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Original scientific article

ANTIOXIDANT VARIABILITY OF WHEAT GENOTYPES UNDER SALINITY STRESS *in situ*

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Crop cultivation under the open-field conditions depends on a variety of biotic and abiotic factors which cause plant stress and deterioration. Due to high soil salinity, some soils can be an unfavourable growing environment for most plants. As a low productivity soil type, solonetz soils are a stressful growing environment, causing plant deterioration. Plants have developed a complex antioxidative defense system as a precaution against oxidative stress caused by high soil salinity. The trial was set up on a halomorphic soil type–solonetz. The research included ten cultivars, one local population of hexaploid wheat (*Triticum aestivum* ssp. *aestivum* L.) and one cultivar of triticale (*Triticosecale* W.). The activity of enzymatic and non-enzymatic antioxidants in plant antioxidative defense system was detected during the trial, as well as lipid peroxidation. The analysis of biochemical markers was done in the flowering stage, and then in the milk maturity phase. Research results of the tested components revealed the highest stress tolerance exhibited by genotypes Banatka and Bankut 1205. Understanding the process of oxygen radical production by the plant tissue contributes to breeding wheat cultivars for better stress tolerance. Selection of genotypes better adapted to growing conditions in solonetz soils could facilitate a more economically

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justifiable wheat production, and promote utilization of the lower-quality soil types in agriculture.

Keywords: wheat, solonetz, abiotic stress, antioxidative defense system

INTRODUCTION

The effect of the natural environment on plant production has caused this economic branch to be very specific in its realization. Cultivation of plants in the open field depends on a number of biotic and abiotic factors, which cause stress in plants and lead to their deterioration. In contrast to biotic stress, caused by the negative impact on plants by microorganisms, phytopathogenic fungi, insects and other living organisms, abiotic stress is defined as the negative effect of temperature, air, and water, as well as physical and chemical properties of the soil, on plant growth and development. Due to high concentrations of salt, soil can be unfavorable environment for most cultivated plants. According to BRAY *et al.* (2000), soil salinization reduces potential yield of the most important crops by more than 50%. Crop development should therefore aim to achieve higher tolerance to soil salinity (BANJAC *et al.*, 2014). Increased saline concentrations in the soil lead to major damage to the plant cells and tissues, resulting in major changes in normal functioning of plant metabolism. Stress caused by high salt concentrations leads to disorders in the transport of electrons in certain cell organelles, creating reactive oxygen species that cause lipid peroxidation, oxidative protein degradation and DNA mutations (ESFANDIARI *et al.*, 2007). Solonetz, as a soil of poor productivity, represents a stressful growing environment for plants because increased concentrations of different types of salt can lead to oxidative stress in plants. Although oxygen is vital for all aerobic organisms, it can also be toxic at higher concentrations. Toxic effect can be explained by reactive oxygen species (ROS), including activated and reduced oxygen species, whose accumulation in plant organism leads to oxidative stress. Research needs to consider the extent to which the cultivation of wheat on solonetz leads to plant deterioration, and how antioxidant protection system helps plants to preserve normal metabolic processes, complete growth, and achieve yield. Oxygen radicals can lead to a number of metabolic disorders in living cells. Due to major damage, the cell is vulnerable without a developed protection mechanism. The amount of free radicals in plant cells is under constant control of antioxidant enzymes: superoxide dismutase, catalase, peroxidase, glutathione reductase, and others. According to GILL *et al.* (2015), superoxide-dismutase (SOD) plays a crucial role in combating oxidative stress caused by different influences. The authors also point to the importance of peroxidase and emphasize that, depending on the type of substrate, different types of enzymes occur glutathione peroxidase, cytochrome c-peroxidase, NADH peroxidase, and others. In addition, phenylalanine ammonium lyase (PAL) is an enzyme that is widely represented in the cells of higher plants and plays a significant role in conservation of plant organisms against oxidative stress (ZHANG and LIU, 2015). Reduced glutathione content (GSH) in the cells is controlled by glutathione reductase. This bond enables normal protein metabolism in the cell because milimolar concentrations of GSH play a key role in protecting protein *cys* groups from ROS. Examination of the influence of abiotic stress (high temperature) on wheat germ, a multiple increase in GSH in relation to control was observed (HASANUZZAMAN *et al.*, 2017). All of these enzymes and compounds constitute a system that maintains reactive oxygen species in low concentrations in cells and tissues and thus prevents oxidative stress formation (KELES and ONCEL, 2002). Understanding the way in which oxygen radicals are formed and disappear from the plant tissue contributes to the development of new, more stress tolerant wheat varieties. The aim of the research is to investigate the behavior of the antioxidant protection system, in order to examine the influence of stress on plants caused by wheat cultivation on halomorphic soils such as solonetz.

