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EFFECT OF SALINITY STRESS ON ANTIOXIDANT ACTIVITY AND GRAIN YIELD OF DIFFERENT WHEAT GENOTYPES

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ABSTRACT

In order to evaluate the antioxidant activity of wheat in salinity stress conditions, an experiment with 27 wheat genotypes grown on two types of soil was conducted: solonetz (increased salinity) and chernozem (control), during two vegetation seasons (2015/2016 and 2016/2017). Analysis of DPPH radical scavenging activity and phenolic content (PC) were performed in different phenophases of wheat (tillering, stem elongation and heading). Genotypes showed significantly higher DPPH radical scavenging activity (9.82 mg trolox equivalents (TE) per mg of dry matter (d.m.)) and PC (8.15 mg gallic acid equivalents (GAE) per mg d.m.) under salinity stress conditions compared to values obtained on control (8.52 mg TE mg^{-1} d.m. and 7.13 mg GAE mg^{-1} d.m., respectively). All analyzed factors (genotype, soil type and year) had the highly significant influence on phenotypic variation of grain yield. Salinity stress reduced grain yield by 30%, whereas drought stress in 2016/2017 vegetation season reduced grain yield by 20%. Highly significant and positive correlations are present between grain yield and parameters of antioxidant activity in all growth stages of wheat and both soil conditions. Therefore, it could be possible to select salinity tolerant genotypes in early growth stages. DPPH scavenging activity and total phenolic content are in highly significant and positive correlation in all growth stages, which indicates that antioxidant activity is highly derived by phenolics.

Key words: Chernozem, correlation, DPPH, phenolic content, solonetz

INTRODUCTION

As one of the staple cereals in human nutrition, wheat has always occupied a central place in agricultural production. Due to the growth rate of the world population, the demand for wheat is expected to increase by 40% by 2030 (Dixon et al., 2009). Soil salinity is one of the main stress factors, especially in arid or semi-arid areas, which can significantly limit agricultural production (Borzouei et al., 2012), reducing the yield of the most important crops up to 50% (Dimitrijević et al., 2012). Approximately one billion hectares (or 7% of the total terrestrial area in the world) is affected by some form of salinity (Hossain, 2019). Soil type of solonetz occupies 150 million hectares in the world, and most of it is located in steppe where climate is characterized by annual precipitation that does not exceed 400 to 500 mm (Chesworth, 2008). Halomorphic soils in the European Union cover a total of 21,585 km^2, where the solonchak takes 11,728 km^2, and the solonetz 9,857 km^2 (Tóth et al., 2008).

Salinity stress could cause the production of Reactive oxygen species (ROS) in the plant (Hasanuzzaman et al., 2021). Production of ROS leads to oxidative stress in the plant, where the formed free radicals can cause cell extinction due to present oxidative processes, such as cell membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage to DNA and RNA (Ashraf, 2009; Azizpour et al., 2010; Hasanuzzaman et al., 2021). All that was noticed affects the inhibition of plant growth and grain yield (Hendawy et al., 2017; Khokhar et al., 2017; Mansour et al., 2020). To avoid harmful effects of ROS production, plants have developed the antioxidant defense systems which contain enzymatic and non-enzymatic antioxidants that reduce the level of oxidative stress in plant cells (Ashraf, 2009; Sharma et al., 2012). Non-enzymatic components of the antioxidant system include major cellular redox buffers, ascorbate, glutathione and other thiol proteins, as well as tocopherols, carotenoids, and phenolic compounds (Sharma et al., 2012). Phenolic compounds are considered to be a major group of compounds that contribute to the antioxidant activity

(Sytar et al., 2018). Analysing the tolerance of wheat and barley genotypes to salinity based on grain yield is expensive and time-consuming, and therefore it's suggested that evaluation of tolerance to salinity is carried out at the early stages of plant development for these species (El-Hendawy et al., 2011; Turkyilmaz et al., 2011). Results obtained by El-Hendawy et al. (2011), Khayatnezhad and Gholamin (2010) and Turki et al. (2012) showed that wheat in the early stages of vegetative growth was sensitive to salinity stress. In addition, parameters in terms of salinity tolerance measured at early growth stages were correlated with salt tolerance parameters at maturity stage (El-Hendawy et al., 2011; Turki et al. 2012). El-Hendawy et al. (2017) state that the establishment of field trials is of great importance in wheat breeding programs aimed to increase the tolerance to soil salinity.

