UDC 575.630
ttps://doi.org/10.2298/GENSR2101219M
Original scientific article

HEAT-INDUCED ACCUMULATION OF PROLINE AND YIELD COMPONENTS IN GENETICALLY DIVERGENT CEREAL VARIETIES

Stefan M. MARKOVIĆ[1*], Desimir KNEŽEVIĆ[2], Nenad M. NEŠOVIĆ[3], Nevena H. DJUKIĆ[1]

[1] University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Kragujevac, Serbia
[2] University of Priština, Faculty of Agriculture, Kosovska Mitrovica, Lešak, Serbia
[3] Agricultural Extension Service Kraljevo Ltd., Kraljevo, Serbia.

Marković S., D. Knežević, N. Nešović. N. Djukić (2021). *Heat-induced accumulation of proline and yield components in genetically divergent cereal varieties*. - Genetika, Vol 53, No.1, 219 -233.

Growth and development of cereals is affected by abiotic factors, particularly by high temperature. An important factor in plant adaptation to abiotic stress can be the proline accumulation. Proline is an amino acid involved in a series of metabolic processes and is important as a protein stabilizer, osmolyte and antioxidant. The aim of this experiment was to determine the effect of high temperature on the proline content and yield elements in different cereal varieties during two vegetative seasons. For the investigation, plant material of 8 genetically divergent winter wheat varieties, 1 variety of triticale and 1 oat variety were used. Samples were collected in days with moderate midday air temperatures of 24-26°C in the milk stage and after a few days in the same phenological stage, in conditions of high midday air temperatures of 34-36°C. Proline accumulation was determined spectrophotometrically. Statistical analyses of data were done in the SPSS program. The results during the first experimental year showed that under moderate air temperature conditions proline content was 0.661 µmol g^{-1} fresh plant, and in the second experimental year 0.777 µmol g^{-1} fresh plant. Under conditions of heat stress during the first year, the content of proline increased to 2.169 µmol g^{-1} fresh plant, and in the second experimental year the average content was 2.510 µmol g^{-1} fresh plant, which confirms the increase of proline accumulations under heat stress. Compared with other cereal varieties, wheat varieties Zvezdana, Pobeda, Simonida and Avenu were characterized by higher contents of proline in conditions of heat stress in both vegetative seasons. Statistical

Corresponding author: Stefan Marković, University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000, Kragujevac, Serbia. E-mail: stefan.markovic@pmf.kg.ac.rs; Phone: +381 34 336223.

analysis and correlation of the results of proline accumulation and yield elements showed that more resistant varieties to heat stress conditions have higher yields.