Selection of genotypes better adapted to growing conditions in solonetz soils could facilitate a more economically justifiable wheat production, and promote utilization of the lower-quality soil types in agriculture.

MATERIAL AND METHODS

Field exams

The research included 12 genotypes, of which 10 varieties and one local population of hexaploid (2n=6x=42) wheat (*Triticum aestivum* ssp. *aestivum* L.) and one variety of triticale (*Triticosecale* W.). Of the wheat varieties examined, eight are winter varieties (Renesansa, Pobeda, Europe 90, NSR-5, Dragana, Rapsodija, Simonida and Cipovka), while Nevesinjka is a facultative variety. The triticale is genotype Odisej. The study included two older wheat genotypes which were present in the experimental area during the last century (Banatka and Bankut 1205). These genotypes were selected based on previous studies of existing genetic variability. They are differ in their tolerance to stressful growing conditions and broader adaptability to abiotic stress. The experiment was set up in Banat (Pannonian basin, country Serbia) at the Kumane site (latitude: 45,539ºN, longitude 20,228ºE and 72m altitude), in a halomorphic soil of the solonetz type, at the sample surface of 2ha, by random block system in three repetitions. Solonetz is a soil type in which the exchanger complex has more than 15% of adsorbed sodium ions-Na^+, so it is alkalized (pH> 9) and unfavorable for cultivation of agricultural plants. Production potential of solonetz is significantly limited by the heavy mechanical composition of the compact and impermeable $B_{t,na}$ horizon. Worldwide, solonetz cover some 135 million ha and major areas are found in Europe (IUSS Working Group WRB, 2006).

Laboratory exams

The analysis of biochemical markers was done in the flowering stage, and then in the milk maturity phase. Flag leaf was used for analysis in both phenological phases. Extraction from the fresh plant material was carried out as follows: 1g of leaf was measured and homogenized in a porcelain mortar, with the addition of 10ml of phosphate buffer KH_2PO_4 (pH=7) and a little quartz sand. The resulting homogenate was quantitatively transferred to a plastic tube and centrifuged for 10 min at 4500 O s^{-1}. The resulting supernatant was used as an extract to determine the activity of the antioxidant enzymes. When dry material was used extraction method was as follows: 0.2g of dry plant material was weighed, frittered, triturated with Erlenmeyer with 10 ml of 70% acetone, and left for 24h in the dark for photosensitive compounds. After 24 hours, the content was filtered through a filter paper, and left in a cold and dark place until use. Compounds of the enzymatic and non-enzymatic antioxidant system were determined by spectrophotometric methods, using a UV/VIS spectrophotometer (model 6105, Jenway, Dunmon, UK).

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined by the BEAUCHAMP and FRIDOVICH (1971) method, based on the ability to inhibit the photochemical reduction of nitroblutetrazolium chloride (nitroblue tetrazolium - NBT). Guaiacol peroxidase (GPx, EC 1.11.1.7) was determined by the CHANCE and MAEHLY (1955) method. Pirogalol peroxidase (PPx, EC 1.11.1.7) was determined by the KAR and MISHRA (1976) method. The method for determining the activity of phenylalanine ammonium lyase (PAL, ES 4.3.1.24), by GERASIMOVA *et al.* (2005), is based on deamination of L-phenylalanine. The amount of reduced glutathione (GSH) was measured by colored reaction of non-protein thiol groups (-SH) in the presence of DTNB (5,5-dithiobis [2-nitrobenzoic acid]), SEDLAK and LINDSAY (1968). The amount of total phenols was determined by the Folin-Ciocalte method, which is based on reductive capability of phenolic compounds (HAGERMAN *et al.*, 2000). The content of total tannins was determined due to being bound by polyvinylpolypyrrolidone (PVPP).Total non-enzymatic activity of the tested samples was determined by LEE *et al.* (1998), which is based on the difference in the activity of DPPH radical (1,1-diphenyl-2-picrylhydrazy) removal between the blind and the workout. The intensity of lipid peroxidation (LP) is determined according to the content of malondialdehyde (MDA), extracted from fresh plant material using a mixture of thiobarbiturone (TBA) and trichloroacetic acid (TCA) acid (PLACER *et al.*, 1966). The content of soluble proteins was determined by the Bradford method (SEDMARK and GROSSBERG, 1977; SPECTOR, 1978) based on binding of the Coomassie blue G-250 to base and aromatic amino acid residues in the protein.

Statistical tools

Mean value, standard deviation, standard error of mean and coefficient of variation were calculated for all biochemical markers. Also, ANOVA and Duncan's multiple range post-hoc test was performed. Statistical data processing was done in statistical program Statistica 13 (Dell).

