maximum yield (kg ha^{-1})	15859	15948	16296
d) Differences of minimum and maximum yield (kg ha^{-1})	4471	4086	4470

kg ha^{-1}), or in the difference interval (4471 kg ha^{-1}, 4086 kg ha^{-1}, 4470 kg ha^{-1}). This means that the evaluation of the hybrids' average obscures the real differences in the plant density response between the different genotypes. According to our study, the optimum plant density of the late 200 FAO and the early 300 FAO hybrids Sarolta and P 9578 was 70 thousand plants ha^{-1}, while the 400 FAO hybrids Kenéz and SY Afinity gave the maximum yield at 90 thousand plants ha^{-1}.

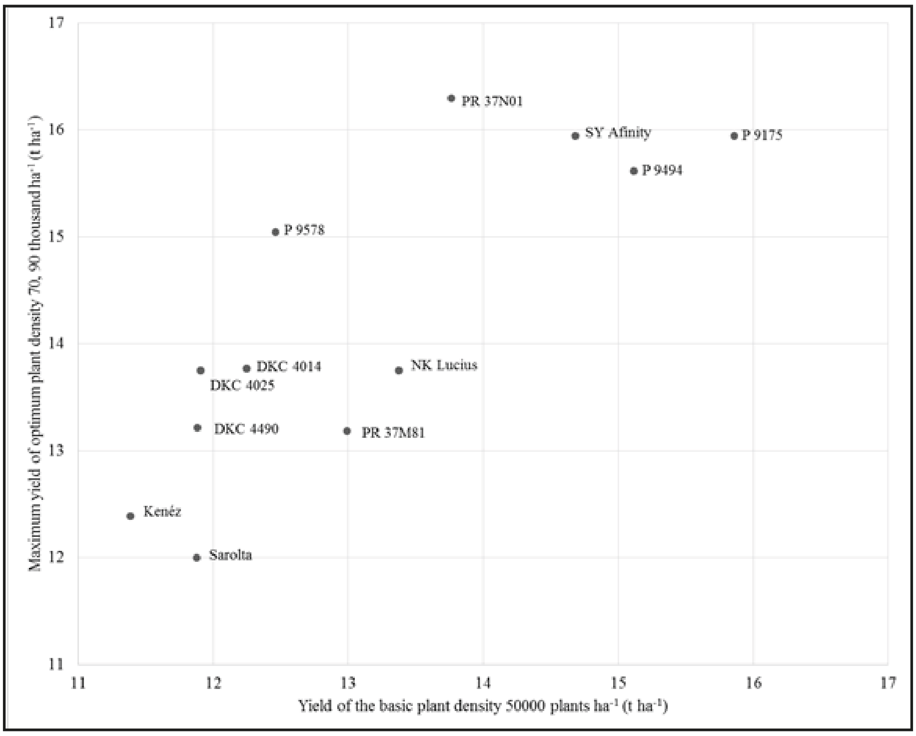
The tested hybrids differed in their yield in the different plant density treatments. In 2013, the hybrids with the highest yield were as follows:

- At 50 thousand plants ha^{-1}: P 9175 (15.9 t ha^{-1}), P 9494 (15.1 t ha^{-1}), SY Afinity (14.7 t ha^{-1});
- At 70 thousand plants ha^{-1}: P 9175 (15.9 t ha^{-1}), P 9494 (15.6 t ha^{-1}), P 37N01 (15.4 t ha^{-1}), SY Afinity (15.4 t ha^{-1}), P 9578 (15.0 t ha^{-1});
- At 90 thousand plants ha^{-1}: PR 37N01 (16.3 t ha^{-1}), SY Afinity (15.9 t ha^{-1}), P 9175 (15.2 t ha^{-1}).

The classification of the hybrids proved that there were hybrids which could tolerate the extreme changes in the plant density (20 thousand plants ha^{-1} lower and higher than the anticipated average 70 thousand plants ha^{-1}) with a very moderate yield fluctuation. These hybrids can be of special value in the production technology as their favourable adaptation ability to the different plant densities is asserted at a high yield level. In our study, such hybrids were P 9175, P 9494 and SY Afinity. However, some genotypes were very sensitive to the plant densities lower or higher than the average (70 thousand plants ha^{-1}), consequently, these hybrids require a very precise sowing and production technology (P 9578, DKC 4490, PR 37N01).

Based on our experimental results, we have developed a special, complex method, which can be used well for determining the actual yield of the hybrids (under the given ecological and agrotechnical conditions) and the plant density response of the maize genotypes (Figure 1). The experimental data were plotted in such a coordinate system in which the yields obtained at the basic plant density (50 thousand plants ha^{-1}) were presented at the horizontal (abscissa) axis, while the yields obtained at the optimum plant density (70 thousand plants ha^{-1} and 90

Figure 1. The plant density response of maize hybrids in a special coordinate system (Debrecen, chernozem soil, 2013)



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thousand plants ha^{-1} depending upon the hybrids) were plotted against the vertical (ordinate) axis. Those hybrids can be regarded favourable which give a high yield at the different plant densities. These hybrids are located in the top right part of the coordinate system (P 9175, PR 37N01, P 9494, SY Afinity). Those hybrids which are less favourable based on their actual yield and plant density response can be found in bottom left part of the coordinate system (Sarolta, Kenéz).

Discussion

The plant density response of 12 maize hybrids of different genotypes was studied on chernozem soil under favourable agrotechnical conditions in 2013. The chernozem soil of excellent water and nutrient management and the significant amount of precipitation in the spring could compensate for the dry and hot weather in July and August. Accordingly, very favourable yields were obtained (11400-16300 kg ha^{-1}) in 2013 depending upon the hybrid and the plant density. Significant differences were found between the hybrids in the maximum yields and optimum plant densities. In 2013, the optimum plant densities were 70 thousand plants ha^{-1} and 90 thousand plants ha^{-1} for the tested hybrids. However, the results showed that the increase of plant density from 70 thousand plants ha^{-1} to 90 thousand plants ha^{-1} resulted only in a minimal yield increment (412-1111 kg ha^{-1}) but it could considerably increase the risk of production under such ecological and agrotechnical conditions which are inferior to the optimal. In the experiments of Dawadi and Sah (2012), Mohseni (2013) and Roekel and Coulter (2011), the maximum yields were obtained at similar plant densities (60-80 thousand plants ha^{-1}), though under different ecological and agrotechnical conditions. The optimum plant density was higher (90 thousand plants ha^{-1}) in the experiments of Widdicombe and Thelen (2002), Gozübenli et al. (2004) and Lashkari et al. (2011). The evaluation of the hybrids' yields at different plant densities proved that the hybrids responded with a different yield fluctuation to the changes in the plant density. Those hybrids were the most favourable which gave a stable, high yield at different plant densities. Such hybrids were P 9175, P 9494 and SY Afinity. As opposed to these, there were hybrids (P 9578, DKC 4490, PR 37N01), which showed a great fluctuation of yield between the different plant density treatments. A special complex evaluation method has been developed for determining the potential yield and plant density response of maize hybrids.

References

Dawadi, D. R., Sah, S. K. (2012): Growth and yield of hybrid maize (Zea mays L. in relation to planting density and nitrogen levels during winter season during winter season in Nepal. Tropical Agricultural Research. 23. (3) 218-227. DOI: http://dx.doi.org/10.4038/tar.v23i3.4659

Gozübenli H., Klinic, M., Sener, O., Konuskan, O. (2004): Effects of single and twin row planting on yield and yield components in maize. Asian Journal of Plant Sciences. **3.** (2) 203-206. DOI: http://dx.doi.org/10.3923/ajps.2004.203.206

Hoshang, R. (2012): Effect of plant density and nitrogen rates on morphological characteristic grain maize. Journal of Basic and Applied Scientific Research. 2. (5) 4680-4683.

Lashkari, M., Madani, H., Ardakani, M. R., Golzardi, F., Zargari, K. (2011): Effect os plant density on yield and yield components of different corn (Zea mays L.) hybrids. American-Eurasian J. Agric. & Environ. Sci. 10. (3) 450-457.

Mohseni, M., Sadarov, M., Haddadi, H. (2013): Study of tillage, plant pattern and plant densities on kernel yield and its component of maiza in Iran. International Journal of Agriculture and Crop Sciences. 5. (15) 1682-1686.

Pepó, P., Vad, A., Berényi, S. (2006): Effect of some agrotechnical elements on the yield of maize on chernozem soil. Cereal Research Communications. (V. Alps-Adria Workshop, Opatija, Crotia) 34. (1.) 1253-1256. DOI: http://dx.doi.org/10.1556/crc.34.2006.1.155

Roekel, J. R., Coulter, A. J. (2011): Agronomic responses of corn top lanting date and plant density. Agronomy Journal. 103. (5.) 1414-1422. DOI: http://dx.doi.org/10.2134/agronj2011.0071

Sárvári, M. (2005): Impact of nutrient supply, sowing time and plant density on maize yields. Acta Agronomica Hungarica. 53. (1.): 59-70. DOI: http://dx.doi.org/10.1556/aagr.53.2005.1.8

Tokatlidis, S. I., Koutroubas, D. S. (2004): A rewiew of maize hybrids' dependence on high plant populations and its implications for crop yield stability. Field Crops Research. 88.:103-114. DOI: http://dx.doi.org/10.1016/j.fcr.2003.11.013

Vad, A., Zsombik, L., Szabó, A., Pepó, P. (2007) Critical crop management factors in sustainable maize (Zea mays L.) production. Cereal Research Communications. (VI. Alps-Adria Scientific Workshop, Obervellach, Austria) 35. (2.): 1253-1256. DOI: http://dx.doi.org/10.1556/crc.35.2007.2.272

Widicombe, D. W. Thelen, D. K. (2002) Row with and plant density effects on corn grain prodution in the Northern Corn Belt. Agronomy Journal. 94. 1020-1023. DOI: http://dx.doi.org/10.2134/agronj2002.1020

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THE EFFECT OF GENOTYPE AND CROPYEAR ON THE YIELD AND THE PHYTOPATHOLOGICAL TRAITS OF SUNFLOWER (*Helianthus annus* L.)

