THE EFFECT OF SALINITY ON THE CHLOROPHYLL CONTENT OF WHEAT

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Abstract

Abiotic stress caused by salinity soil affects the development and plant growth, seed production and quality in a negative way. Salinity is an important factor influencing the quality of water. Saline stress causes negative reactions in plants both morphologically, biochemically and physiologically. The chlorophyll content is an important indicator of photosynthetic capacity of plants. Amount of chlorophyll is very influenced by nutrients reserve and by environment stress. Salinity in soil occurs as a result of the factors such as improper irrigation, lack of drainage, excessive accumulation of soluble salts. The research aims to study the behavior of an assortement of wheat varieties in terms of variability in salinity tolerance. To this end, an indirect test method for salinity tolerance, based on the determination of the influence of saline stress on chlorophyll accumulation, was pursued. Determination of chlorophyll content was performed at 7, 14, 21 days after saline stress induction. The duration of stress had the highest contribution (41.72%) to the variability of the chlorophyll content, followed by the saline (10.88%) and the varieties 7.63% respectively. At the level of the whole experiment it is observed that the chlorophyll content decreased progressively as saline stress prolongation. The change in the concentration from 200 to 240 Mm showed the highest influence on this property, materialized by a significant decrease in photosynthetic capacity. A good way to understand the plant photosynthetic regime is to determine the chlorophyll content as an indirect method. A selection criterion in screening for salt tolerance can be considered the physiological features that are positively associated with production under conditions of saline stress.
Keywords: *chlorophyll content, saline stress, wheat*
JEL Code: *Q19, Q51*

Introduction

Wheat is one of the most important crops and is usually grown with the right amount of nitrogen to get a great deal of production. Therefore, nitrogen content is an important indicator of the level of plant nutrition for autumn wheat (ARANYA et al. 2003).Studies have shown that chlorophyll content in plants has been positively correlated with nitrogen content (FILELLA et al.1995). Thus, the value of leaf chlorophyll content can help understand the nutrient status of the plant and scientifically guide fertilization management to ensure good crop quality and yield (GRATTAN et.al. 1998, MENESATTI et al. 2010). This practice is of great importance for modern precision agriculture. The use of physiological traits as an indirect selection would be important in increasing the effectiveness of yield based selection procedures (KIANI POUYA et al. 2014).

To study the effects of biotic and abiotic stress on plant growth and productivity, numerous physiological parameters can be exploited in various fields of plant science, such as agriculture, agronomy, forestry and horticulture. Some physiological aspects, such as plant photosynthetic efficiency, have begun to be investigated in plant breeding programs to assess crop performance when grown under unfavorable environmental conditions. The chlorophyll fluorescence is one of these parameters indicating the plant's ability to convert light energy into biochemical energy during the photosynthetic process. The advantages of this technique are that it is non-invasive, non-destructive and quickly measured using extremely portable equipment (HAZEM et al. 2008). Studies on relative chlorophyll content in non-destructive testing are mainly focused on measuring the value of chlorophyll (SPAD and Spatial Development Analysis, SPAD), which estimates the relative chlorophyll content by collecting the mean of all values of a SPAD from a point repeatedly measured. The SPAD values express the relative amounts of chlorophyll in the crop leaves and have been demonstrated in several studies. (CAMEN et al. 2017, HUANG et al. 2010, HUANG et al. 2014, SHI et al. 2011, YANG et al. 2009). Direct and indirect methods may be used to investigate primary ecological production. In practice are often used indirect methods for an approximate estimate of the value of organic production, because it is quite difficult to get uses direct methods. Using indirect methods, it is possible to monitor and measure all phenomena and processes related to productivity (ARANYA et al. 2003, KOF et al. 2004, RAYNOLDS et al. 2000). Chlorophyll content is one of the clues in photosynthetic activity (LARCHER et al. 1995). Has special significance for agriculture being considered an indicator of photosynthetic activity. During the growing period, significant changes are associated with the crop reflection spectra with the accumulation and destruction of plant pigments, mainly chlorophyll (JORGENSEN et al. 2006, BROGE et al. 2002).