RESULTS

Enzymatic components of the antioxidant system

Superoxide-dismutase (SOD) is a common name for a group of metalloenzymes that catalyze the reaction of dismutation $O_2^{\cdot-}$ to H_2O_2 and O_2 (SCANDALIOS, 1993). Chain reaction of other reactive oxygen species, the most active being hydrocyl radical (MILLER, 2011), is further initiated. By the action of other enzymatic components of the antioxidant system, the resulting H_2O_2 is neutralized.

The results of this study showed significant differences in the activity of SOD between the investigated genotypes suggesting that the genotypes reacted differently to the conditions of stress caused by breeding on a solonetz, Table 1.

The highest activity of SOD was observed in the Nevesinjka genotype (10727.97 U g^{-1} fresh plant material), while the lowest was in the genotype Pobeda (5925.93 U g^{-1} fresh plant material). Statistically significant differences in the activity of this enzyme were not observed only between Simonida and Bankut 1205, but also between Renesansa and Evropa 90. In addition, SOD activity was significantly higher in Simonida and Bankut 1205 than in the other two genotypes, Table 1.

Peroxidases are enzymes from the oxido-reductase group that catalyze oxidation of various substrates in the presence of peroxide H_2O_2 (POPOVIĆ and ŠTAJNER, 2008). These enzymes are present in a large number of isoenzymes in various tissues and cellular organs.

Examination of peroxidase activity showed significant differences between genotypes when pirogalol peroxidase (PPx) was observed, but there were differences in the activity of guaiacol peroxidase (GPx), Table 1.

The highest activity of guaiacol peroxidase was observed in the Cipovka genotype (20.68 U g^{-1} fresh plant material), whereas the lowest was in the genotype Renesansa (7.00 U g^{-1} fresh plant material). It should also be emphasized that only genotype Renesansa differed statistically from other genotypes.

The genotypes with the highest activity of PPx were Banatka (7106.76 U g^{-1} fresh plant material) and Rapsodija (7102.97 U g^{-1} fresh plant material). They statistically differed significantly from other genotypes. The lowest activity of PPx in the antioxidant system was observed in the Renesansa genotype (2533.39 U g^{-1} fresh plant material). Higher activity of PPx, were also observed in genotypes Odisej, Bankut 1205 and Pobeda, Table 1.

Considering the results of enzymatic components, at the level of the whole sample and in accordance with the values of the variation coefficient, a conclusion is drawn that the activity of PPx was the most variable (33.28%), followed by SOD activity (31.53%) and GPx activity (22.77%), Table 1.

Table 1. Activity of superoxide dismutase (SOD), guaiacol peroxidase (GPx) and pirogalol peroxidase (PPx) in flag leaves of the tested genotypes during the flowering

Genotypes	*SOD* *U g^{-1} fpm**	*GPx* *U g^{-1} fpm**	*PPx* *U g^{-1} fpm**
Renesansa	6590.04^{g**}	7.00^{b}	2533.39^{c}
Pobeda	5925.93^{h}	15.89^{a}	3091.30^{c}
Evropa90	6564.50^{g}	19.42^{a}	4440.54^{bc}
NSR-5	6871.01^{f}	17.93^{a}	4201.43^{bc}
Dragana	3320.56^{j}	13.74^{a}	4116.04^{bc}
Rapsodija	4086.85^{i}	13.58^{a}	7102.97^{a}
Simonida	9987.23^{b}	16.94^{a}	5859.99^{ab}
Cipovka	8480.20^{d}	20.68^{a}	5776.50^{ab}
Banatka	8122.61^{e}	16.72^{a}	7106.76^{a}
Bankut1205	9961.69^{b}	19.12^{a}	3091.30^{c}
Nevesinjka	10727.97^{a}	19.26^{a}	5757.52^{ab}
Odisej	9501.92^{c}	19.46^{a}	3522.07^{c}
Mean	7511.71	16.64	4716.65
Standard deviation	2368.63	3.79	1569.62
Standard error of mean	683.76	1.09	453.11
Coefficient of variation (%)	31.53	22.77	33.28

**fresh plant material*

** *results in the same column marked with different letters indicate significant differences between genotypes at P<0.05 (based on Duncan's multiple range test)*

Activities phenylalanine ammonium lyase

Phenylalanine ammonium-lyase (PAL) is a key enzyme in the synthesis of phenolic compounds, because it catalyses the deamiantion of *L*-phenylalanine into *trans*-cinnamic acid, which is the first step in the phenylpropanoid biosynthesis. PAL represents an important regulatory point between primary and secondary metabolism. Phenolic compounds that are synthesized by PAL catalysis play an important role in the protection of plants from various injuries and attacks of pathogens. First of all, this refers to lignins, a part of cell walls providing firmness to plants (HUANG *et al.*, 2010).