Péter PEPÓ – Adrienn NOVÁK

Institute of Crop Scoience of Agricultural and Food Sciences of Environmental Management, Centre of Agricultural Sciences, University of Debrecen, 138. Böszörményi út, Debrecen H-4032, Hungary
e-mail: novak@agr.unideb.hu

Abstract
The yield and the phytopathological traits of seven sunflower hybrids were studied in two crop protection models with different input levels (extensive=without fungicide treatments, mid-tech=-two fungicide treatments) on chernozem soil in two different years (2012 and 2013). The experimental results proved that the level of infection by Diaporthe (64% in the extensive model, 38% in the mid-tech model as an average of the hybrids), Phoma (53.5% and 34.0%), Sclerotinia (8.4% and 4.6%), Alternaria (76.4% and 48.7%) in the more wet year of 2012. When the precipitation before the season (294 mm from December until March) had filled up the water stock of the chernozem soil and the vegetation period had been dry (79 mm rain during the months of June, July and August), the degree of infection was considerably lower (infection by Diaporthe: 37% in the extensive model and 25% in the mid-tech model, infection by Phoma: 28.5% and 17.4%, infection by Sclerotinia: 1.5% and 0.7%, infection by Alternaria: 35.8% and 22.2% in 2013 as an average of the hybrids). Due to the higher disease infection in 2012, the yield of the hybrids ranged between 3300 – 4200 kg ha^{-1} and between 3900 – 4900 kg ha^{-1} in the extensive (control) model and mid-tech model (two fungicide treatments), respectively. In the more favourable year of 2013 (as regards the weather and the disease infection), the yields of the hybrids varied between 4200 – 5200 kg ha^{-1} in the extensive and between 5000 – 6000 kg ha^{-1} in the mid-tech crop protection model.

Keywords: fungicide treatment, genotype, phytopathological traits, sunflower, yield

Introduction

Sunflower (*Helianthus annus* L.) is the most important oil crop of Hungary, the amount of production is high even when considered at a European level (Treitz, 2003). Nowadays, the extreme weather conditions are increasing the risk of sunflower production. The favourable soil conditions can compensate for the unfavourable effects of the season lack or unfavourable distribution of precipitation (Mijić et al, 2012). However, in order to reduce the unfavourable weather effects as much as possible, the agrotechnical factors need to be optimized (Szabó, 2011). The hybrid selection, the proper sowing technology (sowing date, plant density) and optimized and rational crop protection are of special importance (Szabó, 2012).

The different climate (Borbély and Lesznyák, 2006; Bedő, 2003) and agrotechnical factors (sowing date, plant density etc.) have a strong impact on the yield of sunflower hybrids in the different years (Zsombik, 2006). There is a strong, significant correlation between the climate conditions and the yield (Cerny et al., 2013). The sunflower hybrids respond differently to the changes in the environmental conditions. Those hybrids are regarded less stable which respond to the changes in the environmental conditions with a higher yield fluctuation, while those which can better compensate for the extreme effects of the year are considered stable (Szabó, 2008).

When the weather is cold and wet, lower yields can be expected due to the higher infection by stem and head diseases (Borbély et al., 2007; Szabó and Pepó, 2005), as the yield of sunflower is strongly influenced by the fungal diseases (Mukhtar, 2009). The level and intensity of disease infection in sunflower stands are dependent upon the hybrid selection as there are great differences between the genotypes in their susceptibility to diseases (Borbélyné et al., 2002). The occurrence and spread of diseases are mainly determined by the agroclimatic conditions of the season (temperature, distribution and amount of precipitation (Branimir et al., 2008). According to Ruzsányi and Csajbók (2001), an average and a dry year have similar effects on the yield. A damaging fungal infection does not occur in a dry year and is only of moderate level in a year with average precipitation, thereby, the role of the primary yield-determining factor (disease) becomes negligible. The yield can be increased by protection against fungal pathogens (Szabó, 2013). In a dry year, the yield increasement due to the fungicide treatments as compared to the control was only moderate due to the low disease levels (100-300 kg ha^{-1}). As opposed to this, the fungicide treatments resulted in a yield increment of 100-900 kg ha^{-1} in a rainy year with favourable water supply (Pepó, 2010).

Materials and methods

A field experiment was set up at the experimental farm of the University of Debrecen, Centre of Agricultural Science at Látókép on calcareous chernozem soil. The experimental site is located in Eastern-Hungary, 15 km from Debrecen along the main road No. 33 in the loess region of Hajdúság (N 47°33', E 21°27'). The experimental soil is of good culture-state, medium-hard loam. The soil has good water management characteristics, it has a good water conducting and water-holding capacity.

The yield and the phytopathological characteristics of seven sunflower hybrids of different genetic background were studied in two crop protection models of different intensity (extensive model = no fungicide treatment, mid-tech model = two fungicide treatments) in two different years (2012 and 2013). The tested sunflower hybrids were the followings: NK Neoma (early ripening, low oleic acid [LO], Clearfield ® [IMI]), NK Ferti (early ripening, high oleic acid [HO], normal herbicid tolerant [N]), Tutti (mid-early ripening, HO, N), P64H42 (early ripening, HO, Express-tolerant [E]), P64HE39 (early maturity, HO, N), P63LE13 (early ripening, LO, E), and SY Revelio (early ripening, HO, IMI,).

The experiment was set up in four repetitions in a randomized block design (plot size: 15.2 m^{-2}). The forecrops were winter wheat in 2012 and maize in 2013. Sowing was performed with a seed number of 95 000 ha^{-1} on 10 April in 2012 and 25 April in 2013. After emergence, a plant density of 55 00 plants ha^{-1} was set. The hybrids were treated with the agrotechnique generally applied in the practice. In the mid-tech treatment, fungicides were applied twice (first treatment at the state of 8-10 pair of leaves (BBCH18 – BBCH19), second treatment at the beginning of blooming (BBCH61) (Pictor in a dosage of 0.5 l ha^{-1}, active ingredients: boscalid + dimoxystrobin). The experiment was harvested on 10 September 2012 and 9 September 2013 using a Sampo plot combine harvester with a special adapter.

Table 1. The amount of rainfall and temperature during the investigated crop-years (Debrecen, October, 2011. – September, 2013.)

Months	Oct.	Nov.	Dec.	Jan.	Feb.	Marc.	Apr.	May.	Jun.	Jul.	Aug.	
						Precipitation (mm)						Totally
30 year's average	30.8	45.2	43.5	37.0	30.2	33.5	42.4	58.8	79.5	65.7	60.7	527.3
2012	18.1	0.0	71.1	28.0	17.8	1.4	20.7	71.9	91.7	65.3	4.1	390.1
Difference	-12.7	-45.2	27.6	-9.0	-12.4	-32.1	-21.7	13.1	12.2	-0.4	-56.6	-137.2
2013	22.4	16.6	65.8	38.7	52.9	136.3	48.0	68.7	30.8	15.6	32.2	528.0
Difference	-8.4	-28.6	22.3	1.7	22.7	102.8	5.6	9.9	-48.7	-50.1	-28.5	0.7
						Temperature (°C)						Average
30 year's average	10.3	4.5	-0.2	-2.6	0.2	5	10.7	15.8	18.7	20.3	19.6	9.3
2012	8.6	0.6	1.5	-0.6	-5.7	6.3	11.7	16.4	20.9	23.3	22.5	9.6
Difference	-1.7	-3.9	1.7	2.0	-5.9	1.3	1.0	0.6	2.2	3.0	2.9	0.3
2013	11.1	7.2	-1.2	-1.0	2.3	2.9	12.0	16.6	19.6	21.2	21.5	10.2
Difference	0.8	2.7	-1.0	1.6	2.1	-2.1	1.3	0.8	0.9	0.9	1.9	0.9

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In the critical phenophases, the percentage of disease infection was determined for the major phytopathogens (*Diaporthe helianthi, Phoma macdonaldii, Sclerotinia sclerotiorum, Alternaria helianthi*). The phytopathological data of the hybrids were recorded in four repetitions. In the assessments, 15 plants of average development were selected on each plot. In the case of the stem and head diseases, the level of infection was determined as the percentage of the infected plants. If the disease symptoms were obvious on the studied plant, it was considered infected. The infection was determined as the percentage of infected plants from the 15 selected plants. In the case of leaf diseases, the level of infection was determined as a percentage of the leaf area. At harvest the raw yield of plots and their moisture content were measured. The yields were standardized for a moisture content of 8.0 %.

The meteorological data of the experimental years are included in Table 1. The weather of 2012 was unfavourable for the sunflower's early vegetative and generative development and its yield production. Due to dry April (20.7 mm rainfall compared to the long term average of 42.4 mm), the initiative development of the sunflower plants lagged behind the average. Besides significant rainfalls in May (71.9 mm) and June (91.7 mm), temperature above the average (June: 20.9 °C, July: 23.3 °C) was also favourable. Average precipitation in July (65.3 mm compared to the long term average of 65.7 mm) could only partially satisfy the water demand of the huge vegetative stands. Sunflower stands could only partially tolerate the unfavourable and warm flowering and fertilization period. Extremely dry (4.1 mm) and hot (22.5 °C) August weather had an adverse effect on achene filling processes.

2013 weather conditions significantly challenged the adaptation capability of sunflower hybrids. April and May weather conditions – apart from some short periods – were ideal for the vegetative development of stocks. Sunflower plants with excellent stages of development and significant vegetative sink were able to tolerate the dry (June: 30.8 mm, July: 15.6 mm, August: 32.2 mm) and hot (June: 19.6 °C, July: 21.2 °C, August: 21.5 °C) period from the middle of June till the end of August. The flowering and fertilization of stocks as well as the development and filling of achenes were sufficient. Smaller, but continuous rainfalls prior to the harvesting period set the stock back from drying and hindered harvest.