Material and methods

The biological material was represented by a collection of wheat which had different origins, from Romania and from different European countries, to have as much genetic variation as possible. The varieties were chosen from those cultivated in the area over time. Some of them have been remarked by good productivity, others by the good quality of production. In the laboratory, using saline stress solution (with NaCl), it is possible to test plant salinity tolerance. Seeds of wheat were cultivated in pots containing soil, peat and sand in the ratio of (1:1:1) and grown under green house conditions. Temperature in the green house was 30±2°C during day and 25±2°C at night with relative humidity 50% and a photoperiod of 14 h. The plants were grown under normal conditions (V0-water) and stress conditions (V1-150 mM NaCl, V2-200 mM NaCl, V3-240 mM NaCl).

To determine the chlorophyll content of the leaves was used the portable chlorophilmeter SPAD-502 (Konica Minolta), which measures the absorbance at 650 nm, this being a non-destructive method. Three readings were made on each leaf, the results being expressed in SPAD units. The chlorophyll content of the leaves was deternined after 7 days, 14 days and 21 days from stress induction.

In order to determine the difference between varieties, the statistical processing of the data obtained was done by variance analysis and the t test, for bi- and trifactorial experiences. The meanings of differences between varieties and other graduations of the factors were represented both by symbols (*; **; ***; 0; 00; 000) and by letters; the differences between variants marked with different letters.

Results and discussion

Considering the analysis of variance components (*Table 1*), it is observed that the three sources, namely the period, the concentration of saline stress and the variety, showed a real and statistically assured influence on the chlorophyll content. The period of stress had the highest contribution (41.72%) to the variability of the chlorophyll content, followed by the saline (10.88%) and the varieties 7.63% respectively. The simple interactions between the three factors manifested distinctly significant influences on this character, emphasizing the combined effect of duration and concentration of saline stress (5.28%). The sources of variation related to the studied factors showed a combined influence of 72.45% on the variability of the chlorophyll content. Also, significant variations in chlorophyll content were found in different portions of the foliage, since the upper parts of the leaves were higher than in the basal part of the leaves.

Table 1. Variance analysis for the effect of period and concentration of salt stress on chlorophyll content for different wheat varieties

Source of variation	SP	GL	S^2	F Test
Total	12395.18	191		
Repetition	479.09	2	239.55	17.14*
Period	2113.25	2	1056.63	75.61**
Error period	55.90	4	13.98	
Saline treatement	456.50	3	152.17	19.72**
Period x Treatment	443.02	6	73.84	9.57**
Error treatment	138.90	18	7.72	
Varieties	1578.56	13	121.43	13.82**
Period x Varieties	1625.11	26	62.50	7.11**
Treatment x Varieties	971.34	39	24.91	2.83**
Period x Treatment x Varieties	1792.02	78	22.97	2.61**
Error varieties	2741.50	312	8.79	

Source: own data

At the level of the whole experiment it is observed that the chlorophyll content decreased progressively as saline stress prolongation (*Table 2*). Thus, during the first period there was a small and insignificant variation (-0.37) of the chlorophyll content, equivalent to a rate of -0.053 / day. In the second period, with the prolongation of stress, the salinity effect is increased resulting in a very significant decrease of chlorophyll with an intensity (0.53 / day) net superior to the previous period.

Table 2. Average chlorophyll content for several periods after the salt stress

Period	***chlorophyll content* (SPAD)**		**Relative value (%)**	**Difference/ Signification**
14 days – 7 days	32.78	33.15	98.88	-0.37
21 days – 7 days	29.07	33.15	87.69	-4.08^{000}
21 days – 14 days	29.07	32.78	88.68	-3.71^{000}

$LDS_{5\%}$=1.13 $LDS_{1\%}$=1.88 $LDS_{0,1\%}$=3.51

Source: own data

Considering the unilateral influence of saline treatment (*Table 3*), mean values of chlorophyll content were recorded between 32.70 for untreated variants and 30.78 for the 240 mM concentration, with amplitude of 1.92.