The highest activity of phenylalanine ammonium lyase was observed in genotype Rapsodija (6,63 U g^{-1} fresh plant material), followed by the genotypes Bankut 1205 (6,38 U g^{-1} fresh plant material) and local population Banatka 6.20 U g^{-1} fresh plant material). The three genotypes did not reveal a significant statistical difference. Greater activity of this enzyme in Banatka and Bankut 1205 can be related to better adaptability to the conditions of the examined area. The lowest PAL activity was observed in genotypes Nevesinjka (4.10 U g^{-1} fresh plant material), NSR-5 (4.28 U g^{-1} fresh plant material) and Simonida (4.50 U g^{-1} fresh plant material), Table 2.

Table 2. Activity of phenylalanine ammonium lyase (PAL) in flag leaves of the tested genotypes during flowering

Genotypes	*PAL*U g^{-1} fpm*
Renesansa	5.04^bc**
Pobeda	4.89^c
Evropa90	4.78^c
NSR-5	4.28^c
Dragana	4.99^bc
Rapsodija	6.63^a
Simonida	4.50^c
Cipovka	6.01^ab
Banatka	6.20^a
Bankut1205	6.38^a
Nevesinjka	4.10^c
Odisej	5.18^bc
Mean	5.25
Standard deviation	0.85
Standard error of mean	0.25
Coefficient of variation (%)	16.20

*fresh plant material

** *results in the same column marked with different letters indicate significant differences between genotypes at P<0.05 (based on Duncan's multiple range test)*

Non-enzymatic components of the antioxidant system in the stages of flowering and milk maturity
Reduced glutathione content

The investigated genotypes differed significantly in the content of reduced glutathione. Genotype with the highest average GSH content was Banatka (5.10 µmol GSH g^{-1} fresh plant material). Considering this component of the antioxidant system, a conclusion is drawn that Evropa 90 and Dragana were the most damaged by breeding on solonetz, given the significantly lower average GSH content from other genotypes (2.42 and 2.65 µmol GSH g^{-1} fresh plant material), Table 3.

Table 3. The content of reduced glutathione (GSH), total phenols, tannins and DPPH test in flag leaf extraction in the tested genotypes during flowering

Genotypes	*GSH* µmol GSH g^{-1} fpm*	*Phenols* mg catechin g^{-1} fpm*	*Tannins* mg catechin g^{-1} fpm*	*DPPH test* % of neutralized radicals
Renesansa	3.26^{cdef**}	2595.01^{a}	1944.07^{a}	25.40^{bc}
Pobeda	2.86^{def}	2187.26^{ab}	1687.77^{abc}	24.20^{bc}
Evropa90	2.42^{f}	2210.56^{ab}	1625.16^{abc}	21.00^{c}
NSR-5	3.12^{cdef}	2156.68^{ab}	1601.86^{abc}	41.93^{abc}
Dragana	2.65^{ef}	2133.38^{ab}	1622.24^{abc}	46.33^{ab}
Rapsodija	4.65^{ab}	2123.19^{ab}	1527.59^{abc}	32.47^{abc}
Simonida	2.83^{def}	2044.55^{b}	1483.90^{bc}	32.73^{abc}
Cipovka	3.70^{bcde}	2047.46^{b}	1521.76^{abc}	46.00^{ab}
Banatka	5.10^{a}	2115.91^{ab}	1488.27^{bc}	49.27^{a}
Bankut1205	3.96^{bc}	2324.15^{ab}	1821.75^{ab}	45.73^{ab}
Nevesinjka	3.46^{cdef}	1906.21^{b}	1377.60^{c}	52.47^{a}
Odisej	3.89^{bcd}	2019.80^{b}	1396.53^{bc}	52.33^{a}
Mean	3.49	2155.35	1591.54	39.16
Standard deviation	0.81	174.07	166.17	11.41
Standard error of mean	0.81	50.25	47.97	3.29
Coefficient of variation (%)	23.27	8.08	10.44	29.13

*fresh plant material;
**results in the same column marked with different letters indicate significant differences between genotypes at P<0.05 (based on Duncan's multiple range test)

Content of total phenols

Testing samples taken at the flowering stage showed that Renesansa had the highest phenol content (2595.01 mg of catechin g^{-1} fresh plant material) and statistically significantly differed from Simonida, Cipovka, Nevesinjka and Odisej, but not others which had a lower average value of the content of phenolic compounds and did not statistically differ from one another. The lowest content was recorded in Nevesinjka (1906.21 mg catechin g^{-1} fresh plant material), Table 3.