Results and discussion

Considerable changes have been occurring in Hungarian sunflower production for the past 10-15 years. One of the most important factors in generating these changes was the cardinal change in the biological bases. The number of domestic, registered hybrids has tripled, nowadays it is around 100. The quality transformation was even more significant than the quantitative change, as a consequence of which the yielding capacity of sunflower hybrids considerably increased, for the exploitation of which a more intensive mid-tech agrotechnique of higher input level is needed as compared to the earlier extensive and low-input production technologies. Consequently, the new hybrids created by breeding require the application of more intensive production technology elements with special regards to fertilization, sowing technology and crop protection. For the latter, the proper application of fungicides is of special importance.

Seven sunflower hybrids of different genetic backgrounds were studied in two crop protection models of different intensity (extensive model = no fungicide treatment, mid-tech model = two fungicide treatments [first treatment at the stage of 8-10 pair of leaves - BBCH18/BBCH19, second treatment at the beginning of flowering - BBCH61]) in two different years (2012 and 2013). The weather was totally different in the two years, the effect of which was manifested both in the phytopathological characteristics and in the yield in the studied genotypes. In the months preceding the season of 2012 (from

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Table 2. The effect of crop year, genotypes and fungicide treatment on the yield of sunflower (Debrecen, chernozem soil, 2012- 2013.)

Hybrids (A)	Fungicide treatment (B)	2012. year (kg ha^{-1})	2013. year (kg ha^{-1})	Difference in yield (kg ha^{-1})
NK Neoma	control	3972	4237	265
	fungicide 2x	4550	5103	553
P 63 LE 13	control	4240	4686	446
	fungicide 2x	4907	5639	732
NK Ferti	control	3437	4621	1184
	fungicide 2x	3910	5282	1372
Tutti	control	3605	5218	1613
	fungicide 2x	4260	5970	1710
SY Revelio	control	3619	4490	871
	fungicide 2x	4196	5004	808
P 64 HE 39	control	3336	4382	1046
	fungicide 2x	4061	5184	1123
PR 64 H 42	control	3381	4196	815
	fungicide 2x	3968	5090	1122
Average	**control**	**3656**	**4547**	**891**
	fungicide 2x	**4265**	**5325**	**1060**
$LSD_{5\%}$	Hybrid (A)	1158	969	
	Fungicide treatment (B)	203	250	
	Interaction (A x B)	537	663	

the beginning of December until the end of March), the amount of precipitation was very low (118.3 mm). The amount of precipitation during the season was higher (253.7 mm) but its distribution was unfavourable (71.9 mm in May, 91.7 mm in June, 65.3 mm in July). The precipitation from May until the end of June created favourable microclimate conditions for the relatively early occurrence of the leaf, stem and head diseases of sunflower and for the development of a significant infection. The average temperature of June, July and August was considerably, 2.2 – 3.0 ^{o}C higher than the 30-year average which was unfavourable for the yield formation. These factors had a negative influence on the fertilization, seed development and seed filling of sunflower. As a result of these effects, the yield of the sunflower hybrids varied between 3300 and 4900 kg ha^{-1} in 2012.

The weather and phytopathological conditions of 2013 and the preceding period were totally different from those of 2012. The amount of precipitation from December 2012 until March 2013 (293.7 mm) was very favourable, it enabled the fill-up of the water stock of the chernozem soil. With its enormous root system, sunflower is able to utilize the significant available water stock of the chernozem soil. The amount of precipitation (195.3 mm) in the vegetation period of 2013 (April-August) was considerably lower than in 2012. Consequently, the sunflower diseases occurred later in 2013 and their spread was moderate in the stands. The water supply of sunflower on the whole was more favourable in 2013 (489.0 mm between December 2012 and August 2013) than in 2012 (372.0 mm). The monthly mean temperature of the critical months also had a favourable effect on the fertilization and seed development in 2013 (the mean monthly temperatures of June, July and August were moderately 0.9 – 1.9 ^{o}C higher than the 30-year average). The joint effect of these positive factors was that the yields of the sunflower genotypes were much more favourable in 2013 than in 2012, they varied between 4200 and 6000 kg ha^{-1} (Table 2).

When studying the different fungicide models separately (Table 2), it can be stated that the average yield of the hybrids was 3656 kg ha^{-1} in 2012 and 4547 kg ha^{-1} in 2013 in the extensive model, which increased considerably in the mid-tech model in both years (4265 kg ha^{-1} in 2012

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Table 3. The effect of crop year, genotypes and fungicide treatment on the lodging and phytopathological traits of sunflower (average of hybrids) (Debrecen, chernozem soil, 2012- 2013.)

Crop year		2012. year		2013. year	
Disease	Fungicide treatment	Average	Min. - Max.	Average	Min. - Max.
Lodging %	control	18,8	14,4 - 26,3	8,9	7,2 - 10,8
	fungicide 2x	10,4	8,2 - 16,1	4,5	2,9 - 6,3
Diaporthe helianthi %	control	64	58 - 74	37	30 - 42
	fungicide 2x	38	32 - 49	25	21 - 28
Phoma macdonaldii %	control	53,5	43,8 - 61,2	28,5	25,0 - 32,8
	fungicide 2x	34	27,3 - 40,3	17,4	12,2 - 20,6
Sclerotinia sclerotiorum %	control	8,4	7,3 - 9,5	1,5	1,1 - 2,1
	fungicide 2x	4,6	4,0 - 5,9	0,7	0,4 - 1,1
Alternaria helianthi %	control	76,4	67,9 - 84,6	35,8	31,0 - 41,3
	fungicide 2x	48,7	41,8 - 56,8	22,2	18,9 - 27,2

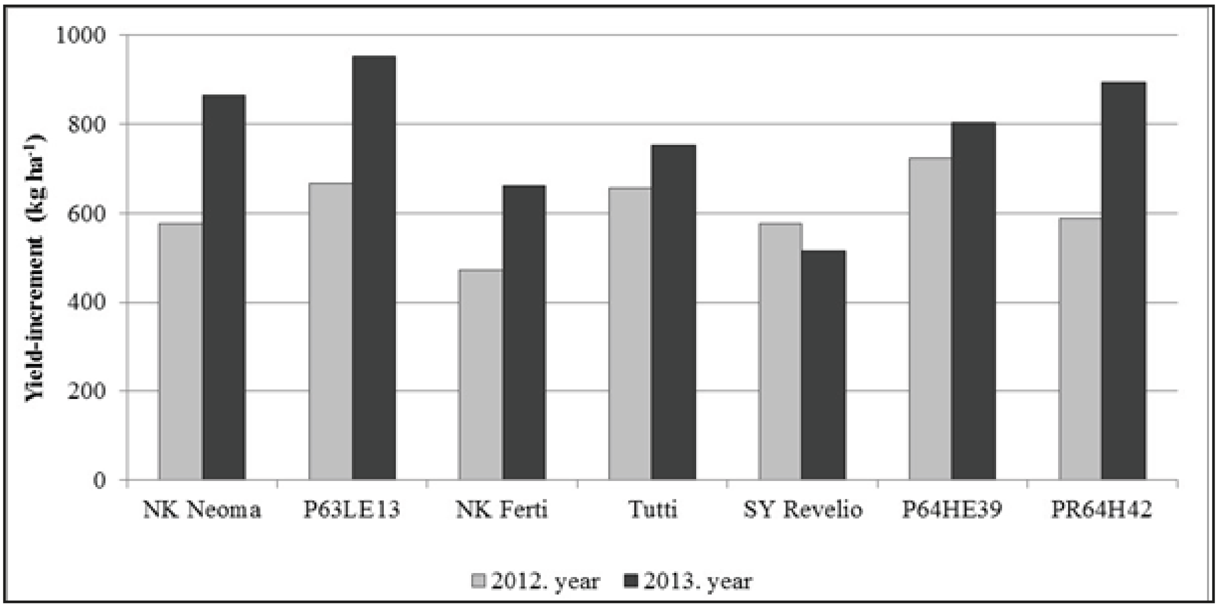
and 5325 kg ha^{-1} in 2013). The sunflower hybrids differed in their adaptation to the different years. In both fungicide models, the highest difference in yield between the two years was found for the hybrid Tutti (1613 kg ha^{-1} in the extensive and 1710 kg ha^{-1} in the mid-tech model), that is the adaptation ability of the hybrid was low. In the favourable years of 2013, the hybrid Tutti gave the highest yield of the tested hybrid sortiment (5970 kg ha^{-1}). The lowest difference in yield between the years was found in the case of NK Neoma (265 kg ha^{-1} and 553 kg ha^{-1}) and P63LE13 (446 kg ha^{-1} and 732 kg ha^{-1}) , which indicated a good adaptation ability of the hybrids. In addition to their good adaptation ability, these hybrids gave a higher than average yield in the mid-tech model. In the mid-tech model, the yields of NK Neoma and P63LE13 were 4550 kg ha^{-1} and 4907 kg ha^{-1} in 2012 and 5103 kg ha^{-1} and 5639 kg ha^{-1} in 2013, respectively.

The experimental results showed that the differences between the potential yield of the different sunflower hybrids were manifested strongly in the untreated control (extensive model). In both years, the smallest yields without fungicide treatment were obtained for the hybrids PR64H42 (3381 kg ha^{-1} in 2012 and 4196 kg ha^{-1} in 2013) and P64HE39 (3336 kg ha^{-1} and 4382 kg ha^{-1}) (Table 2). In both years, the highest yields were obtained in the mid-tech model. From among the tested hybrids, the highest yields were measured for P63LE13 (4907 kg ha^{-1}) and NK Neoma (4550 kg ha^{-1}) in 2012, while Tutti (5970 kg ha^{-1}) and P63LE13 (5639 kg ha^{-1}) gave the highest yields in 2013.

In the two experimental years, the major stem, leaf and head diseases of sunflower were studied (Table 3). The disease infection levels had a strong effect on the lodging and consequently on the yield loss. The lodging and infection data clearly represent the differences between the weather of 2012 and 2013.