The aim of this study was to determine the influence of salt stress on grain yield and parameters of antioxidant activity analyzed in different growth stages. Furthermore, the goal is to establish the connection between the antioxidant activity and grain yield in different growth stages of wheat and to investigate the possibility of selecting salt tolerance genotypes at early growth stages.

MATERIALS and METHODS

Experiment design and plant material

Field experiment was obtained in Randomized Complete Block Design with three replications during 2015/2016 and 2016/2017 vegetation seasons at two localities: Kumane (Banat, Vojvodina, 45.522ºN, 20,195ºE) and Rimski Šančevi (Novi Sad, Bačka, Vojvodina, 45,322ºN, 19,836E). The locality of Kumane was chosen due to solonetz soil type, while locality of Rimski Šančevi, where chernozem soil type was present, was chosen as a control locality. The experimental material consisted of 27 wheat genotypes (*Triticum aestivum* ssp. *vulgare*). The genotypes included in this study (local landrace Banatka, old Hungarian variety Bankut 1205, varieties created at Institute for Field and Vegetable Crops in Novi Sad: Jugoslavija, NS-rana 5, Renesansa and Pesma) were chosen on the basis of previous studies that tested the adaptability of these genotypes to abiotic stress, expressed at the Kumane locality. In order to increase genotypic variability, this research included old and modern varieties of the Centre for Small Grains in Kragujevac, as well as the local landrace Grbljanka grown in Montenegro (Table 1).

Table 1. Wheat genotypes included in investigation

Genotype code	Genotype	Year of approval	Genotype code	Genotype	Year of approval
G1	Banatka	Local landrace	G15	Jugoslavija	1980.
G2	Grbljanka	Local landrace	G16	Oplenka	1982.
G3	Bankut 1205	1953.	G17	Ljubičevka	1985.
G4	Kragujevačka 75	1966.	G18	Srbijanka	1986.
G5	Šumadija	1968.	G19	Šumadinka	1988.
G6	Kosmajka	1971.	G20	NS-rana 5	1991.
G7	Gružanka	1972.	G21	Renesansa	1994.
G8	Morava	1972.	G22	Pesma	1995.
G9	Zastava	1973.	G23	Aleksandra	2007.
G10	Kragujevačka 56	1975.	G24	Perfekta	2009.
G11	Orašanka	1976.	G25	Harmonija	2012.
G12	Kragujevačka 58	1977.	G26	Rujna	2013.
G13	Kragujevačka 78	1978.	G27	Premija	2013.
G14	Lepenica	1980.			

At both soil types, the analyzed genotypes were sown with continuous sowing, where the arrangement of genotypes was organized according to a randomized complete block design. The size of the basic plot was 2 m^2, the space between rows was 10 cm, and the distance between the plots was 25 cm. The usual agronomic practices for wheat production were applied at both localities. Harvesting was performed at the stage of physiological maturity, when the grain moisture was below 14%. The grain yield was determined by measuring the grain weight per square meter, on each plot, and converting into t ha^{-1}.

Soil conditions

Solonetz is an alkaline soil type with high clay content in the subsurface horizon and a high proportion of adsorption of sodium and / or magnesium ions (Tóth et al., 2008). The highest content of adsorbed Na+ was found in Bt,na horizon (13.45 cmol kg^{-1}), at a depth of 58 to 85 cm, while the lowest content of adsorbed Na was recorded in the surface layer (0.88 cmol kg^{-1}), Belic *et al*. 2014. Due to the high sum of exchangeable cations, and content of clay and exchangeable Na (containing more than 15% of adsorbed Na), as well as the alkalinity (pH>9) in Bt,na horizon, the solonetz is characterized as a soil of unfavorable physical and chemical properties (Belić et al., 2006). On the other hand, chernozem is considered an ideal soil type for agricultural production with a favorable, loamy, mechanical composition (Miljković, 1996). It is characterized by a good crumbly structure, stable aggregates and good water permeability. Also, it has a sufficient organic matter (3 to 4%) and plant nutrients and is characterized by a favorable water-air and heat regime (Hadžić et al., 2002).