The content of total phenols was reduced in flag leaf samples taken in the milk maturity phase, compared to the previous development phase. However, the Duncan's test did not show statistically significant differences between genotypes. In relation Genotype rank changed according to the flowering stage. Genotype Bankut 1205 (1416.92 mg of catechin g^{-1} dry plant material) contained several more phenolic compounds than other genotypes (Table 4).

The content of total tannins

Since the total tannin content was obtained from the difference in the content of total phenols and the content of the non-tannin phenols, a distribution of the content similar to total phenols was obtained (Table 3 and Table 4).

In the first sampling phase, statistically significant differences were found between genotypes. The average value of total tannin varied from 1377.60 mg of catechin g^{-1} fresh plant material (Nevesinjka) to 1944.07 mg of catechin g^{-1} fresh plant material (Renesansa), Table 3.
As with total phenols, the content of total tannin was reduced in the milk maturity phase, relative to the flowering stage. No statistically significant differences were found between the genotypes by Duncan test. The NSR-5 genotype had the lowest average tannin value in the milk maturity stage of 637.83 mg of catechin g^{-1} dry plant material), while the highest value was achieved with the Simonida genotype (1089.26 mg of catechin g^{-1} dry plant material), Table 4.

Table 4. The content of total phenols, tannins and DPPH test in flag leaves of the tested genotypes during milk maturity

Genotypes	*Phenols* mg catechin g^{-1} dpm*	*Tannins* mg catechin g^{-1} dpm*	*DPPH test* % of neutralized radicals
Renesansa	1064.51^{a**}	728.12^{a**}	33.50^{bcd**}
Pobeda	1048.49^{a}	690.25^{a}	18.67^{d}
Evropa90	1322.26^{a}	942.18^{a}	39.17^{abcd}
NSR-5	1004.80^{a}	637.83^{a}	42.17^{abc}
Dragana	1214.50^{a}	844.62^{a}	26.83^{cd}
Rapsodija	1192.66^{a}	908.69^{a}	50.00^{abc}
Simonida	1376.14^{a}	1089.26^{a}	30.00^{bcd}
Cipovka	1055.77^{a}	773.26^{a}	45.17^{abc}
Banatka	1143.14^{a}	697.54^{a}	44.00^{abc}
Bankut1205	1416.92^{a}	1004.80^{a}	60.17^{a}
Nevesinjka	1111.11^{a}	707.73^{a}	37.83^{abcd}
Odisej	1380.51^{a}	948.01^{a}	52.83^{ab}
Mean	1194.23	831.02	40.03
Standard deviation	146.73	145.61	11.66
Standard error of mean	42.36	42.03	3.37
Coefficient of variation (%)	12.29	17.52	29.12

*dry plant material

** results in the same column marked with different letters indicate significant differences between genotypes at $P<0.05$ (based on Duncan's multiple range test)

DPPH test

The total non-enzymatic antioxidant activity, i.e. the possibility of removing DPPH radicals observed at the level of the whole sample, showed no difference between the flowering phase and the milk maturity phase. The tested genotypes had an average test value of 39.16% (flowering stage), i.e. 40.03% (phase of milk maturity), Table 3 and Table 4.

However, when genotypes are observed separately, significant differences are observed. The percentage of neutralized radicals ranged from 21.00 (genotype Evropa 90) to 52.47 (Nevesinjka genotype), for the first sampling. Samples taken from the milk maturity phase had different values of the DPPH test. Genotype Bankut 1205 had the best result (60.17%) and statistically significantly differed from other genotypes. Genotype Pobeda had the smallest antioxidant activity, which amounted to 18.67% of neutralized radicals.

Analysis of variability of total phenols, tannins and % of neutralized radicals, revealed certain differences, Table 3 and Table 4. The coefficient of variation of total phenols and total tannins was lower than the value of this parameter for the DPPH test in both sampling phases. Genotypes were more homogeneous in view of lower coefficient of variation for phenol content (8.08% in the flowering stage) and the content of tannins (10.44% in the flowering stage) of % neutralized radicals (29.13% in the flowering stage).

A similar result was obtained in the milk maturity phase, with the difference that the DPPH test remained unchanged relative to the previous maturity phase, and the content of total phenols and tannins became more variable, which means that the resulting differences were due to maturation.

Lipid Peroxidation (LP)

Different types of abiotic stress can lead to lipid peroxidation, which is manifested by the formation of a direct product, malondialdehyde (MDA). Since it is caused by the formation of ROS, lipid peroxidation, i.e. MDA content in leaves is considered to be a prominent indicator of membrane damage caused by increased salt content in soil (RADI *et al.*, 2013).