Keywords: proline, heat stress, cereals, yield, genetic variability

INTRODUCTION

Cereals represent an important food and economic resource. A good indicator are modern nutrition guidelines where cereals are placed as the most important food for everyday use. The World Health Organization has made recommendations within the CINDI nutrition guide (the countrywide integrated noncommunicable disease intervention program and the program for nutrition), according to which cereals and their products are recommended as the basis for everyday nutrition (RAĐEN, 2012). In addition to the significant amount of carbohydrates, proteins and fibers, cereals are also significant due to their mineral composition (P, K, Fe, Mg, Se, Zn, Mn) and rich vitamins content (B2 - riboflavin, B6 - pyridoxine, B5 - pantothenic acid, as well as vitamins E, A and in smaller quantities of K) (SINGH *et al.*, 2012). Cereals and cereal products may also contain a range of bioactive substances and there is an increasing interest in the potential health benefits that these substances can provide. In addition to nutritional and economic importance of cereals, the importance of their medicinal properties is increasingly emphasized in recent times (MALENČIĆ *et al.*, 2016). Therefore, numerous studies have examined the impact of cereals on human health, and research has confirmed numerous positive medical properties (ADOM and LIU, 2002; THOMPSON, 1994). Due to the high importance of cereals as well as the growth of the human population, it is important to maintain and increase the production of cereals in order to ensure their quantity is sufficient. In the last decades are more frequent abiotic factors which negatively affect the production of cereal crops worldwide (ROY *et al.*, 2011). The amount of all cereals produced is significantly influenced by high temperature and drought, which could in the future cause major problems (CIAIS *et al.*, 2005; BARNABÁS *et al.*, 2008). The growth and development of plants is influenced particularly by temperature (PORTER and MOOT, 1998). Temperatures higher than 35°C reduce the enzyme activity and significantly limit photosynthesis (GRIFFIN *et al.*, 2004). Due to heat stress, there is an increase in reactive oxygen species in the plant (hydrogen peroxide, hydroxy radical, superoxide radical), which cause oxidative damage to many cellular components and structures, cause metabolic disorders, and can lead to lipid peroxidation (GILL and TUTEJA, 2010). Lipid peroxidation leads to lipid damage in cell membranes, resulting in cell death and plant damage (LIU and HUANG, 2000). The combined effect of these disorders leads to a decrease in total crop yields (KUMAR *et al.*, 2002). Plants have developed through evolution mechanisms of protection against abiotic stress. When the ambient temperature is 5°C higher than the optimal conditions for the plant, the characteristic set of cell and biochemical processes necessary for surviving the life functions of plants under high temperature conditions are activated (GUY, 1999). Reduced normal protein synthesis is triggered and accelerated transcription and translation of heat shock proteins occurs (BRAY *et al.*, 2000), as well as the production of phytohormones and antioxidants (MAESTRI *et al.*, 2002). There is also a change in the organization of cellular structures, including cytoskeleton, organelles, and membrane functions (WEIS and BERRY, 1988). An important factor in the adaptation to heat stress is the accumulation of components with a lower molecular weight, such as amino acids (SIMON-SARKADI *et al.*, 2006) including proline (KAVI-KISHOR *et al.*, 2005). Proline is an amino acid involved in several metabolic processes, although it is important as a protein stabilizer and osmolyte, it also participates in an antioxidant response as one of the non-enzymatic antioxidants and helps to remove free radicals (ASHRAF and FOOLAD, 2007). Proline can stabilize subcellular structures and membranes including photosystem II (SZABADOS and SAVOURÉ, 2010). Proline also acts as a lipid peroxidation inhibitor. In normal physiological conditions, the proline makes up less than 5% of the free amino acids in the plant, while this amount due to stress can increase to over 80% (VERBRUGGEN and HERMANS, 2008). In the upcoming period, a trend of temperature rise is predicted (NELSON *et al.*, 2010), so its impact will be significant and can lead to a decrease in cereals yield in the next decade (WHEELER and BRAUN, 2013). For this reason, it is important to find cereal varieties adapted to the conditions of heat stress, in order to ensure production and provide enough food in the future.

The aim of this study was to determine the effect of high air temperature on the content of proline and yield traits in the different wheat, triticale and oat varieties during the two vegetation seasons (2017 and 2018) and to determine if there is a correlation between the accumulation of proline and the yield traits in different cereals varieties. To compare different varieties of cereals according to proline content, as well as to determine in which cereal varieties the highest increase of proline in the conditions of high air temperature occurred compared to moderate air temperature conditions, in order to distinguish them as varieties that are more resistant to conditions of abiotic stress.

MATERIAL AND METHODS

Plant material and field data

For research, plant material of 8 genetically divergent wheat varieties (Simonida, Zvezdana, Pobeda, NS40S, Nikol, Avenu, Ortegus, Hystar), 1 variety of triticale (Odisej) and 1 oat variety (Jadar) were used. The varieties used in the experiment were planted on the experimental field of the Agricultural Station in Kraljevo (43°47′26″N; 20°38′07″E) on the meadow-valley black soil type. Sowing was done at the end of October 2016 and 2017. Leaf samples used for the analyzes were collected from the experimental field in midday (11-12 h) in June 2017 and June 2018. Samples were collected in days with moderate midday air temperatures of 24-26°C (samples were collected after a few days with air temperatures below 27°C), and after a few days in conditions of high midday air temperatures of 34-36°C (samples were collected after a few days with air temperatures exceeding 30°C). The timing of leaf gathering between moderate and high air temperatures was 6-8 days. The flag leaves of the sampled cereal varieties were used for the analysis.

Meteorological conditions

Meteorological conditions during the two vegetative seasons are shown in Table 1. During the January/June period the average air temperature values were 9.9ºC during the first and 11.3ºC during the second year of the experiment. During the second season, precipitation was more abundant with a sum of 537.4 mm compared to the first season, where it was 353.3 mm. During the phenological phase of milking development when samples were collected, the average air temperature was higher during the second season (19.9ºC) compared to the first season (19.2ºC).