Meteorological conditions

The mean monthly temperature at both localities (Kumane and Rimski Šančevi) during analyzed vegetation seasons (2015/2016 and 2016/2017) was similar. In both vegetation seasons, the average sum of monthly precipitation was higher at the locality of Rimski Šančevi (603 mm in 2015/2016 and 428 mm in 2017/2018), compared to amount of precipitation recorded at locality of Kumane (527.3 mm in 2015/2016 and 289.9 mm in 2016/2017). Warmer weather and small amount of precipitation affected the earlier ripening of plants in the 2016/2017 vegetation season, especially at Kumane locality (RHMZ, 2020), Figure 1.

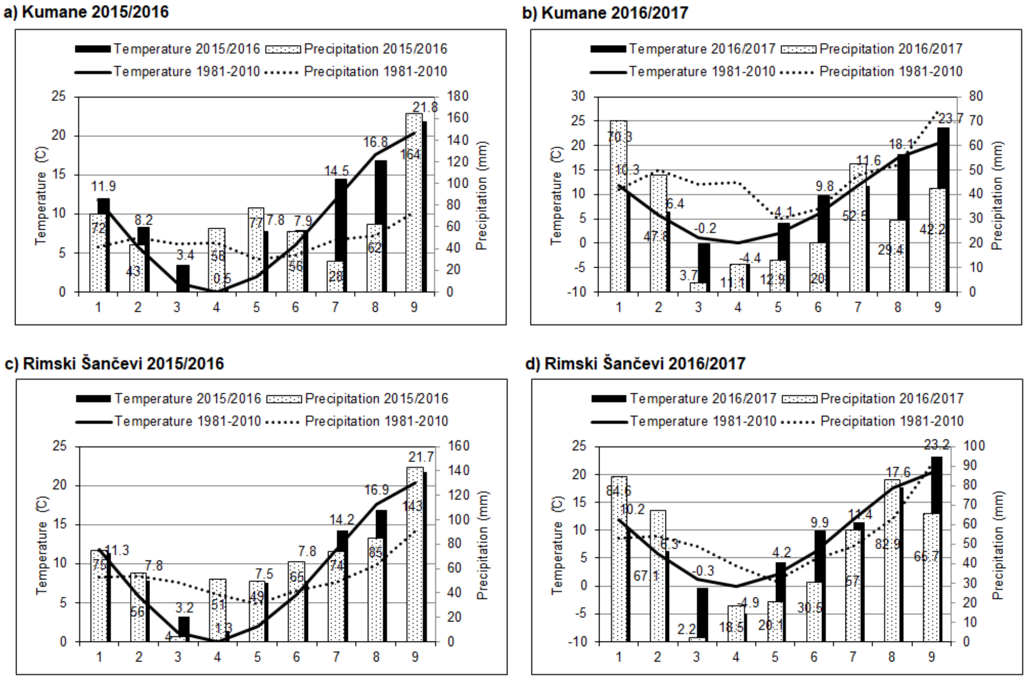

Figure 1. Monthly mean temperatures and sum of precipitation in Kumane (a, b) and Rimski Šančevi (c, b) in two vegetation seasons

Methods to evaluate the antioxidant activity

The antioxidant activity was analyzed in dry plant material. Sampling of the leaves from whole plant was carried out in three different wheat phenophases: tillering, stem elongation and heading. The average sample was made from each plot.

The plant material (0.2 g) was extracted with 70% acetone (10 mL) during 24 h in a dark. After extraction, the macerate was filtered through a qualitative filter paper. The extracts are stored in a refrigerator at a temperature of a 4°C, during 24h (until use for further analysis).

Determination of DPPH˙ scavenging activity. Free radical scavenging ability was measured using the stable DPPH (2.2-diphenyl-1-picrylhydrazine) radical (Lee et al., 1998). Extract (40 µL) was mixed with DPPH reagent (2 mL). The absorbance was measured at 517 nm, using UV/VIS spectrophotometer (Thermo Scientific Evolution 220, USA). Calibration curve was made using trolox as standard. The activity of removing the DPPH radical is expressed in mg equivalents of trolox per g of dry matter (mg TE g^{-1} d.m.). All measurements were performed in triplicate.