Table 3. The effect of saline treatment concentration on the chlorophyll content in wheat

Treatment (mM NaCl)	*Chlorophyll content* (SPAD)		Relative value (%)	Difference/ Signification
150 - 0	31.89	32.70	97.52	-0.81^{0}
200 - 0	31.30	32.70	95.72	-1.40^{000}
240 - 0	30.78	32.70	94.13	-1.92^{000}
200 - 150	31.30	31.89	98.15	-0.59
240 - 150	30.78	31.89	96.52	-1.11^{00}
240 - 200	30.78	31.30	98.34	-0.52

$LDS_{5\%}$=0.74 $LDS_{1\%}$=1.01 $LDS_{0,1\%}$=1.37

Source: own data

The NaCl concentration had a negative effect on the chlorophyll content, causing a gradual and significant reduction of it at a rate of 0.005/mM between the first two treatments to 0.048mM between the last two treatments. As such, the intensity of stress action on this property was proportional to the increase in NaCl concentration. Average values of chlorophyll content recorded by the 14 varieties (*Table 4*) showed amplitude of 4.91, with the limits of 29.11 in the case of Esperia variety up to 34.02 in the Glosa variety, amid a medium interpopulation variability (15.74%). The most common values of these characteristics were 30-32.5. Compared to the average of the experience, the Glosa, Solehio, Cubus and Exotic varieties showed significantly higher chlorophyll of 1.49-2.35. For Capo and Cerere varieties, the values of this attribute were significantly lower.

The Glosa variety has accumulated a significantly higher amount of chlorophyll than 10 of the varieties, associated with relative spores ranging from 1.52 to Alex and 4.91 relative to Esperia. It is also noted that the Solehio, Exotic and Cubus varieties showed significantly more photosynthetic capacity than eight of the remaining varieties. Against a lower tolerance to saline stress, Cere, Capo and Esperia varieties recorded significantly lower chlorophyll content than most other varieties.

Considering the effect of the period and concentration of saline treatment on the chlorophyll content (*Table 5, Fig. 1*), it was observed that with the use of concentrations of 150 and 200 mM NaCl the photosynthetic capacity of plants was progressively reduced with prolonged stress duration. Thus, at the end of the experiment after 21 days of stress the content of chlorophyll was significantly lower than the values recorded in the first two weeks. The effect of stress duration was more intense when using 200 mM NaCl.

In the absence of saline stress, there is a significant change in chlorophyll content from one week to another, with the highest value after 14 days and the lowest at the end.

Due to the use of the 240 mM NaCl concentration, an intense stress action is observed during 7-14 days, resulting in a significant reduction in chlorophyll content, after which the intensity of the stress diminishes so that the photosynthetic capacity varies insignificantly.

After seven days of saline stress, the chlorophyll content ranged between 32.36 for the 240 mM concentration and 33.94 for the 200 mM NaCl concentration, with an insignificant variation up to a stress level of 200 mM NaCl. The negative salinity effect was more pronounced by altering the NaCl concentration from 200 to 240 mM, resulting in a significant reduction in chlorophyll content.

Table 4. Average chlorophyll content for studied wheat varieties

No	Varieties	Chlorophyll content (SPAD) $\bar{x} \pm s_{\bar{x}}$	$s_{\%}$	Relative value (%)	Signification of difference
	Average of experience	31.67±0.22	15.74	100	Control
1	**Alex**	32.50±0.80 bcd	14.70	102.63	0.83
2	**Glosa**	34.02±0.85 a	15.05	107.43	2.35***
3	**Esperia**	29.11±0.78 f	16.10	91.93	-2.56^{00}
4	**Capo**	29.48±1.32 f	26.85	93.10	-2.19^{00}
5	**Josef**	32.05±0.57 cd	10.62	101.21	0.38
6	**Cerere**	30.00±1.03 f	20.51	94.74	-1.67^{0}
7	**Genesi**	31.61±0.46 de	8.65	99.82	-0.06
8	**Apache**	32.46±0.96 bcd	17.80	102.51	0.79
9	**Soissons**	30.38±0.46 ef	9.11	95.94	-1.29
10	**Exotic**	33.18±0.89 abc	16.19	104.78	1.51*
11	**Solehio**	33.60±0.58 ab	10.43	106.11	1.93**
12	**Zephyr**	30.39±0.67 ef	13.20	95.97	-1.28
13	**Cubus**	33.16±0.71 abc	12.81	104.72	1.49*
14	**Calisol**	31.39±0.67 de	12.89	99.13	-0.28