Table 1. Monthly and average air temperatures and monthly and cumulative values of precipitation during vegetative seasons

Period	January	February	March	April	May	Jun	Average	Sum
Temperature, ºC								
2017	-5	4.5	10.3	11.2	16.2	22.2	9.9	59.4
2018	2.7	1.9	6.5	16.6	19	20.8	11.3	67.5
Precipitation, mm								
2017	22.1	35.3	57.7	82.1	99.9	56.2	58.9	353.3
2018	51	80.9	111.2	40.6	84.4	169.3	89.6	537.4

Proline determination

The content of the proline was determined spectrophotometrically according to the method described by BATES *et al.* (1973). The plant tissue was homogenized with a solution of 3% sulfosalicylic acid. The ninhydrin reagent and glacial acetic acid were added after filtration. Incubation was carried out at 100°C for 1h. The reaction was terminated by transferring tubes to ice, followed by addition of toluene. After separation of the toluene layer from the aqueous phase, the toluene layer with the proline was separated, transferred to the cuvette, and spectrophotometrically measured the absorbance at a wavelength of 520 nm. As a blank, pure toluene was used. The proline concentration was determined from the standard curve prepared with known proline concentrations, in the same manner as the sample. Results for content of proline of analyzed wheat, oat and triticale varieties were obtained by reading the absorbance at spectrophotometer (λ=520 nm) for each of the three test tubes.

Yield associated traits

Harvest of wheat in the experimental field was performed at the stage of full maturity in late June and early July 2017 and 2018, respectively. After harvest, the weight of dry grain and grain number per spike were measured. Yield traits were determined on a plant sample collected from an area of 1 m^2, in three repetitions with standard methods (KALUĐERSKI and FILIPOVIĆ, 1998).

Statistical analysis

Statistical analysis of data was performed using the SPSS program (IBM SPSS Statistics, Version 20, Inc. 1989-2011, USA). Proline concentrations were subjected to a two-factor analysis of variance (ANOVA) with variety and temperature as factors, followed by a Fisher's LSD multiple-range test at the significance level $p \leq 0.05$. The analysis of the correlation between proline accumulation and yield traits with the degree of significance of $p \leq 0.05$ was performed. The obtained values of proline concentration were subjected to a paired t-test with a significance level $p \leq 0.05$ (Figure 3).

RESULTS

Proline content in June 2017

In the analyzed cereal varieties under moderate air temperature (MT) conditions (24°C) in June 2017, the proline content ranged from 0.089 μmol g^{-1} (oat variety Jadar) to 0.947 μmol g^{-1} of fresh plant (fp) (wheat variety Zvezdana) (Figure 1). High proline contents under MT conditions were found in the wheat variety Pobeda (0.936 µmol g^{-1} fp), Ortegus (0.856 µmol g^{-1} fp) and Simonida (0.854 µmol g^{-1} fp). Varieties in which lower proline content was found under conditions of MT are wheat variety Avenu (0.679 µmol g^{-1} fp) and triticale variety Odisej (0.660 µmol g^{-1} fp). Low proline content in MT conditions was observed in varieties of wheat Hystar, NS40S and Nikol. Average proline content in the conditions of MT in June 2017 was 0.661 µmol g^{-1} fp.