The results of lipid peroxidation analysis showed statistically significant differences between the majority of the tested genotypes on solonetz. The average MDA value in the flag leaf during the flowering period varied from 8.75 nmol MDA equivalents g^{-1} fresh plant material (genotype Nevesinjka) to 11.57 nmol of MDA equivalents g^{-1} fresh plant material (genotype Odisej). Genotype Odisej was ranked by the Duncan's test in a group with an average of the highest MDA content and statistically did not differ significantly from Bankut 1205 genotype (Table 5), suggesting that these two genotypes caused the greatest damage to membranes due to abiotic stress at the site.

The content of soluble proteins

The results of the test of soluble proteins suggest statistically significant differences between most examined genotypes. The values of soluble protein amount ranged from 90.37 mg of g^{-1} dry plant material in Renesansa to 98.53 mg of g^{-1} dry plant material in genotypes Cipovka and Nevesinjka (Table 5).

Table 5. The intensity of Lipid Peroxidation (LP) and content of soluble proteins in flag leaves of the tested genotypes during flowering

Genotypes	*LP* nmol MDA equivalent g^{-1} fpm*	*Soluble proteins* mg proteins g^{-1} fpm*
Renesansa	10.74[b**]	90.37[h]
Pobeda	10.62[b]	91.15[g]
Evropa90	10.11[bc]	91.25[g]
NSR-5	9.33[cd]	97.55[b]
Dragana	9.90[bc]	95.92[c]
Rapsodija	10.50[abc]	94.43[f]
Simonida	9.82[bcd]	96.13[c]
Cipovka	10.89[b]	98.53[a]
Banatka	10.28[bc]	95.04[e]
Bankut1205	11.51[a]	98.40[a]
Nevesinjka	8.75[d]	98.53[a]
Odisej	11.57[a]	95.28[d]
Mean	10.34	95.22
Standard deviation	0.83	2.94
Standard error of mean	0.24	0.85
Coefficient of variation (%)	8.00	3.09

**fresh plant material;*

*** results in the same column marked with different letters indicate significant differences between genotypes at P<0.05 (based on Duncan's multiple range test)*

DISCUSSION

According to ZHU (2003) and CHEN *et al.* (2011), soil salinity restricts plant growth by acting in four directions:

1. inhibition of plant growth and development, leading to osmotic stress and limiting water intake and reduction of assimilation, which in turn leads to
2. oxidative stress, which causes it
3. ionic toxicity, and
4. disorder in nutrient absorption.

For the abovementioned reasons, the need for more intense research into the activities of enzymatic and non-enzymatic components of the antioxidant system is obvious. The obtained results are of great importance for examining the response of wheat genotypes to stress conditions.

Analysis of the SOD content is significant when plants are subjected to abiotic stress caused by various factors. Increased SOD activity was observed in wheat grown in drought conditions (GORJI *et al.*, 2011). Also, increasing soil salinity leads to a greater activity of this enzyme (ESFANDIARI *et al.*, 2007; PERVEEN *et al.*, 2011). According to DEY *et al.* (2007), the activity of superoxide-dismutase is also increased in soil contaminated with heavy metals. SOD activity is a very important indicator of oxidative stress in wheat, since many authors agree that increase in the activity of the enzyme and reduced oxidative damage are closely related (ESFANDIARI *et al.*, 2007; ZHAO *et al.*, 2007; BHUTTA, 2011). Considering various activities of superoxide-dismutase identified in this study, a conclusion is drawn that the investigated genotypes reacted differently to stress conditions caused by growing on solonetz. For example, genotype Nevesinjka showed the highest tolerance to stress conditions, while genotype Pobeda was the most sensitive to increased salt concentrations in the soil. This suggests that the older wheat selection is better adapted to the newer genotypes. SAIRAM *et al.* (2002) recorded results similar to these, i.e. increased activity of SOD, examining the impact of long-term stress caused by different soil salt concentrations. These authors have determined a 50% higher superoxide-dismutase activity in a genotype that is resistant to high salt concentrations in the soil than in those with moderate resistance. A different reaction of durum wheat genotypes to stress caused by high salt content in soil was shown in the study by KAHRIZI *et al.* (2012).

The study of peroxidase is very important, because these molecules play an important role in the lignification of the cell walls during phenol polymerization. Peroxidases catalyze the oxidation reaction of phenol and the formation of phenoxy radicals, which polymerize and produce lignin (PASSARDI *et al.*, 2005). The activity of this group of enzymes has been confirmed in many plant species, including wheat. Peroxidase analysis is very important in wheat breeding because the gene pool where high enzymatic activity is determined can be a desirable genetic material for creating varieties tolerant to high soil salt content.