$LDS_{5\%}$=1.38 $LDS_{1\%}$=1.81 $LDS_{0,1\%}$=2.32

Source: own data

Table 5. The effect of period and concentration of salt stress on chlorophyll content in wheat

Concentration (mM NaCl)	Period 7 days	14 days	21 days	$\bar{x} \pm s_{\bar{x}}$	s%
0	y 32.99 ab	x 35.02 a	z 30.08 a	32.70±0.42	14.55
150	x 33.32 ab	x 32.39 b	y 29.97 a	31.89±0.41	14.51
200	x 33.94 a	x 32.95 b	y 27.02 b	31.30±0.51	18.40
240	x 32.36 b	y 30.76 c	y 29.21 a	30.78±0.40	14.79
$\bar{x} \pm s_{\bar{x}}$	33.15±0.31	32.78±0.34	29.07±0.41	31.67±0.22	
s%	12.14	13.55	18.41	15.74	

Period - $LDS_{5\%}$=1,40 $LDS_{1\%}$=1.92 $LDS_{0,1\%}$=2.62 (x,y,z,)

Concentration - $LDS_{5\%}$= 1.27 $LDS_{1\%}$=1.75 $LDS_{0,1\%}$=2.38 (a,b,c,)

Source: own data

Stress extension up to 14 days caused an increase in its effect on plant photosynthetic capacity, given the high amplitude (4.26) of the four variants. Thus, there is a significant reduction in the chlorophyll content under conditions of use of 150 mM NaCl. Subsequently, increasing the concentration to 200 mM caused an insignificant variation of this property. The change in the concentration from 200 to 240 mM showed the highest influence on this property, materialized by a significant decrease in photosynthetic capacity.

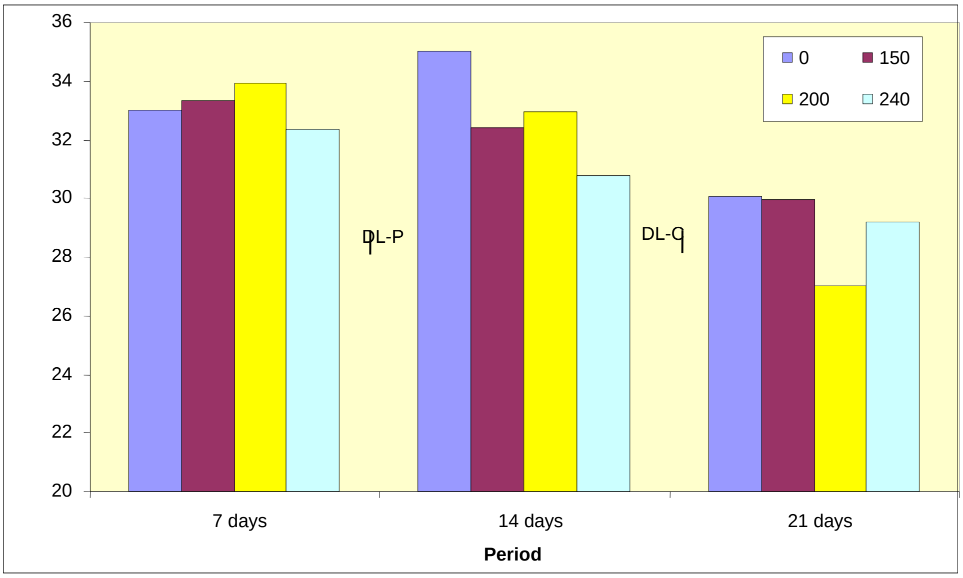

Figure 1. The chlorophyll content in wheat under different period and concentration of salin stress

Source: own data

At the end of the study, the amplitude of the treatments was intermediate for the other two periods, with the limits of 27.02% for the 200 mM to 30.08 concentrations for saline untreated variant. The differences between the four treatments were lower than in the previous period, but statistically ensured. Saline stress led to a significant reduction in chlorophyll content when applying a 200mM concentration, while deviations from the other variants were lower and statistically uninsured.

Regarding the effect of the period of the treatment and the variety on the chlorophyll content (*Table 6, Figure 2*), it was observed that after seven days between varieties a variation amplitude of 6.11 was recorded with values between 30.5 for Soissons variety and 36.61 in Exotic, on the background of a medium interpopulation variability. Most of the varieties (71%) presented statistically undifferentiated values of this characteristic of 30.5-34.