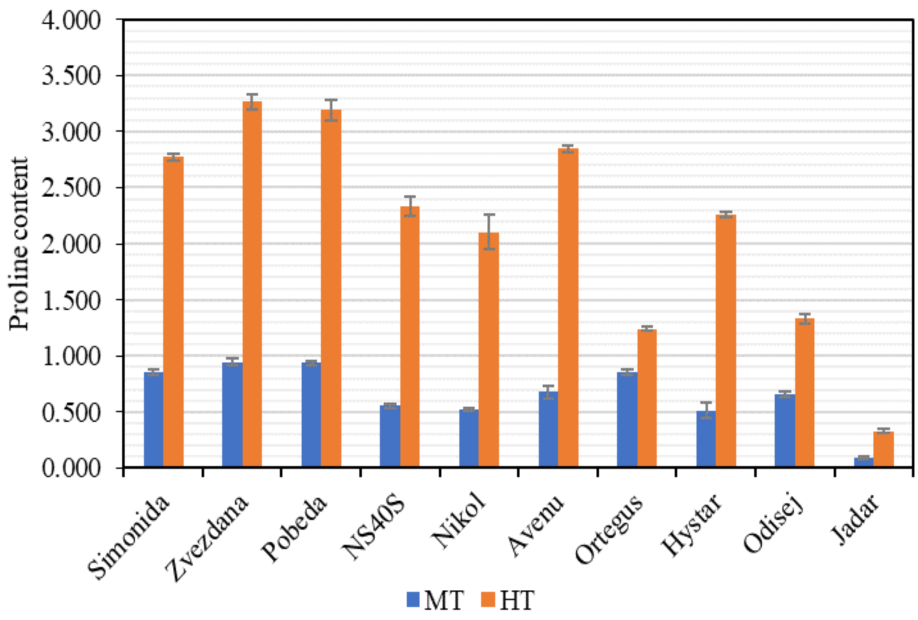

Figure 1. Proline content in leaves of 10 cereal varieties, after exposure to high (34°C) or moderate (24°C) air temperatures in June 2017, expressed in µmol g^{-1} fresh plants. MT moderate air temperature, HT high air temperature.

Under conditions of high air temperature (HT) (34°C) in June 2017, in analyzed cereal varieties, the proline content ranged from 0.332 µmol g^{-1} fp to 3.267 µmol g^{-1} fp. The highest proline content among analyzed cereal varieties under HT conditions was found in wheat variety Zvezdana, with a content of 3.267 µmol g^{-1} fp, while the lowest content of proline was found in oat variety Jadar with the content of 0.332 µmol g^{-1} fp. In other analyzed cereal varieties in conditions of HT, high content of proline was observed in the wheat varieties Pobeda (3.192 µmol g^{-1} fp), Avenu (2.845 µmol g^{-1} fp) and Simonida (2.772 µmol g^{-1} fp). Varieties in which lower proline content was found under conditions of HT are wheat varieties NS40S (2.337 µmol g^{-1} fp), Hystar (2.264 µmol g^{-1} fp) and Nicole (2.105 µmol g^{-1} fp). Low proline content in HT conditions was observed in triticale variety Odisej and wheat variety Ortegus. Average proline content in the conditions of HT in June 2017 was 2.169 µmol g^{-1} fp.

Proline content in June 2018

In the analyzed cereal varieties under MT conditions (26°C) in June 2018, the proline content ranged from 0.258 µmol g^{-1} (oat variety Jadar) to 1.046 µmol g^{-1} fresh plant (fp) (wheat variety Zvezdana). High proline contents under MT conditions were found in wheat variety Pobeda (1.016 µmol g^{-1} fp) and Simonida (0.953 µmol g^{-1} fp). Varieties in which lower proline content was found under conditions of MT are wheat variety Ortegus (0.887 µmol g^{-1} fp), Avenu (0.836 µmol g^{-1} fp) and triticale variety Odisej (0.812 µmol g^{-1} fp). Low proline content in MT conditions was observed in varieties of wheat Hystar, NS40S and Nikol. Average proline content in the conditions of MT in June 2018 was 0.777 µmol g^{-1} fp (Figure 2).

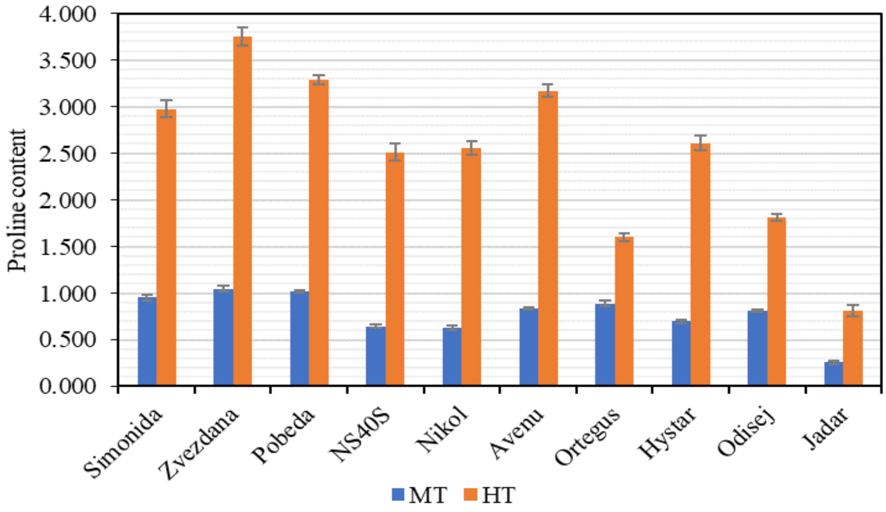

Figure 2. Proline content in leaves of 10 cereal varieties, after exposure to high (36°C) or moderate (26°C) air temperatures in June 2018, expressed in µmol g^{-1} fresh plants. MT moderate air temperature, HT high air temperature.