Table 3 contains the average, minimum and maximum lodging and infection data of the tested seven hybrids. In the control treatment in the extensive model, the degree of lodging was 18.8% in 2012 and 8.9% in 2013, while the disease infection values were as follows: infection by Diaporthe 64% and 37%; infection by Phoma 53.5% and 28.5%; infection by Sclerotinia 8.4% and 1.5%; infection by Alternaria 76.4% and 35.8%. The two fungicide treatments applied in the mid-tech model considerably reduced the degree of infection in both years, as a result of which lodging was also diminished. In the mid-tech technology, the degree of lodging was 10.4% in 2012 and 4.5% in 2013. The disease infection levels in the mid-tech model in 2012 and 2013 were as follows: infection by Diaporthe: 38% and 25%; infection by Phoma: 34.0% and 17.4%; infection by Sclerotinia: 4.6% and 0.7%; infection by Alternaria: 48.7% and

Figure 1. The yield-increment of the mid-tech fungicide plant protection model at the different sunflower hybrids (Debrecen, chernozem soil, 2012- 2013.)



22.2% as an average of the tested hybrids. The minimum and maximum lodging and disease infection data for 2012 and 2013 presented in Table 3 proved that there are great differences between the hybrids in both the extensive model and the mid-tech fungicide model, that is the genotypes has different phytopathological tolerance.

The experimental data (Figure 1) proved the yield-increasing effect of the mid-tech model as compared with the extensive model for all tested sunflower hybrids in both years (2012 and 2013). As an average of the hybrids, the yield increasement due to the two fungicide treatments (mid-tech model) was 609 kg ha^{-1} in 2012 and 777 kg ha^{-1} in 2013 as compared to the control treatment (extensive model). This means that it is worth applying the mid-tech fungicide model for all sunflower hybrids in years with a higher (2012) and lower infection pressure. The yield increasement of hybrids due to the two fungicide treatments varied between 473 and 725 kg ha^{-1} in 2012 and between 514 and 953 kg ha^{-1} in 2013 depending upon the genotype.

Conclusion

The yield and the phytopathological characteristics of seven sunflower hybrids were studied under favourable agrotechnical conditions on chernozem soil in years with different weather conditions. The year of 2012 was unfavourable as regards the water supply of sunflower and the disease infection. The amount of precipitation before the season (December-March) was low (118.3 mm), while the amount (253.7 mm) and distribution of precipitation in the season (April-August) advanced the occurrence of the leaf, stem and head diseases of sunflower. The temperatures of June, July and August were 2.2 – 3.0 ^{o}C higher than the 30-year average which was unfavourable for yield formation. The weather conditions were favourable in 2013. The significant amount of precipitation from December until March (253.7 mm) increased the water stock of the chernozem soil, which had a positive influence on the vegetative and generative development of sunflower stands. Due to the low amount of precipitation in the season of 2013 (195.3 mm) disease infections were moderate in sunflower. The temperatures of the summer months, which were moderately higher than the 30-year average (by 0.9 – 1.9 ^{o}C) also advanced the yield formation. Due to the different weather conditions in the experimental yields, there were great differences in the yields of the tested hybrids. The yields of the hybrids

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varied between 3300 and 4900 kg ha^{-1} in 2012 and between 4200 and 6000 kg ha^{-1} in 2013 depending upon the fungicide treatment. The significant effect of weather during the season on the yield of sunflower was also proved by Borbély et al. (2007), Szabó and Pepó (2005).

The experiment proved that the hybrids differed in their adaptation ability to the weather (and indirectly to the phytopathological) conditions, which was also observed by Mukhtar (2009). The hybrids NK Neoma and P63LE13 showed a good adaptation ability and produced high yields in both years. From among the hybrids, the highest yields were given by P63LE13 (4907 kg ha^{-1}) and NK Neoma (4550 kg ha^{-1}) in 2012 and by Tutti (5970 kg ha^{-1}) and P63LE13 (5639 kg ha^{-1}) in 2013. These results draw attention to the importance of hybrid selection in modern sunflower production. According to the experimental results, the disease infection values and the degree of lodging were considerably higher in the year of 2012 with a wet season and unfavourable distribution of precipitation as compared to the drier year of 2013. In the experiments of Ruzsányi and Csajbók (2001), the primary yield-determining factor was the disease infection level. As a result of this, the yields of the hybrids were 256 – 1613 kg ha^{-1} higher in the control (extensive model) and 553 – 1710 kg ha^{-1} higher in the mid-tech model (two fungicide treatments) in 2013 than in 2012.

The yield-increasement of the mid-tech model as compared to the extensive model was 473 – 725 kg ha^{-1} (609 kg ha^{-1} as an average of the hybrids) in 2012 and 514 – 953 kg ha^{-1} (average: 777 kg ha^{-1}) in 2013. Our results proved that the mid-tech technology model (two fungicide treatments in the season) can be applied with favourable agrotechnical efficiency in different years for all the tested hybrids.

References

Bedő, Z. (2003): Development of crop production technologies for multifunctional agriculture. Acta Agronomica Hungarica. 51. 83–90. DOI: http://dx.doi.org/10.1556/aagr.51.2003.1.11

Borbélyné, HÉ., Csajbók, J., Lesznyák, M. (2007): Relations between the yield of sunflower and the characteristics of the cropyear. Cereal Research Communications. 35:2. 285-288. DOI: http://dx.doi.org/10.1556/crc.35.2007.2.30

Borbélyné, HÉ., Lesznyák, M. (2006): Investigating yield and phenological stages of sunflower varieties in agro-ecological environment. Cereal Research Communications. 34:1. 417-420. DOI: http://dx.doi.org/10.1556/crc.34.2006.1.104

Borbélyné, HÉ., Pepó P., Kutasy, E., Zsombik, L. (2002): Az évjárat hatása különböző napraforgó genotípusok termésére, minőségére és agronómiai jellemzőire. In: Jávor A. (szerk.) Innováció, a tudomány és a gyakorlat egysége az ezredforduló agráriumában: Plenáris ülés. 2002.04.10-11. Debrecen. 257-262.

Branimir, S., Jasenka, C., Ruza, P., Karolina, V. (2008): Influence of climate conditions on grain yield and appearance of white rot (Sclerotinia sclerotiorum) in field experiments with sunflower hybrids. Cereal Research Commuications. 36. 63-66.

Cerny, I., Veverková, A., Mátyás, M., Kovár, M. (2013): Evaluation of year weather conditions and hybrids impact on the sunflower (Helianthus Annuus L.) achene yield and fat content. Journal of Microbiology, Biotechnology and Food Sciences. 2. 1846-1855.

Mijic, A., Liovic, I., Kovacevic, V., Pepó, P. (2012): Impact of weather conditions on variability in sunflower yield over years in eastern parts of Croatia and Hungary. Acta Agronomica Hungarica. 60:4. 397–405. DOI: http://dx.doi.org/10.1556/aagr.60.2012.4.10

Mukhtar, I. (2009) Sunflower Disease And Insect Pests In Pakistan: A Review. African Crop Science Journal. 17:2. 109 - 118 pp. DOI: http://dx.doi.org/10.4314/acsj.v17i2.54204

Pepó, P. (2010): A napraforgó növényvédelme. Magyar mezőgazdaság. 22-25.

Ruzsányi, L., Csajbók, J. (2001): Termésstabilitás és az évjárat kölcsönhatása a fontosabb szántóföldi növényeinknél. Agrártudományi Közlemények. 2. 41-46.

Szabó, A. (2008): Napraforgó hibridek tőszámreakciójának értékelése regresszió- és stabilitásanalízissel. Agrártudományi Közlemények = Acta Agraria Debreceniensis. 32. 101-109.

Szabó, A. (2011): Study of agrotechnical response in sunflower (Helianthus annuus L.) production. Analele Universitatii din Oradea. Fascicula: Protectia Mediului 17 Oradea: University of Oradea Publishing House. 147-154

Szabó, A. (2012): Az integrált napraforgó termesztés néhány kritikus agrotechnikai tényezőjének értékelése. In: Lehoczky É. (szerk) I. Talajtani, Vízgazdálkodási és Növénytermesztési Tudományos Nap: Talaj, víz, növény kapcsolatrendszer a növénytermesztési térben. 2012.11.23., Debrecen. MTA Talajtani és Agrokémiai Kutatóintézet, Budapest. 217-220. 12.

Szabó, A. (2013): Evaluation on the critical agrotechnical factors of the integrated sunflower production on chernozem soil. Növénytermelés. 62:Suppl. 115-118.

Szabó, A., Pepó, P. (2005): Effect of agrotechnical and meteorological factors on yield formation in sunflower production. Cereal Research Communications. 33:1. 49-52. DOI: http://dx.doi.org/10.1556/crc.33.2005.1.11

Treitz M. (2003): Napraforgó hibridek rezisztencia-vizsgálata a Diaporthe helianthi (Muntanola-Cvetkovic et al.) kórokozó gombával szemben. Doktori értekezés. Keszthely

Zsombik, L. (2006): Effect of sowing time on yield and oil content of different sunflower hybrids. Cereal Research Communications. 34:1. 725-728. DOI: http://dx.doi.org/10.1556/crc.34.2006.1.181

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THE FREQUENCY OF GENETIC REARRANGEMENTS DURING CARROT (*Daucus carota*) SOMATIC EMBRYOGENESIS IS DEPENDENT ON 2,4-D LEVELS AND DIMINISHED IN ITS ABSENCE

Ottó TOLDI – Kitti TÓTH-LENCSÉS – László KOVÁCS – Zsófia TÓTH – Ákos MENDEL – Adrienn KEREKES – Gábor GYULAI

Institute of Genetics and Biotechnology, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő 2103, Hungary

e-mail: toldi.otto@mkk.szie.hu

Abstract

For quantification of genetic variations occurring in plant tissue cultures, DNA sequence alterations and replicon-size changes were monitored through subsequent phases of the model carrot tissue culture system, from the 2,4-D-induced proembryiogenic cell cultures to regenerated plantlets, by RAPD and flow cytometry techniques. Banding patterns of random amplified DNA fragments and ploidy-level distributions of cultured cells were significantly different in the presence and in the absence of 2,4-D. In addition, there were marked differences between the cells induced with lower (1.0 mg/l) and higher (2.5 mg/l) doses of 2,4-D. Among those samples that were cultured in the absence of 2,4-D, the epicotyl (C2), hypocotyl control (H1) and the morphologically normal regenerants (IV2) showed identical banding patterns overlapping with the true-to-type seedling controls (N1, N2). In contrast, treatment of starting explants (H2) with 1.0 mg/l 2,4-D resulted in a marked 82% increase in the number of amplified fragments associated with an appearance of cell lines possessing unusual haploid-like and aneuploid-like DNA contents. The induction with 2.5 mg/l 2,4-D resulted in even greater increase in the number of DNA fragments (~100%) amplified from proembryiogenic cell cultures, while it had no further effect on ploidiy-level distributions compared to the treatment with 1.0 mg/l 2,-D. After the withdrawal of the synthetic auxin, banding patterns and ploidy-level distributions were gradually shifted back to the levels of controls resulting in true-to-type regenerants. Conclusions are in short, the combined use of RAPD and flow cytometry can make quantification of genetic variations typical of dedifferentiated plant cells possible. Quantification opens the window for comprehensive evaluation of different methods, treatments and bioactive compounds.