The Exotic variety presented a photosynthetic capacity significantly superior to the other varieties with over 4.64. A high value of this characteristic was also observed in the Glosa variety (36.12), associated with significant increases of 4.32-5.62 compared to the Josef, Alex, Experia and Soisons varieties. After two weeks of stress, the 14 varieties showed chlorophyll content ranging from 29.72 to Esperia and 36.68 to Glosa, with amplitude of 6.96 and an intergenotipic variability of 13.55%. The Glosa variety manifested the highest tolerance to saline stress at this time, recording significantly higher chlorophyll content than Genesi, Calisol, Soissons, Zephyr and Esperia.

At the end of the experiment, the variability (18.41%) and the amplitude (10.46) of the chlorophyll content of the varieties were superior to the previous periods, on the background of a more distinct differentiation of varieties in terms of their tolerance to saline stress.

Regarding the effect of the period of saline stress on the chlorophyll content in each variety, it is observed that in most of them the prolongation of the stress period did not cause significant variations in the photosynthetic capacity.

Table 6. The effect of salin stress period and variety on the chlorophyll content in wheat

Varieties	Period		
	7 days	14 days	21 days
Alex	x 31.27 d	x 33.21 abcd	x 33.01 a
Glosa	x 36.12 ab	x 36.68 a	y 29.28 abcd
Esperia	x 30.91 d	x 29.72 d	x 26.70 de
Capo	x 33.02 abcd	x 32.88 abcd	y 22.55 e
Josef	x 31.80 cd	x 32.40 abcd	x 31.96 ab
Cerere	x 34.06 abcd	x 32.89 abcd	y 23.06 e
Genesi	x 31.97 bcd	x 31.98 bcd	x 30.88 abcd
Apache	x 35.64 abc	x 34.15 abc	y 27.60 cd
Soissons	x 30.50 d	x 31.24 bcd	x 29.39 abcd
Exotic	x 36.61 a	x 34.58 ab	y 28.35 bcd
Solehio	x 33.96 abcd	x 33.89 abcd	x 32.95 a
Zephyr	x 32.31 abcd	x 30.24 cd	x 28.61 bcd
Cubus	x 34.11 abcd	x 33.53 abcd	x 31.85 abc
Calisol	x 31.86 bcd	x 31.55 bcd	x 30.78 abcd
$\bar{x} \pm s_{\bar{x}}$	33.15±0.31	32.78±0.34	29.07±0.41
s%	12.14	13.55	18.41

Period- $LDS_{5\%}$=4.16 LDS $_{1\%}$=5.49 LDS $_{0,1\%}$=7.05 (x,y,z,)
Varieties - LDS $_{5\%}$=4.32 LDS $_{1\%}$=5.70 LDS $_{0,1\%}$=7.31 (a,b,c,)
Source: own data