Determination of phenolic content. Phenolic content (TPC) of the wheat extract was determined according to the Folin-Ciocalteu method (Hagerman et al., 2000). Plant extract (20 µL) was mixed with 1.8 mL of deionized water, 0.2 mL of 20% sodium carbonate and 0.1 mL of Folin-Ciocalteu reagent, which was previously diluted (1:2) with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 720 nm using an UV/VIS spectrophotometer (Thermo Scientific Evolution 220, USA). The phenolic content was determined using a standard curve with gallic acid (0.005 to 0.05 mg ml^{-1}). The data were expressed in mg of gallic acid equivalents (GAE) per g of dry matter (mg GAE g^{-1} d.m.). All measurements were performed in triplicate.

Statistical analysis

The data obtained in this study were expressed as the mean of triplicate determinations. The analysis of variance was performed, with genotype and environment (phenophase, year and soil type) as fixed effects. All data and means were compared by the LSD test, at $p<0.01$ and $p<0.05$ levels of significance. Correlations between analyzed characteristics were calculated in each environment and each phenophase and both localities (agro-ecological environments). Statistical analysis was performed using IBM SPSS Statistics 22.0, trial version (https://www.ibm.com/analytics/spss-trials).

RESULTS

DPPH$^{\bullet}$ scavenging activity and phenolic content

DPPH$^{\bullet}$ scavenging activity and phenolic content significantly varied under the influence of soil type (Figure 2). In average for both vegetation seasons, the significantly higher ($p<0.01$) DPPH$^{\bullet}$ scavenging activity and phenolic content was obtained on solonetz (9.82 mg TE g^{-1} d.m. and 8.15 mg GAE g^{-1} d.m.) in relation to the chernozem (8.53 mg TE g^{-1} d.m. and 7.13 mg GAE g^{-1} d.m).

Factor of genotype had a significant effect on analyzed parameters. The highest DPPH$^{\bullet}$ scavenging activity and phenolic content were examined by the genotypes Orašanka (G11) (13.41 mg TE g^{-1} d.m, 10.24 mg GAE g^{-1} d.m.) and Bankut 1205 (G3) (12.71 mg TE g^{-1} d.m., 12.74 mg GAE g^{-1} d.m.) when grown on solonetz. On chernozem soil type, the highest DPPH$^{\bullet}$ scavenging activity and phenolic content were measured in genotypes Jugoslavija (G15) (12.42 mg TE g^{-1} d.m., 9.89 mg GAE g^{-1} d.m.), Renesansa (G21) (11.22 mg TE g^{-1} d.m., 9.30 mg GAE g^{-1} d.m.) and Harmonija (G25) (11.22 mg TE g^{-1} d.m., 9.91 mg GAE g^{-1} d.m.), Figure 2.