Keywords: genetic alterations, replicon size, ploidy-level distribution, plant tissue culture, somatic embryogenesis, genetic identity

Abbreviations: 2,4-D – 2,4-dichlorophenoxi acetic acid; DAPI – 4',6-diamidino-2-phenylindole; R_o plants – regenerated plants originating directly from in vitro tissue culture; RAPD - random amplification of polymorphic DNA technique; N1, N2, H1, H2, P1, P2, E1, E2, IV1, IV2 – sample set of the culture level RAPD and flow cytometry detailed in Table 1; mbn – mean band number per random amplified DNA sample

Introduction

Although a wide scale of genetic alterations can be obtained in plant tissue cultures, from point mutations to replicon-level rearrangements (for review see Smulders and de Klerk 2010), the occurrence of genetically non-identical off-types (somaclones) is relatively rare (Jain 2001, Gyulai et al. 2003, Joshi and Dhawan 2007). Among the components of a typical plant tissue culture medium mainly synthetic growth regulators were suggested as inducers of genetic alterations (Kaeppler et al. 2000) together with different abiotic stressors manifested typically in tissue cultures (Phillips et al. 1994, Guo et al. 2006). Our aim was to develop a technically simple and fast method, which, in theory, covers all potential genetic variations from sequence to replicon-size changes in order to allow estimations of the frequency of genetic rearrangements. Quantification of the frequency of genetic rearrangements can make different treatments

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and effects comparable. There are evidences to support that 2,4-D, previously was used as an ingredient of Agent Orange herbicide to defoliate plants, induces irregular cell divisions (Fujimura and Komamine 1982, Nuti Ronchi et al. 1992ab,) an elevated genetic translocation frequency (Kaeppler et al. 2000) and reversible changes in the number of repetitions of the repetitive DNA sequences in plant cell cultures (Arnholdt-Schmitt, 1995), without severe negative impacts on the genetic identity of regenerants. Therefore, the carrot (*Daucus carota cv*. 'Nantes Duke') tissue culture system was chosen as model, where proembryogenic cell cultures are induced by 2,4-D at different concentrations (1.0 mg/l and 2.5 mg/l), and the subsequent plant regeneration via somatic embryogenesis starts after the withdrawal of 2,4-D. If our hypothesis is true, differences in the frequencies of genetic changes can be estimated comprehensively between 2,4-D-induced and non-induced cells and tissues, even finer variations could be identified in cell and tissue samples treated with 2,4-D at different concentrations. To test this hypothesis RAPD (Random Amplification of Polymorphic DNA) and flow cytometry techniques were combined for parallel monitoring of sequence and ploidy-level alterations.

Materials and methods

Plant material, culture conditions and sampling

Surface-sterilized carrot (*Daucus carota cv*. 'Nantes Duke') seeds were used as starting explants. After *in vitro* germination on growth regulator-free solid MS medium (Murashige and Skoog 1962), hypocotyl sections were excised and divided to 3 groups. The control group was placed into growth regulator-free liquid MS medium, the second group of excised hypocotyls was placed into liquid MS medium supplemented with 1.0 mg/l 2,4-D, while the third group was transferred into liquid MS medium supplemented with 2.5 mg/l 2.4-D. The list of samples was shown in Table 1. The control hypocotyl segments of the first group were labelled as H1, the second group incubated with1.0 mg/l or 2.5 mg/l 2,4-D were labelled as H2.The 2,4-D-induced hypocotyl segments were removed after sufficiently dense embryogenic suspension cultures were formed in liquid MS medium containing 2,4-D. Young proembryogenic suspensions (P1) were incubated for 15 days in either 1.0 mg/l or 2.5 mg/l 2,4-D. In the case of old proembryogenic suspensions (P2) the duration of the incubation was 30 days. Subculturings were performed weekly.

To induce embryogenesis and plant regeneration, embryogenic cell clusters (P1) were separated from 2,4-D-containing induction media and were transferred into growth regulator-free liquid MS media. Somatic embryos at the stage of heart-torpedo (E1) were obtained 15 days after the withdrawal of the 2,4-D, while elongated-torpedo stage embryos (E2) appeared after 30 days-incubation in growth regulator-free media. For plant regeneration, the mature somatic embryo cultures were placed to darkness without subculturing and shaking. Morphologically normal (IV2) and abnormal plantlets showing symptoms of undetermined polarity (IV1) were selected for further analysis as R_0 generation. Besides these morphological alterations, the most important difference between the IV1 and IV2 plantlets was that the IV1 plantlets were non-viable after the removal from tissue culture.

Flow cytometry

Cell nuclei were isolated mechanically as described by Dolezel et al. (1989) and Mitykó et al. (1995). Approximately 10 mg tissues were chopped with a scalpel in a glass petri dishes containing 2 ml lysis buffer LB01 (pH 7.5) supplemented with 2 µg/ml DAPI. Settled suspension cells were resuspended in lysis buffer LB02 and left at room temperature for 15 min. The cell nuclei were released from the cells by syringing twice through a 25-gauge needle. Cell fragments were filtered through a 21 µm nylon filter and kept on ice until the analysis. The flow cytometer was adjusted so that the diploid-like peak of the nuclei isolated from the control was set as channel 100. Flow cytometric

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Table 1. Description of the sample set examined by RAPD and flow cytometry techniques. Liquid or agar solidified MS media were used for *in vitro* maintenance of tissue cultures and 2,4-D was used at 1.0 mg/l or 2.5 mg/l concentrations for inducing the growth of proembryogenic mass

Sample set	Treatment types (1.0 mg/l or 2.5 mg/l 2,4-D)
N1 - seedling for control N2 - seedling for control	*in vivo*, greenhouse conditions
C2 - epicotyls excised H1 - hypocotyls (not-treated)	*in vitro*, growth regulator-free liquid MS
H2 - hypocotyls (treated) P1 - young proembryogenic suspension (treated) P2 - old proembryogenic suspension (treated)	*in vitro*, liquid MS + 1.0 mg/l or 2.5 mg/l 2,4-D
E1 - young embryo suspension E2 - old embryo suspension	*in vitro*, growth regulator-free liquid MS
IV1 - somatic embryo derived abnormal plantlets IV2 - somatic embryo derived true-to-type plantlets (S_1-S_{12})	*in vitro*, growth regulator-free solidified MS

measurements were performed with a Partec CA-II computerized compact flow cytometer. The amounts of nuclear DNA were calculated on the basis of the areas under the plotted graphs by using SIS Soft Imaging Software.

DNA extraction

Total DNA was isolated by homogenizing 1 g FW of plant materials with phenol/chloroform/isoamyl alcohol (25/24/1; v/v/v) in liquid nitrogen containing pre-chilled mortars as described by Dellaporta et al. (1983). The extracted DNA was treated with RNAase A (10 mg/ml) for 1h at 37°C. The DNA was then extracted again with phenol/chloroform/isoamyl alcohol (25/24/1; v/v/v), following isopropanol precipitation and 70% ethanol wash. The pellet was dissolved in 50 µl sterile ddH_2. The final concentration of DNA (~20 ng μl^{-1}) was measured by a Hoefer TKO-100 fluorimeter.

RAPD conditions

Fifty 10-mer RAPD primers (kits A, B from Operon Technologies, Almeda, California) were used for PCR amplifications. RAPD reactions were performed in a volume of 50 µl containing 100 ng of extracted DNA, 1.0 µM of primer, 200 µM of dATP, dTTP, dGTP, dCTP (Pharmacia), 1/10 volume 10xPCR-buffer (Boehringer Mannheim). Taq DNA polymerase (Boehringer Mannheim) concentration was 3.0 units per sample. For the RAPD cycling conditions, samples were first heated to 94 °C for 3 min. before entering a 40-cycle PCR procedure of 94 °C for 1 min., 36 °C for 2 min. and 72 °C for 3 min., followed by 72 °C for 5 min. and closed at 4 °C. Amplifications were carried out using a PCT-100/60 thermal cycler (MJ Research Inc). Amplified DNA fragments were separated by gel electrophoresis at 100V for 7h in a 1.5-2% agarose gel with a TBE buffer. Gels were stained with ethidium bromide and fragment patterns were photographed under UV light for further analysis. RAPD patterns were analyzed by the *restml* program of the PHYLIP

3.5c software package. The mean band number (*mbn*) for each sample was calculated.

Results

Genotype identity of somatic embryo originated carrot plants

Genotype identity of 35 randomly selected R_0 carrot plants regenerated from somatic embryos was tested by RAPD technique. Of the 35 regenerants, results of 12 are presented in Figure 1 (lanes S1-S12). The negative control group was formed by 2 greenhouse grown carrot plantlets (lanes N1, N2), while the positive control group consisted of T-DNA-inserted carrots (lanes T1, T2) and a tobacco sample (lane C3) as a taxonomically non-related species. Amplifications were obtained with all the primers, between 2-11 bands per primer, showing a homogenous genotype identity both in the control and in tissue culture-originated carrot plants, while tobacco always showed a different banding pattern. In *Agrobacterial* T-DNA carrying carrots only one primer, the OPB-11 (5'-GTAGACCCGT-3'), amplified a different banding pattern compared to control and tissue culture-originated plants.