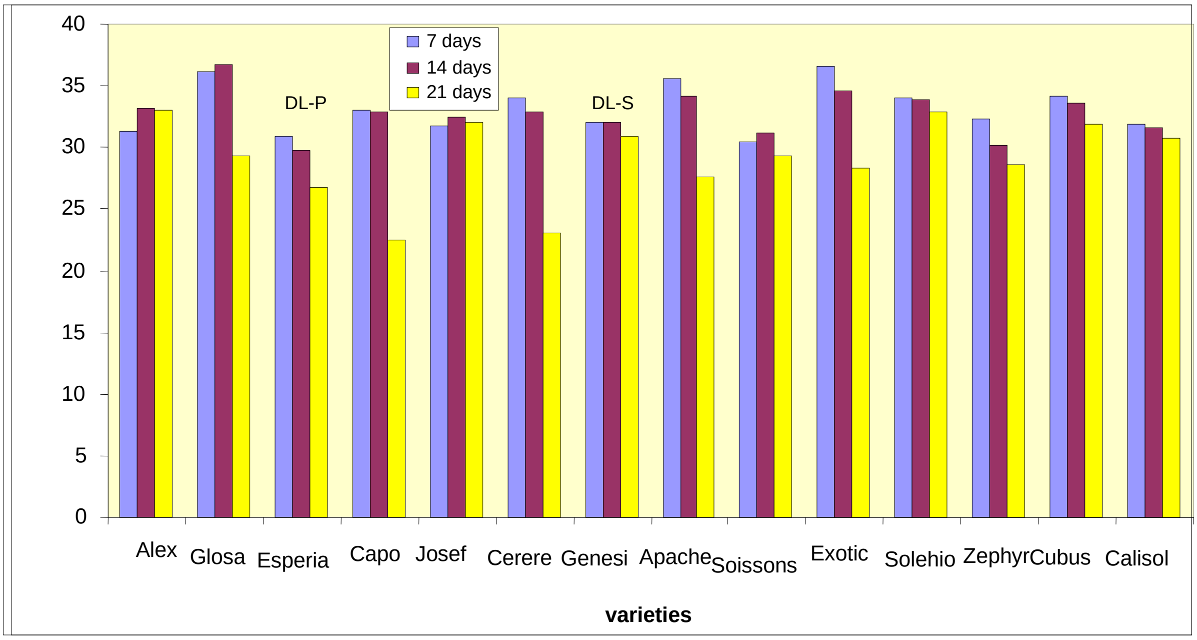

Figure 2. The chlorophyll content of wheat varieties for several periods after salin stress
Source: own data

From the point of view of the combined effect of salin stress concentrations and variety on chlorophyll content (*Table 7*), the highest amplitude variation between genotypes (9.58) was recorded at the 240mM concentration, while amplitude was lower compared to the concentration of 200mM (4.78).

Table 7. The effect of salin stress concentration and variety on the chlorophyll content in wheat

Variety	Concentration (mM NaCl)			
	0	150	200	240
Alex	x 35.33 ab	x 29.23 b	x 30.41 a	x 35.02 a
Glosa	x 33.05 ab	x 36.84 a	x 33.01 a	x 33.20 ab
Esperia	x 30.70 b	x 31.03 ab	x 29.26 a	x 25.44 d
Capo	x 29.63 b	x 29.77 b	x 28.23 a	x 30.30 abcd
Josef	x 32.10 ab	x 32.13 ab	x 32.24 a	x 31.75 abc
Cerere	x 30.79 b	x 28.72 b	x 28.64 a	x 31.86 abc
Genesi	x 32.91 ab	x 31.32 ab	x 32.14 a	x 30.05 abcd
Apache	x 33.73 ab	x 32.21 ab	x 31.50 a	x 32.42 abc
Soissons	x 30.65 b	x 30.81 ab	x 31.92 a	x 28.12 bcd
Exotic	x 34.61 ab	x 33.58 ab	x 32.25 a	x 32.27 abc
Solehio	x 35.25 ab	x 33.66 ab	x 32.90 a	x 32.57 abc
Zephyr	x 30.38 b	x 32.21 ab	x 32.29 a	x 26.67 cd
Cubus	x 36.96 a	x 32.82 ab	x 31.97 a	x 30.90 abcd
Calisol	x 31.66 ab	x 32.17 ab	x 31,48 a	x 30.27 abcd
$\bar{x} \pm s_{\bar{x}}$	32.70±0.42	31.89±0.41	31.30±0.51	30.78±0.40
s%	14.55	14.51	18.40	14.79

Concentration - $LDS_{5\%}$=6.11 LDS $_{1\%}$=8.05 LDS $_{0,1\%}$=10.33 (x,y,z,)
Varieties - LDS $_{5\%}$=6.11 LDS $_{1\%}$=8.05 LDS $_{0,1\%}$=10.34 (a,b,c,)
Source: own data

Conclusion

- The NaCl concentration had a negative effect on the chlorophyll content, causing a gradual and significant reduction of it at a rate of 0.005/mM between the first two treatments to 0.048mM between the last two treatments.
- The use of the 240 mM NaCl concentration, an intense stress action is observed during 7-14 days, resulting in a significant reduction in chlorophyll content, after which the intensity of the stress diminishes so that the photosynthetic capacity varies insignificantly.
- The duration of saline stress was the highest contribution (41.72%) to the variability of chlorophyll content, followed by the salinity (10.88%) and the variety (7.63%) respectively.

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The lecture was delivered at the 5th International Scientific Conference on Water on 22-24. March 2022 in Szarvas, Hungary.

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