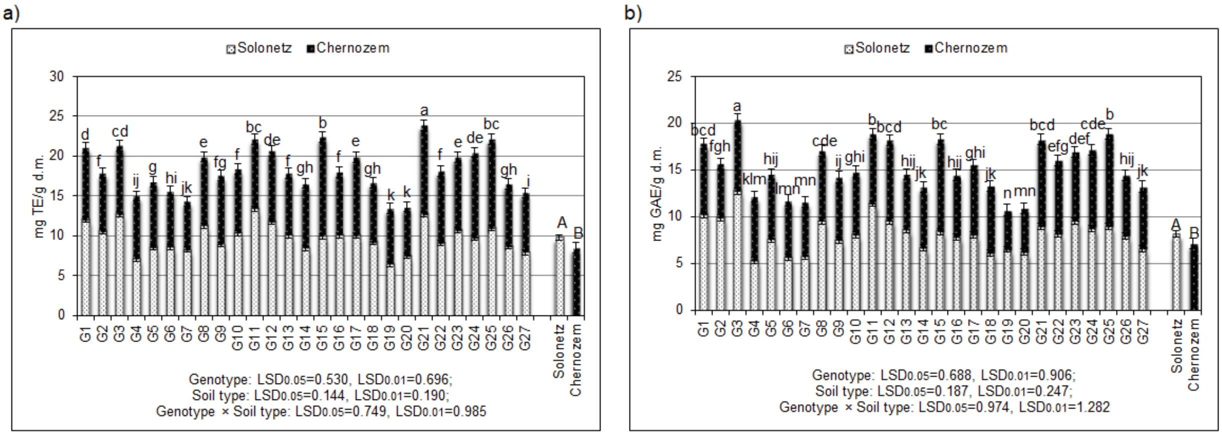

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Figure 2. Influence of genotype, soil type and genotype × soil type interaction on variation of DPPH$^{\bullet}$ scavenging activity (a) and phenolic content (b)

Factor of year had a dominant effect ($p<0.01$) on the variation of DPPH$^{\bullet}$ scavenging activity, while the phenolic content was not affected by this factor ($p>0.05$). In 2016/2017 vegetation season, DPPH$^{\bullet}$ scavenging activity was 12.05 mg TE g^{-1} d.m. and was twice as high as the value measured in 2015/2016 vegetation season (6.30 mg TE g^{-1} d.m.), while almost equal values of phenolic content were recorded in both vegetation seasons (Figure 3).

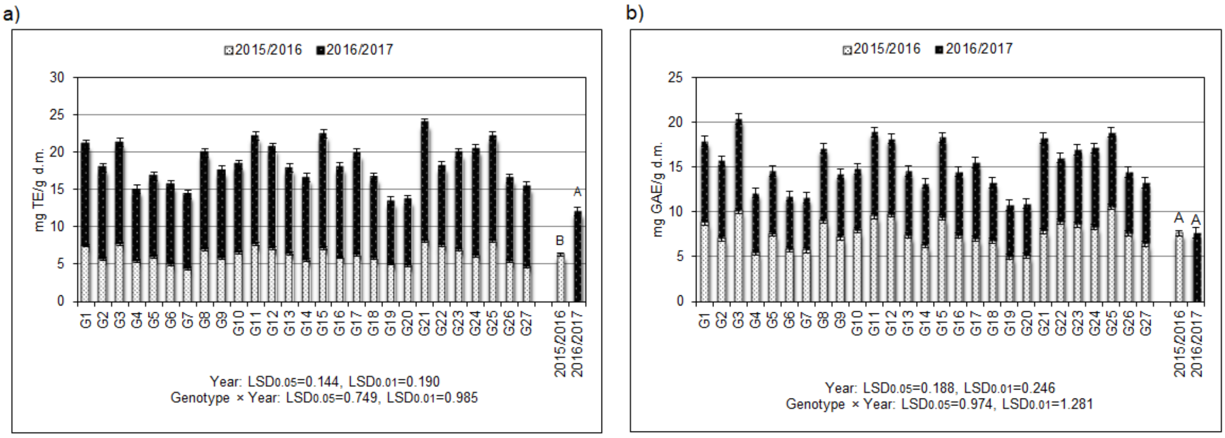

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Figure 3. Influence of year and genotype × year interaction on variation of DPPH$^{\bullet}$ scavenging activity (a) and phenolic content (b)

Factor of phenophase had siginificant effect on DPPH$^{\bullet}$ scavenging activity and phenolic content variation. The highest DPPH$^{\bullet}$ scavenging activity was measured in phenophase of stem elongation (10.87 mg TE g^{-1} d.m.), while the highest value of phenolic content was recorded in phenophase of heading (7.86 mg GAE g^{-1} d.m.). Also, interactions of phenophase × soil type and phenophase × year were statistically significant ($p<0.01$) for both analyzed parameters (Figure 4). The highest differences between soil types in measured values of analyzed parameters were observed in phenophase of stem elongation, while the lowest differences were recorded in phenophase of tillering. The highest difference in DPPH$^•$ scavenging activity between vegetation seasons was established in phenophase of tillering, while the highest difference in phenolic content was observed in phenophase of heading. The DPPH$^•$ scavenging activity significantly ($p<0.01$) varied under the influence of soil type × year ineraction, where the difference between soil conditions in DPPH$^•$ scavenging activity was more pronounced in the 2015/2016 vegetation season. Interaction of soil type × year did not significantly ($p>0.05$) affect the variation of phenolic content (Figure 4).