The potential anomalies in the ploidy-level distributions of tissue culture-originated carrots were tested by flow cytometry using the same R_0 plant material as in RAPD analysis. No differences were found between tissue culture-originated R_0 and greenhouse grown control carrot plants (Figure 4AB sample group no. I., Table 2).

Quantification of the frequency of genetic alterations in 2,4-D-induced and control carrot tissue cultures

The whole *in vitro* plant tissue culture process was screened by RAPD and flow cytometry from the establishment of the embryogenic cell culture to the growth of the Ro regenerants. Twenty-five primers (OPA-01, OPA-08, OPA-12, OPA-16, OPA-17, OPA-19, OPA-20, OPB-01, OPB-02, OPB-03, OPB-05, OPB-11, OPB-12, OPB-16, OPB-18), which were randomly chosen from the 50 primers confirming homogenous uniformity of the R_0 plants, were used for fingerprinting. Amplifications were obtained with all the 25 primers between 11-26 bands per primer (Figure 2). Both the epicotyl (C2) and the hypocotyl controls (H1) incubated in the

Figure 1. Testing genotype identity of tissue culture-originated carrot plantlets by RAPD technique using 50 ten-mer OPB primers of which banding pattern of OPB-11 is presented. Lanes Mw - size marker in Kbp (lambda phage DNA digested with PstI); lanes N_1 and N_2 – greenhouse grown carrot plantlets; lane DNA$^-$ - no-template DNA; lanes S_1 to S_{12} - carrot plantlets regenerated via somatic embryo genesis; lane Taq$^-$ - no-Taq DNA polymerase; lanes T_1 and T_2 - T-DNA-carrying carrot controls; lane Tobac - tobacco (Nicotiana tabacum) control plants

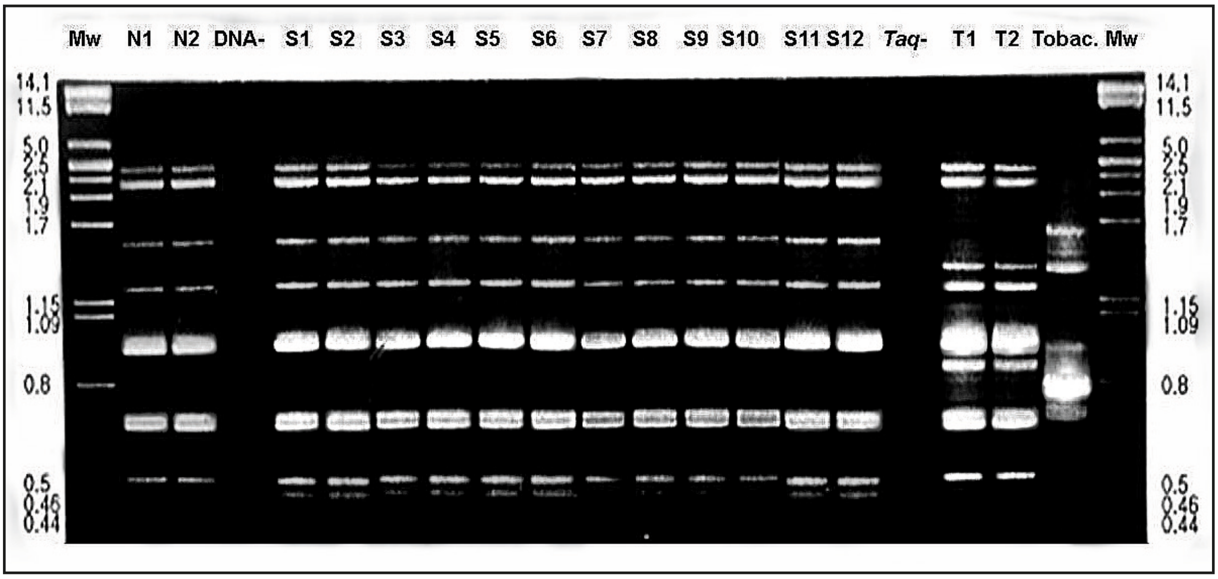

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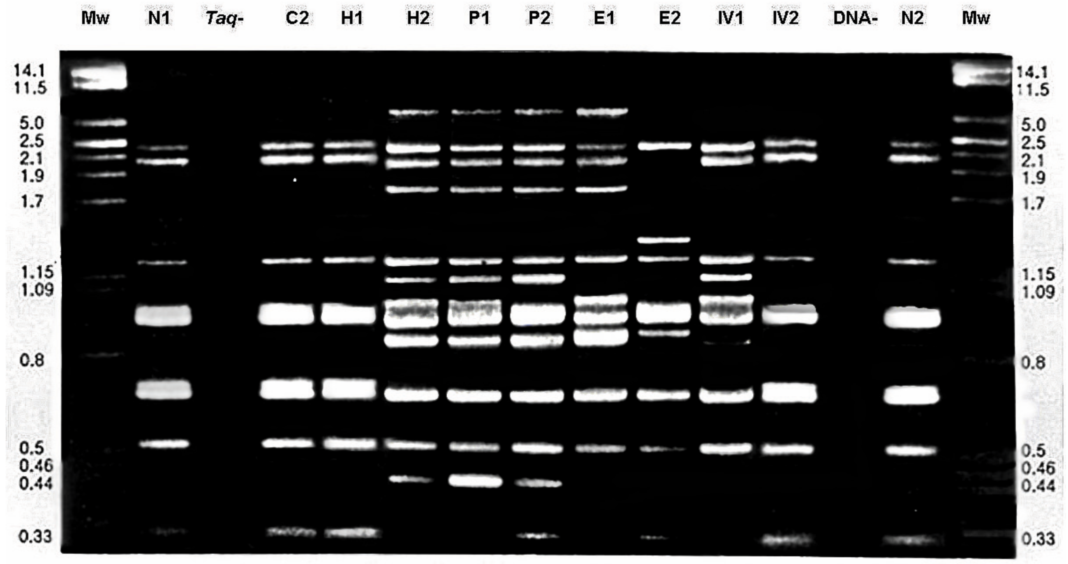
Table 2. Ploidy-level (replicon size) distributions (% of analyzed nuclei) and sequence-level genetic alterations, quantified by changes in *mbn*, during carrot plant regeneration via somatic embryogenesis. The standard deviations were less than 5% of the mean value in each case.

Tissue culture and individual samples	DNA content per sample (% of the analyzed samples)				Mean band number (*mbn*)	
	1C haploid-like	1C-2C aneuploid-like	2C diploid-like	tetraploid-like cells	1.0 mg/l 2,4-D	2.5 mg/l 2,4-D
N_1 (---)	-	-	100.0	-	11.33	11.33
N_2 (---)	-	-	100.0	-	11.33	11.33
C2 (*grf*)	-	-	100.0	-	11.33	11.33
H1 (*grf*)	-	-	100.0	-	11.33	11.33
H2 (+2,4-D)	64.9	24.9	10.2	-	20.00	24.00
P1 (+2,4-D)	58.3	30.9	10.8	-	20.00	25..00
P2 (+2,4-D)	58.3	30.9	10.8	-	18.66	25.66
E1 (*grf*)	28.1	45.4	26.5	-	17.00	19.00
E2 (*grf*)	-	-	88.8	11.2	15.66	16.66
IV1 (*grf*)	-	-	88.8	11.2	13.66	13.66
IV2 (*grf*)	-	-	100.0	-	11.33	11.33

(---): greenhouse grown seedlings;
(*grf*): tissue culture incubated on growth regulator-free media;
(+2,4-D): tissue culture incubated on 1.0 mg/l or 2.5 mg/l 2,4-D containing media;

absence of 2,4-D, as well as the morphologically normal regenerants (IV2), showed identical banding patterns corresponding to each other and to the greenhouse grown, individual controls (N1, N2). For statistical analysis, the banding pattern values of the sample group consisting of sample N1, C2, H1, IV2 and N2, incubated in the absence of 2,4-D, was anchored as the root of the similarity tree generated by the *restml* program of PHYLIP 3.5c software (Figure 3AB). In this sense, the cultures incubated with 1.0 mg/l 2,4-D (H2, P1, P2) represented the top of the similarity tree. Between these two sample groups, which were the least similar to each other in terms of the number of amplified DNA fragments, torpedo-stage embryo cultures (E1, E2) and the morphologically deviant regenerants (IV1) formed a statistically intermediate sample group that settled down on the stem of the similarity tree. Based on the comprehensive banding pattern analysis by the '*restm*l' program of PHYLIP 3.5c, *the in vitro* carrot plant regeneration process could be divided into proembryogenic or 2,4-D induction phase (H2, P1, P2), somatic embryo differentiation phase (E1, E2) on growth regulator-free tissue culture medium, and finally plant regeneration phase again in the absence of 2,4-D (Figure 3A). We have stated that the mean band number (*mbn*) of the control sample group (N1, C2, H1, IV2, N2) was identical within the sample group and the lowest compared to the other two groups (Table 2). The *mnb* of proembryogenic cultures induced with either 1.0 mg/l or 2.5 mg/l 2,4-D showed a dramatic increase 82% (1.0 mg/l 2,4-D) and 100% (2.5 mg/l 2,4-D) respectively. In the intermediate sample group, the *mbn* gradually decreased (E1=17.0 bands; E2=15.66 bands; IV1=13.66 bands) until the number of amplified fragments returned to the starting value in regenerated plantlets (IV2) which corresponds to the parameters of the individual controls (N1, N2). In each case the increased *mbn* characteristic of the 2,4-D-induced (H2, P1, P2) and the intermediate (E1, E2, IV1) sample groups appeared in a way that the amplified fragment sets of these samples included all the bands of the N1, C2, H1, IV2, N2 control sample group and some extra amplificates were found besides them. Even within the 2,4-D-treated cultures the 2.5 mg/l 2.4-D-induced ones showed significantly

Figure 2. Monitoring the whole tissue culture process by RAPD technique from the establishment of proembryogenic cell cultures with 1.0 mg/l or 2.5 mg/l 2,4-D to regeneration of carrot plantlets via somatic embryogenesis on growth regulator-free tissue culture media. Banding patterns amplified with OPB-11 primer were presented as in Figure 1. The loading order of samples followed the characteristic stages of tissue culturing given in Table 1. *Lanes Mw* - size marker in Kbp (lambda phage DNA digested with *Pst*I); *lanes* N_1 *and* N_2 – greenhouse grown carrot plantlets; *lane DNA*$^-$ - no-template DNA; *lanes C2* to *IV2* – cell culture-level samples described in Table 1; *lane Taq*$^-$ - no-Taq DNA polymerase.



higher *mnb* value matching over a 20% increase compared to 1.0 mg/l 2.4-D induced ones (Table 2, Figure 3B).