Studying of peroxidase activity is very important, given that the direct indices of H_2O_2 accumulation in cells in a state of stress caused by increased salt content in the soil. This study determined the increased activity of the investigated peroxidases (PPx and GPx) suggesting that stress induced the production of ROS while the plant was able to neutralize them. In terms of this biochemical marker, the local population of Banatka ranks as the best for PPx, whereas the newer genotype Cipovka is better considering the GPx. In the end, it allows the selection of genotypes that are more resistant to abiotic stress conditions and their use in further selection. Such conclusions are in line with the work of SAIRAM *et al.*, (2002); SAKR and EL-METWALLY, (2009); MARVI *et al.*, (2011).

Strengthening of the defense cellular mechanism depends on the activity of PAL. PAL activation was noticed in the genotypes cultivated at the Kumane site. TIAN and LEI (2006) indicates that PAL activation under various stress conditions contributes to better plant protection.

Glutathione is tripeptide (γ-L-glutamyl-L-cysteinyl-glycine) and the most commonly known thiol compound in most plant species (POPOVIĆ and ŠTAJNER, 2008). As an electron donor, reduced glutathione (GSH) can act in two directions to protect the cell. First, it complexes the toxic ions of heavy metals and keeps them in vacuoles. The second implies the activity of GSH in the removal of reactive oxygen species (CHEN J. *et al.*, 2011) which translates into an oxidized form, glutathione disulfide (POPOVIĆ and ŠTAJNER, 2008). This is significant for wheat genotypes grown on a solonetz soil, where increased soil concentrations of sodium salt can damage the root system, and therefore the entire plant. Indeed, if genotypes in these conditions show greater GSH activity, the impact of this abiotic stress type can be significantly reduced.

The study of biological and antioxidative properties of phenols in wheat under the conditions of increased salt concentrations in soil showed that the content of total phenols in wheat leaves gradually increased with increasing salinity levels in the soil (SAKR and EL-METWALLY, 2009). This suggests that genotypes with higher content of total phenols can reduce the presence of free radicals more efficiently, or tolerate stressful conditions more easily. Analyzing the results obtained by examination of the total phenol content in genotypes grown at the Kumane site, a significant difference was observed in their metabolism during the two phases of the study (flowering and milk maturity). Differences are expected since each genotype responds differently to stress, but was due to other factors, above all the climate. It especially applies to daily air temperatures, extremely high in the period from the flowering stage to harvest, which accelerated maturation and resulted in the decrease of the total phenol content. Results published by ASHRAF *et al.* (2010) also emphasize that plant growth phase leads to change in phenol content. If phenol content is designated as an adaptive mechanism for reducing free oxygen radicals accumulated in the body during stress, then the preferred genes for this protection system might be present in genetic constitution of the old variety of Bankut 1205. Although the recent selection of Renesansa, had a higher content of phenolic compounds in the flowering stage than Bankut 1205, this genotype maintained a high level of phenols at later stages of development, distinguishing it from others as the best adapted to the stress induced by solonetz. Studies by other authors show that various abiotic stresses increase the percentage of neutralized radicals (RAO *et al.*, 2013). According to LEE *et al.* (1998), extracts of plants can be divided into three groups according to their antioxidant activity, depending on the% of the DPPH-neutralized neutrals:

1. Active-percentage inhibition greater than 80%
2. Medium active-percentage inhibition from 50 to 80%
3. Inactive-percentage inhibition of less than 50%

It can therefore be concluded that wheat genotypes were inactive in the neutralization of radicals, with the exception of Nevesinjka and Odisej, which are classified as medium-active when the flowering stage is observed. In the milk maturity phase, the active genotypes were Bankut 1205, Odisej and Rapsodija, while the other examined genotypes belonged to a group of the inactive ones. In both phases of the study, the result suggests that the older variety Bankut 1205, as well as the synthetic species (triticale Odisej), were better adapted to stress conditions in Kumane than other tested varieties, because of better neutralization of the radicals accumulated in their cells.

According to CHEESEMAN and SLATER (1993), lipid peroxidation at the cellular level directly damages cell membranes, while indirectly there is damage to other cellular parts resulting from the action of aldehydes, secondary products of the reaction.

Most authors agree that the characteristics of the genotype, the environmental factors and crop development phase are crucial for antioxidant enzymatic activity and the intensity of lipid peroxidation. Adverse conditions of the middle and later plant growth phases lead to a decrease in enzymatic activity and an increase in lipid peroxidation, resulting in degradation and wounding of plant tissues (HONGBO *et al.*, 2005). The results of the study are in line with the above, since the flag leaf completely wilted in the second sampling phase (milk maturity), so lipid peroxidation and activity of the antioxidant enzymes were impossible to determine. The analysis of this biochemical parameter thus gains importance during high metabolic activity in the early stages of plant development. The obtained results may be a significant criterion for the selection of genotypes more resistant to cultivation on solonetz soils.