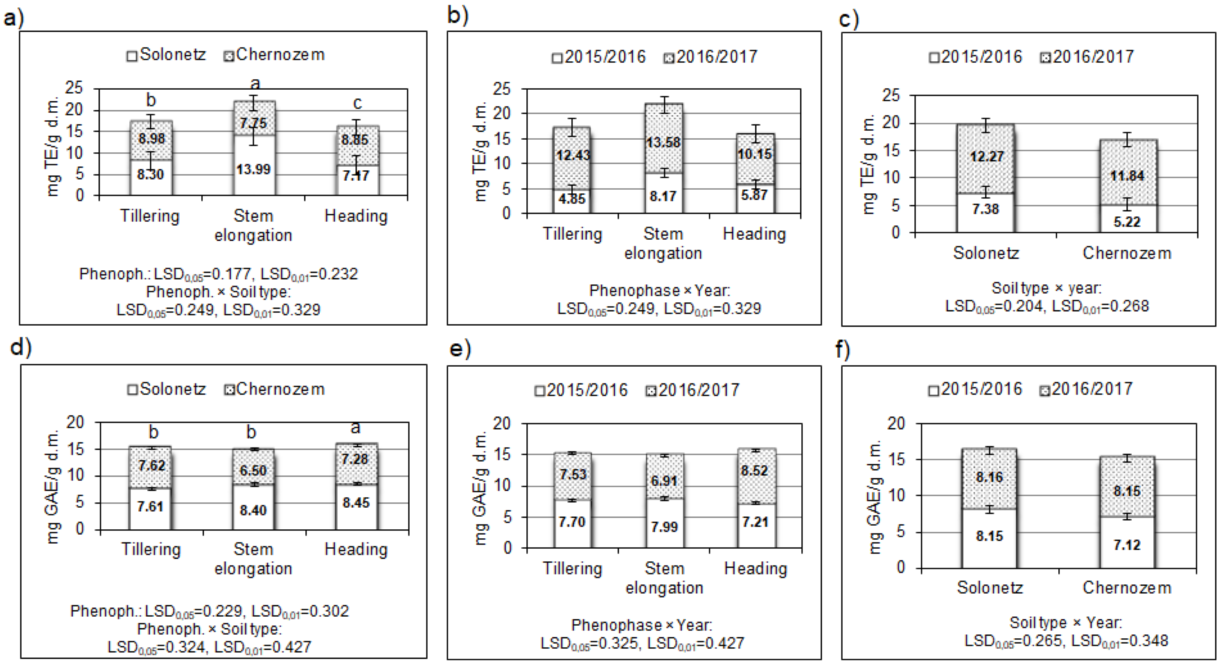

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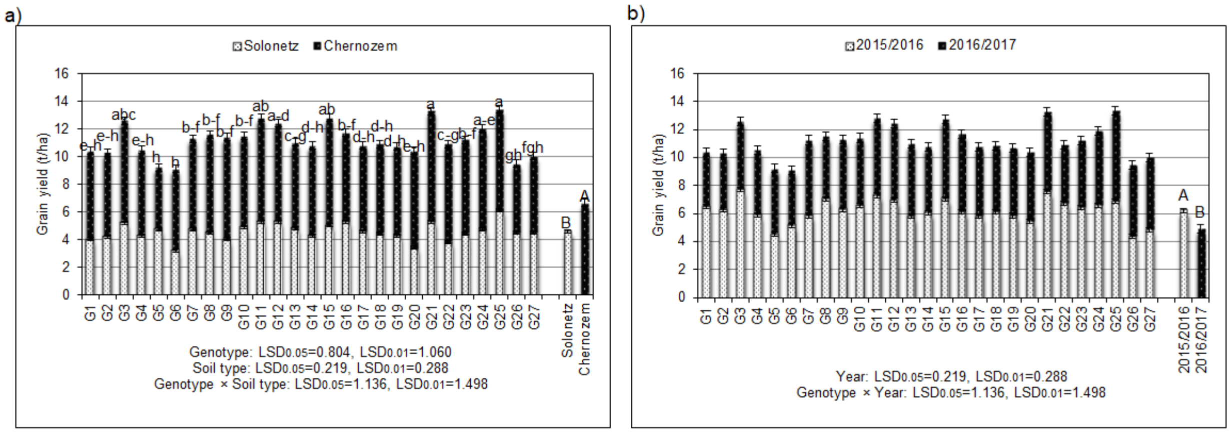
Figure 4. Influence of phenophase, phenophase × soil type, phenophase × year (b) and soil type × year on variation of DPPH$^•$ scavenging activity (a, b, c) and phenolic content (d, e, f)