Alterations in ploidy-level distributions correlate not only with the absence and presence of 2,4-D, but also with its concentrations

For the flow cytometry, the same sample set was used as for the RAPD. Similarly to the controls (N1, N2), epicotyl- (C2) and non-induced hypocotyl cultures (H1) incubated in the absence of 2,4-D showed a diploid 2C DNA content (Figure 4AB sample group no.I., Table 2). A common characteristic of the cultures incubated with 2,4-D (H2, P1, P2) was the coexistence of the continuously present 2C DNA containing (G0/G1 phase cells), 2C-4C DNA containing (S phase cells), and 4C DNA containing cell lines (G2+M phase cells) with the aberrant 1C DNA containing (58.3-64.9% of the cells), and 1C-2C DNA containing (24.9-30.9% of the cells) ones (Figure 4AB sample groups no. II., III., Table 2). In those cell cultures in which the higher concentration of 2,4-D (2.5 mg/l) was used for inducing the growth of proembryogenic mass, the ploidy-level distributions showed even more extreme rearrangements (Figure 4B sample groups no. II., III, Table 2). However, after the withdrawal of 2,4-D, the 1C DNA containing cell line gradually disappeared (E1=28.1%, E2=0%, IV1=0%, IV2=0%) together with the 1C-2C DNA containing aneuploid-like cell line (E1=45.4%, E2=0%, IV1=0%, IV2=0%) (Figure 4AB sample groups no. IV., V., Table 2). At the same time, the diploid 2C DNA containing cell line gradually became dominant (E1=26.5%, E2=88.8%, IV1=88.8%) after the withdrawal of 2,4-D, during differentiation of somatic embryo-originated plantlets. As a result of the above mentioned process, the regenerated plants (IV2) showed the same 2C DNA contents as the individual control plants independently of the applied 2,4-D concentrations (Figure 4AB sample group no. I., Table 2).

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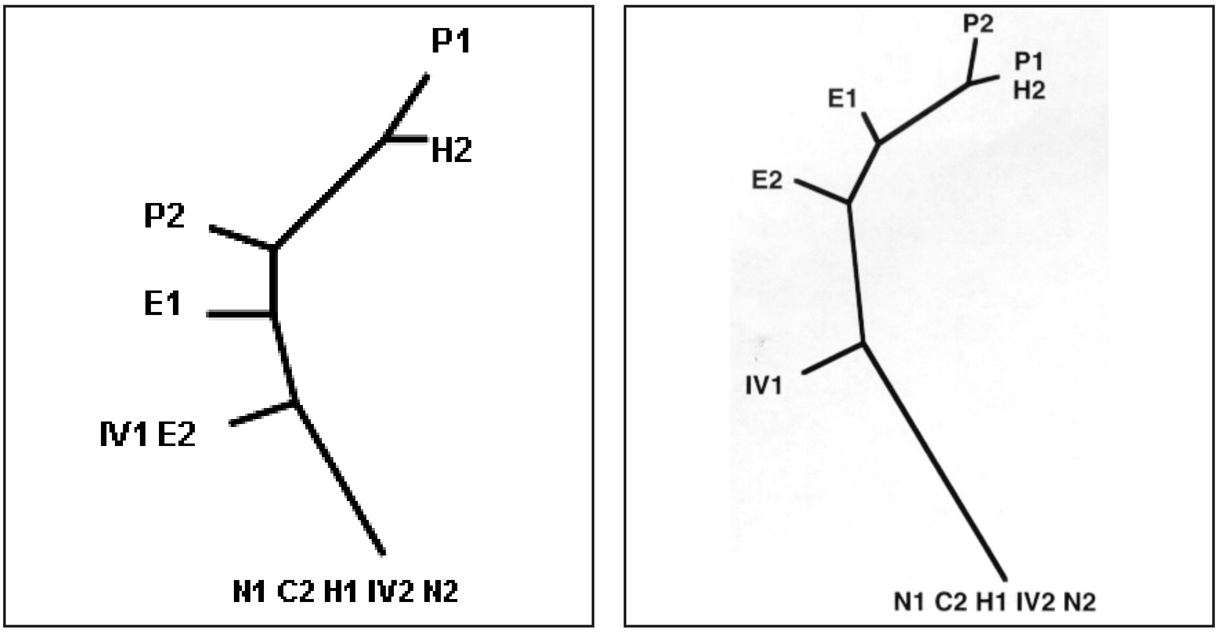
Figure 3. Statistical distances between carrot tissue cultures (see Table 1) incubated with 1.0 mg/l 2,4-D **(A)** or 2.5 mg/l 2,4-D **(B)** calculated from the mean band numbers generated by RAPD analyses and processed with the *restml* program of PHYLIP 3.5c software package. Roots of dendrograms were fixed to samples showing genetic alterations at the lowest frequency (N1, C2, H1, IV2).



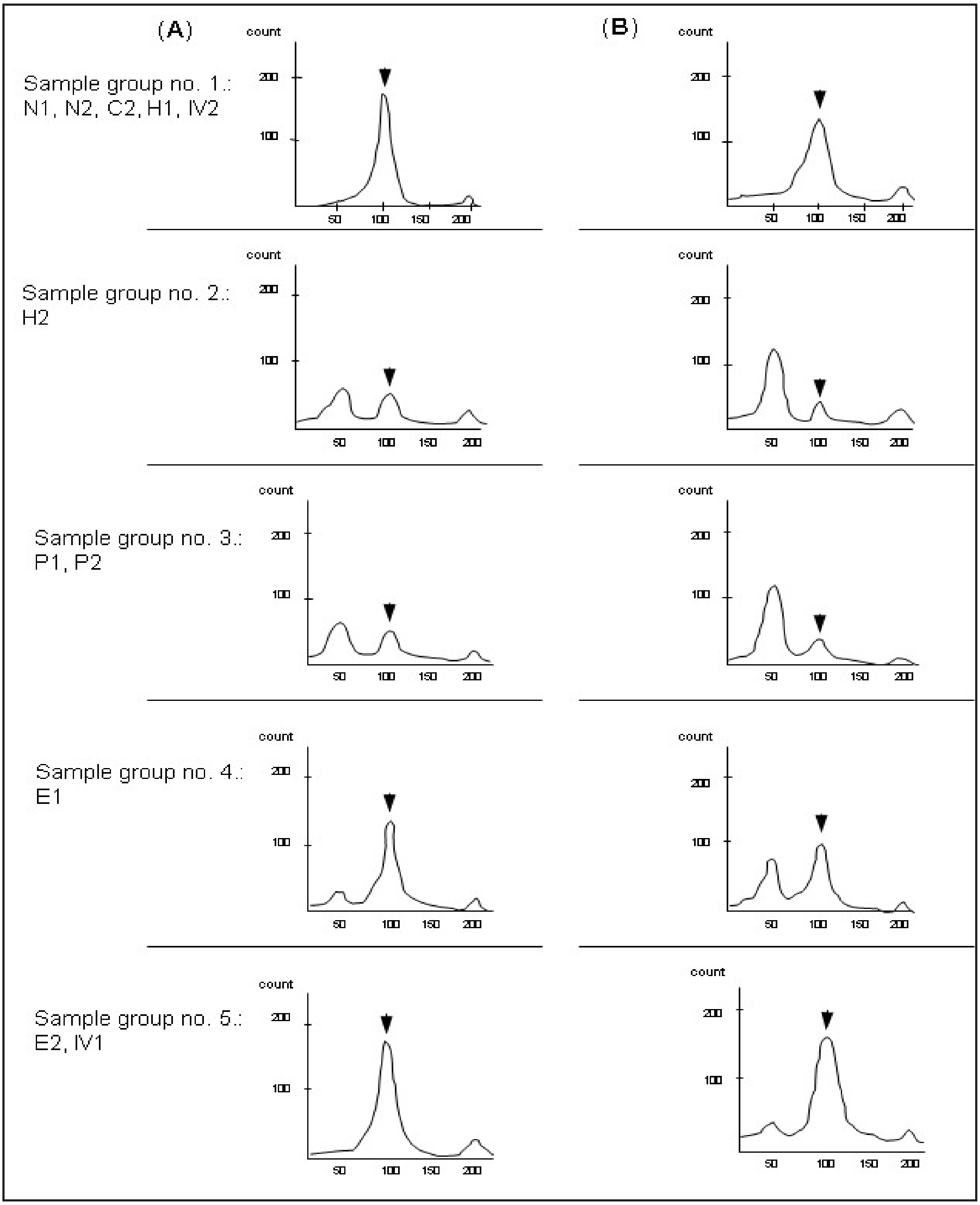
Discussion

Comparative analysis of tissue culture-originated plants based on RAPD technique is a widely applied method for the evaluation of genotype identity (Martins et al. 2004, Saker et al. 2006, Guo et al. 2007). Our examinations were different from the foregoings in one aspect; it was not a stationary relation comparing banding patterns of two treatments or sample groups, but a dynamic approach analysing two complete plant regeneration systems. The theory behind this approach came from the realisation that if the RAPD fragment patterns of individual mother plants were identical, the fragment patterns of bulk samples of tissue cultures originating from them should be also identical. If an *in vitro* culture became different from its individual mother-plant, it means that the *in vitro* tissue culturing resulted in such genetic changes that are detectable by culture-level application of RAPD technique.