The study of oxidative stress caused by growing on solonetz and the activities of the antioxidant defense system components showed different tolerance to stress between varieties, and consequently different activity of the tested biochemical parameters. Genotypes Banatka and Bankut 1205 were the best in view of most investigated components. Given that these two genotypes have a long breeding tradition in the investigated area of Banat, they were better adapted to the existing conditions than other genotypes. Since the activity of a large number of enzymatic and non-essential components of the antioxidant system is under the control genes, genetic component of these two genotypes should be taken into consideration because they could be a potential source of desirable genes in breeding for the desired properties. Considering genotypes obtained in the modern breeding programs, variety Rapsodija is distinguished from others due to a good reaction when enzymatic components are examined, while variety Renesansa exhibited the greatest non-enzymatic activity. These two varieties may also be desirable parent material in the development of varieties better adapted to growing on a solonetz.

CONCLUSION

Due to permanent degradation of arable land caused by various modern-day factors, the results of the research contribute to raising the economic value of soils which belong to a lower bonitet class. Since solonetz soils fail to provide favourable conditions for wheat cultivation and are mainly used as pastures, the research has contributed to the prospect of growing wheat on a solonetz soil. The results of the research provide a clear overview of wheat reaction under the conditions of global climate change. This way, the findings contribute to obtaining new genetic variation, but they also indicate that wheat cultivation on poor-quality soils has a bioremediation role, which raises the economic value of soils and results in expansion of the wheat growing area.

The results obtained in the study can be of importance in further breeding for abiotic stress tolerance, as well as obtaining stable wheat genotypes with a developed antioxidant defense system against solonetz induced stress.

Industrial and technological progress of the modern-day society, supported by the global climate change, leaves fewer areas of fertile soil and raises further issues concerning environment preservation discovery of new food sources. Knowing that wheat feeds the largest part of world population, the goals set by the breeders are reduced to the most important and most complex one - to produce higher-yielding and more nutritional genotypes. Numerous steps are taken on this path , especially towards the examination of adaptability and stability of genotypes under different agroecological conditions. Of particular benefit are the findings that contribute to open-field cultivation of wheat on less fertile soils, where plants are exposed to various biotic and abiotic stresses. Solving the problem of providing enough food for the present and future generations is thereby certain.

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ANTIOKSIDANTNA VARIJABILNOST PŠENICE GAJENE U *in situ* USLOVIMA STRESA IZAZVANOG SOLIMA

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Izvod

Gajenje biljaka na otvorenom polju, zavisno je od niza živih i neživih činilaca, koji kod biljaka izazivaju stres i dovode do slabljenja njihovog organizma. Nepovoljnu sredinu, za većinu gajenih biljaka predstavljaju zemljišta, koja se odlikuju visokim koncentracijama različitih vrsta soli. Solonjec, kao zemljište slabije produktivnosti, predstavlja proizvodnu sredinu koja može da se smatra stresnom za biljke, a povećane koncentracije različitih vrsta soli u zemljištu, mogu da dovedu do pojave oksidativnog stresa biljaka. Kao meru borbe protiv uslova stresa u kojem žive, biljke su razvile vrlo složen antioksidativni sistem zaštite. Ogled je postavljen na halomorfnom zemljištu tipa solonjec. Istraživanja su obuhvatila 10 sorti i jednu lokalnu populaciju heksaploidne pšenice (*Triticum aestivum* ssp. *aestivum* L.) i jednu sortu tritikalea (*Triticosecale* W.). Utvrđene su enzimske aktivnosti antioksidativnog sistema, neenzimske komponente antioksidativnog sistema, kao i lipidna peroksidacija. Analiza biohemijskih parametara je izvršena kada su se biljke nalazile u fazama cvetanja i mlečne zrelosti. Sa stanovišta obe grupe ispitivanih komponenti, genotipovi Banatka i Bankut 1205 su najbolje podneli stres. Razumevanje načina na koji se kiseonični radikali stvaraju u biljnom tkivu i nestaju iz njega, doprinosi stvaranju novih sorti pšenice, koje će biti tolerantnije prema stresu. Izbor što bolje adaptiranih genotipova na uslove gajenja na solonjecu može da omogući ekonomski opravdanu proizvodnju pšenice i intenzivnije iskorišćenje ovakvog zemljišta u poljoprivrednoj proizvodnji.

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