Grain yield

The grain yield significantly varied under the influence of genotype, soil type, year and their interactions (Figure 5). The factor of soil type had the highest effect on grain yield variation. The result indicates that salinity led to reduction in grain yield by 30% (4.5 t ha^{-1} on solonetz, 6.6 t ha^{-1} on chernozem). Similar effect on grain yield variation had factor of year, where grain yield in 2015/2016 vegetation season (6.2 t ha^{-1}) was significantly higher ($p<0.01$) in relation to the grain yield achieved in 2016/2017 vegetation season (4.9 t ha^{-1}). Interaction of genotype × soil type and genotype × year significantly affected the grain yield variation as well. On solonetz, genotype Harmonija achieved the highest grain yield (6.1 t ha^{-1}). This genotype had the highest value in 2016/2017 vegetation season, while in 2015/2016 the highest value of grain yield was observed at genotype Jugoslavija (7.1 t ha^{-1}). On chernozem soil type, the highest average value of grain yield was found in genotype Renesansa (G21) (8.0 t ha^{-1}), Figure 5.



Bars marked with different letters differ significantly at the level of 5%

Figure 5. Influence of genotype, soil type, genotype × soil type (a) and year, genotype × year (b) on variation of grain yield

Correlation between antioxidant activity parameters and grain yield

In order to analyse the relationship of grain yield and parameters of antioxidant activity, single correlations were conducted for both soil conditions separately during two vegetation seasons (Table 2). The values of grain yield and DPPH scavenging activity were in a significant and positive correlation, in all phenophases and in both soil conditions. On solonetz, the highest value of correlation coefficient between grain yield and DPPH scavenging activity is present in the phenophase of stem elongation (0.528**), followed by the value in phenophase heading (0.481**) and tillering (0.444**). On chernozem, grain yield had the strongest and the most positive correlation with DPPH scavenging activity in phenophase of heading (0.633**), then in phenophase of stem elongation (0.485**) and in phenophase of tillering (0.329*), Table 6. Significant and positive correlations between grain yield and phenolic content were found in all phenophases and on both soil types during two vegetation seasons (Table 2). The highest value of correlation coefficient was observed in stem elongation (0.456** on solonetz and 0.485** on chernozem), while the lowest correlation was found in growth stage of tillering (0.383* on solonetz and 0.322* on chernozem), Table 2.

Table 2. Correlation analysis between grain yield and parameters of antioxidant activity in three phenophases of wheat genotypes grown on two soil types and two vegetation seasons

Solonetz		**Chernozem**		**Solonetz**		**Chernozem**	
DPPH scavenging activity	Grain yield	DPPH scavenging activity	Grain yield	Phenolic content	Grain yield	Phenolic content	Grain yield
Tillering	0.444**	Tillering	0.329*	Tillering	0.383*	Tillering	0.322*
Stem elongation	0.528**	Stem elongation	0.485**	Stem elongation	0.456**	Stem elongation	0.485**
Heading	0.481**	Heading	0.633**	Heading	0.423**	Heading	0.411**

*Significant correlation per $p<0.05$; **Significant correlation per $p<0.01$

DISCUSSION

In order to avoid harmful effects of salinity, due to the production of reactive oxygen species (ROS), plants have developed an antioxidant defense system which includes enzymatic and non-enzymatic components. Therefore, changes in the content of antioxidant levels may act as a signal for ROS scavenging processes (Hasanuzzaman et al., 2021). Phenolic compounds are among the main non-enzymatic components of the plant antioxidant system (Ashraf, 2009; Sytar et al., 2018). Accumulation of phenolic compounds could be one of the cellular adaptive mechanisms for scavenging oxygen free radicals during stress (Mohamed and Aly, 2008). The analyzed genotypes showed significantly higher DPPH scavenging activity and phenolic content when grown on high saline soil, such as solonetz, compared to chernozem. Furthermore, the results obtained by Kumar et al. (2017) and Kiani et al. (2021), confirmed that the salinity significantly increases phenolic content in the wheat plant. Also, salinity stress caused significant increases the DPPH scavenging activity in wheat leaf (Kiani et al., 2021).