The increased frequency of point mutations and tranlocation events, the irregular cell divisions caused by 2,4-D (Fujimura and Komamine 1982, Nuti Ronchi et al. 1992ab), as well as the decreased replicon size due to a 2,4-D-induced reversible reduction in repetitive DNA sequences (Arnholdt-Schmitt, 1995) have already been proven (Kaeppler et al. 2000, Smulders and de Klerk 2010). In our case, it is also probable that the different RAPD patterns in the proembryogenic (H2, P1, P2) and somatic embryo cultures (E1, E2) were stimulated by the 2,4-D treatment, because of two reasons. First, other factors of the *in vitro* culture had no effect on these parameters, becasue genetic alterations could not be detected in cell cultures maintained in the absence of 2,4-D. Second, the quantification of the frequency of genetic alterations showed a dose effect of 2,4-D, namely, incubation of cell cultures with higher concentration of 2,4-D resulted in an increase in the number of random amplified DNA fragments compared to incubation at lower 2,4-D concentrations (Table 2, Figure 3AB).

Based on the quantified results of RAPD analyses, a hypothesis was devised to describe the most likely mechanism associated with

Figure 4. Flow cytometry plots of carrot tissue cultures (see Table 1) incubated with 1.0 mg/l 2,4-D **(A)** or with 2.5 mg/l 2,4-D **(B)**. The peak of diploid (2n) DNA volume was adjusted to the channel 100. Arrow heads indicate diploid (2n) genome.



reversible changes in DNA sequences and ploidy-level distributions detected in our carrot tissue culture system. The results of culture-level RAPD and flow cytometry showed that the diploid starting genotype became a mixture of different ploidy-level deviant cell lines consisting of a 1C haploid-like, 1C-2C aneuploid-like and 2C diploid cell lines (Figure 4AB sample

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groups no. II., III.). Meanwhile, the number of random amplified DNA fragments of these cell lines, possissing unusual ploidy-level distributions, contained the amplified DNA fragments characteristic to the starting 'genotype' and different numbers of additional fragments depending on the presence and concentration of 2,4-D (Figure 2, 3AB). The appearance of 1C haploid-like and 1C-2C aneuploid-like cell lines in 2,4-D-induced proembryogenic cultures might be a consequence of the reversible loss of a marked proportion of repetitive DNA sequences descibed by Arnholdt-Schmitt (1995). Although the chromosome number of these cell lines is diploid, their DNA contents (replicon size) are varied and can be reduced up to half (1C DNA content). In spite that the molecular machinery of the above process still unknown, these data are concordant with and may explain our flow cytometric results. Omitting 2,4-D from culture media led to the disappearance of aberrant cell lines, which resulted in a disarrangement of the original ploidy-level distribution and RAPD pattern (Figure 2, Figure 4AB sample groups no. IV., V.).

We suppose that 2,4-D plays a central role in triggering these genetic alterations in two ways. First, as a direct inducer of irregular cell divisions (Fujimura and Komamine 1982, Nuti Ronchi et al. 1992ab) and reduction of the number of repetiitions within repetitive DNA sequences (Arnholdt-Schmitt 1995). Second, 2,4-D acts also as auxinic herbicide triggering oxidative strees, abnormal growth, senescence, and plant death (Song 2014). Physiological stress associated with *in vitro* tissue culturing, thought to be the second most important among the potential inducers of genetic alterations, exacerbating with the fact that 2,4-D has no catabolism in plants, making it capable for prolonged actions (Wright et al. 2010). However, genetic alterations can be stimulated not only by induction with exogenous effectors, but also by disturbing endogenous repaire mechanisms through a defficiency in suppression of repeat-induced point mutations (Phillips et al. 1994) and activation of plant retrotransposons (Grandbastien 1998).

The sensitvity and the resolution of our approach, the paralel use of RAPD (Random Amplification of Polymorphic DNA) and flow cytometry techniques supported by PHYLIP 3.5c and SIS Soft Imaging softwares, allowed a complete cover of all genetic alterations that, in theory, can be perceived in tissue cultures. This combined approach made quantification of genetic variations typical of dedifferentiated plant cells possible. opening a window for comprehensive evaluation of different methods, treatments and bioactive compounds.

References

Arnholdt-Schmitt B, Physiological aspects of genome variability in tissue culture. II. Growth phase-dependent quantitative variability of repetitive *BstN*I fragments of primary cultures of *Daucus carota* L., Theor Appl Genet 91, 1995, 816–823. DOI: http://dx.doi.org/10.1007/bf00220965

Dellaporta SL, Wood J and Hick JB, A plant DNA minipreparation: Version II, Plant Mol Biol Rep 1, 1983, 19-21. DOI: http://dx.doi.org/10.1007/bf02712670

Dolezel J, P Binarova, S Lucretti, Analysis of nuclear DNA content in plant cells by flow cytometry, Biologia Plantarum 31/2, 1989, 113–120. DOI: http://dx.doi.org/10.1007/bf02907241

Fujimura T, A Komamine: Molecular aspects of somatic embryogenesis in a synchronous system. In: Plant tissue culture 1982: Proceedings, 5th International Congress of Plant Tissue and Cell Culture held at Tokyo and Lake Yamanake, Japan, July 11-16, 1982, edited by Akio Fujiwara, p. 105–106.

Grandbastien M-A, Activation of plant retrotransposons under stress conditions. Trends in Plant Science 3(5), 1998, 181–187. DOI: http://dx.doi.org/10.1016/s1360-1385(98)01232-1

Guo WL, Gong L, Ding ZF, Li YD, Li FX, Zhao SP and Liu B, Genomic instability in phenotypically normal regenerants of medicinal plant *Codonopsis lanceolata* Benth. et Hook. f., as revealed by ISSR and RAPD markers, Plant Cell Reports 25(9), 2006, 896-906. DOI: http://dx.doi.org/10.1007/s00299-006-0131-8

Guo WL, Wu R, Zhang YF Liu XM, Wang HY, Gong L, Zhang ZH and Liu B, Tissue culture-induced locus-specific alteration in DNA methylation and its correlation with genetic variation in *Codonopsis lanceolata* Benth. et Hook.f, Plant Cell Reports 26, 2007, 1297-1307. DOI: http://dx.doi.org/10.1007/s00299-007-0320-0

Gyulai G, Z Mester, J Kiss, Szemán L, Heszky L, A Idnurm, Somaclone breeding of reed canarygrass (*Phalaris arundinacea* L), Grass and Forage Sci 58, 2003, 210–215. DOI: http://dx.doi.org/10.1046/j.1365-2494.2003.00372.x

Jain SM, Tissue culture-derived variation in crop improvement, Euphytica 118, 2001, 153-166.

Joshi P and Dhawan V, Assessment of genetic fidelity of micropropagated *Swertia chirayita* plantlets by ISSR marker assay, Biologia Plantarum 51(1), 2007, 22-26. DOI: http://dx.doi.org/10.1007/s10535-007-0005-0

Kaeppler SM, Kaeppler HF and Rhee, Epigenetic aspects of somaclonal variation in plants. Plant Molecular Biology 43, 2000, 179-188. DOI: http://dx.doi.org/10.1007/978-94-011-4183-3_4

Larkin PJ, WR Scowcroft, Somaclonal variation - a novel source of variability from cell cultures for plant improvement, Theor Appl Genet 60, 1981, 197–214. DOI: http://dx.doi.org/10.1007/bf02342540

Martins M, Sarmento D and Oliveira MM, Genetic stability of micropropagated almond plantlets as assessed by RAPD and ISSR markers, Plant Cell Reports 23, 2004, 492-496. DOI: http://dx.doi.org/10.1007/s00299-004-0870-3

Mitykó J, Andrásfalvy A, Csilléry G, Fári M, Anther- culture response in different genotypes and F1 hybrids of pepper (*Capsicum annuum* L.). Plant Breeding, 1995, 114:78–80. DOI: http://dx.doi.org/10.1111/j.1439-0523.1995.tb00764.x

Murashige T, F Skoog, A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol Plant 15, 1962, 473–497. DOI: http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nuti Ronchi VN, L Giorgetti, M Tonelli, G Martini, Ploidy reduction and genome segregation in cultured carrot cell lines, I. Prophase chromosome reduction, Plant Cell Tiss Org Cult 30, 1992a, 107–114. DOI: http://dx.doi.org/10.1007/bf00034303

Nuti Ronchi VN, L Giorgetti, M Tonelli, G Martini G, Ploidy reduction and genome segregation in cultured carrot cell lines. II. Somatic meiosis, Plant Cell Tiss Org Cult 30, 1992b, 115–119. DOI : http://dx.doi.org/10.1007/bf00034304

Phillips RL, Kaepplert SM and Olhof P, Genetic instability of plant tissue cultures: Breakdown of normal controls. Proc. Nati. Acad. Sci. USA 91, 1994, 5222-5226.

Saker MM, Adawy SS, Mohamed AA and El-Itriby HA, Monitoring of cultivar identity in tissue culture-derived date palms using RAPD and AFLP analysis. Biologia Plantarum 50(2), 2006,198-204. DOI: http://dx.doi.org/10.1007/s10535-006-0007-3

Smulders MJM and de Klerk GJ, Epigenetics in plant tissue culture. Plant Growth Regulation 63(2), 2011, 137-146. DOI: http://dx.doi.org/10.1007/s10725-010-9531-4

Song Y, Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide, Journal of Integrative Plant Biology 56(2), 2014, 106–113. DOI: http://dx.doi.org/10.1111/jipb.12131

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K, Russell SM, Cicchillo RM and Peterson MA, Simpson DM, Zhou N, Ponsamuel J, Zhang Z, Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes, Proc Natl Acad Sci USA 107, 2010, 20240–20245. DOI: http://dx.doi.org/10.1073/pnas.1013154107

 DOI: 10.18380/SZIE.COLUM.2014.1.2.109

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GYURICZA Csaba, editor-in-chief

PhD /agric/, dean of the the Faculty of Agricultural and Environmental Sciences of the Szent István University, Gödöllő, Hungary, member of the Soil Science, Water Management and Crop Production Committee of the Hungarian Academy of Sciences. Professional fields: crop production and soil tillage.

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.