The highest share in total variation of DPPH radical scavenging activity had vegetation season.

In the dry 2016/2017 vegetation season, antioxidant activity was twice as high as the antioxidant activity in the more favorable 2015/2016 vegetation season. Sarker and Oba (2012) noted that DPPH radical scavenging activity significantly increased in *Amaranthus tricolor* with the increased of drought stress. The variation in phenol content was mostly influenced by the factor of genotype, while the factor of vegetation season did not have a significant influence. Predominant effect of genotype on the accumulation of total phenolic acid in wheat grains, under elevated temperatures, was found by Shamloo et al. (2017).

In condition of salinity stress, the highest values of DPPH radical scavenging activity and phenolic content were shown in the leaves of old varieties Bankut 1205 and Orašanka, such as in local landrace Banatka. On the other hand, newer varieties Harmonija, Renesansa and Jugoslavija had the highest DPPH radical scavenging activity and phenolic content on chernozem. Banjac (2015) got similar results at the same locality, on the solonetz soil type, where he stated that the genotype Bankut 1205 had the highest phenolic content in the phenophase of heading and the highest DPPH scavenging activity in the phase of milk maturity. The same author states that the local landrace Banatka was characterized by higher neutralization ability of free DPPH radicals in relation to newer varieties.

Soil salinity significantly reduced the grain yield of the examined genotypes by 30%. This is in accordance to the results obtained by Dimitrijević et al. (2012), where they found that increased soil salinity on solonetz, reduced grain yield by 35 to 50% in relation to yield achieved on nonsaline soil (chernozem). Also, Khokhar et al. (2017), Nadeem et al. (2020) and Mansour et al. (2020) found a significant reduction in wheat grain yield under the influence of salinity stress. Phenolic components are the main group of compounds that contribute to the antioxidant activity of cereals (Zendehbad et al., 2014). Therefore, the difference in antioxidant activity between wheat genotypes may be due to the different composition of the phenolic compounds present.

Parameters of antioxidant activity were in highly significant and positive correlations with grain yield in all analyzed phenophases. Therefore, these antioxidant parameters could be used as selection criteria, even in the early growth stages of plant growth, especially in conditions of high salinity. Similar results have been reported by El-Hendawy et al. (2011), where they found that rank of wheat genotypes for salt tolerance in terms of parameters measured at seedling stages matched with their ranking in terms of grain yield at final harvest. The same authors emphasized that analyzed genotypes were very sensitive to salinity stress at early growth stage, and that it is possible to select salt tolerance of wheat genotypes in seedling stage. Turki et al. (2012) reported similar results, where they found a highly positive correlation between measured parameters in maturity and tillering stage of wheat at high salinity conditions.

CONCLUSION

The analyzed genotypes had a significantly higher values of DPPH scavenging activity and the phenolic content on solonetz soil type (in conditions of increased salinity), compared to the values achieved on chernozem soil type. The old genotypes Bankut 1205, Orašanka and Banatka had the highest antioxidant activity on solonetz, while on chernozem, the highest antioxidant activity was shown by newer genotypes - Harmonija, Renesansa and Jugoslavija. Thus, this shows that even older genotypes can serve as a desirable genetic resource for breeding for salinity tolerance. Also, this is of practical importance for wheat producers in areas of increased soil salinity. Highly significant and positive correlations are present between grain yield and parameters of antioxidant activity measured in all phenophases of wheat. The correlation between grain yield and antioxidant activity in early growth stages indicates that the parameters of antioxidant activity could be used as a selection criteria, especially in stressful conditions due to salinity. The association of phenolic content and the DPPH radical scavenging activity in tested genotypes indicates that phenolic compounds have a large share in the antioxidant activity of wheat.

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