Under conditions of HT (35°C) in June 2018, in analyzed cereal varieties, the proline content ranged from 0.812 µmol g^{-1} fp to 3.754 µmol g^{-1} fp. The highest proline content among analyzed cereal varieties under HT conditions was found in wheat variety Zvezdana, with a content of 3.754 µmol g^{-1} fp, while the lowest content of proline was found in oat variety Jadar with the content of 0.812 µmol g^{-1} fp. In other analyzed cereal varieties in conditions of HT, high content of proline was observed in the wheat varieties Pobeda (3.294 µmol g^{-1} fp), Avenu (3.169 µmol g^{-1} fp) and Simonida (2.974 µmol g^{-1} fp). Varieties in which lower proline content was found under conditions of HT are wheat varieties Hystar (2.611 µmol g^{-1} fp), Nicole (2.555 µmol g^{-1} fp) and NS40S (2.514 µmol g^{-1} fp). Low proline content in HT conditions was observed in triticale variety Odisej and wheat variety Ortegus. Average proline content in the conditions of HT in June 2018 was 2.510 µmol g^{-1} fp.

After exposure to high (34-36°C) or moderate (24-26°C) air temperatures in June 2017 and June 2018, average value of proline content in leaves of 10 cereal varieties were calculated. Data obtained for the varieties were compared and analyzed by two-factor ANOVA, followed by the Fisher's LSD multiple-range test at significance level $p \leq 0.05$ (Figure 3).

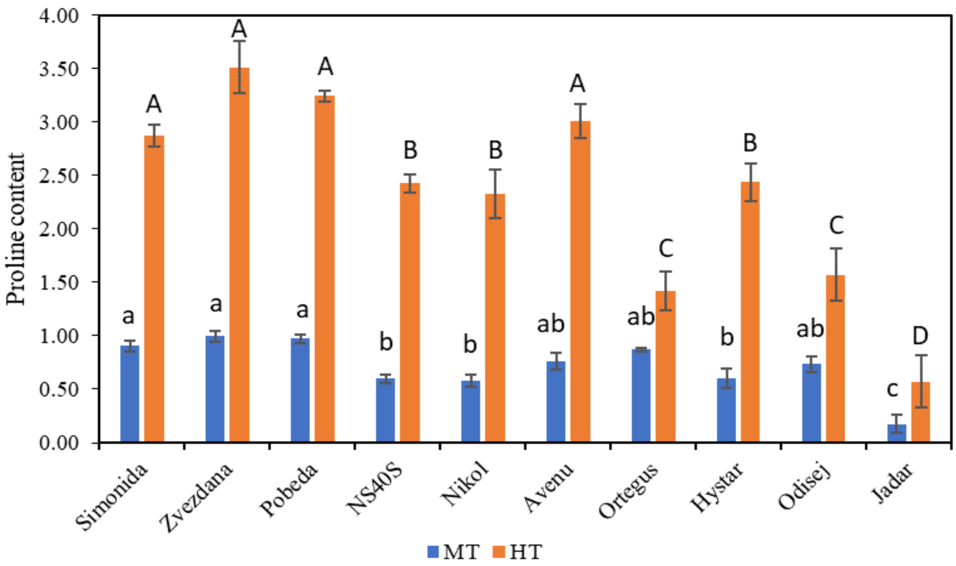

Figure 3. The average value of proline content in leaves of 10 cereal varieties, after exposure to high (34-36°C) or moderate (24-26°C) air temperatures in June 2017 and June 2018, expressed in μmol g^{-1} fresh plants. Data were analyzed by two-factor ANOVA, followed by the Fisher's LSD multiple-range test. Means labeled with different letters in the conditions of heat stress (uppercase letters) and in moderate temperature conditions (lowercase letters) are significantly different ($p \leq 0.05$). MT moderate air temperature, HT high air temperature.

In conditions of MT the obtained values of proline concentration (Figure 3) were subjected to a two-factor analysis of variance (ANOVA test) with variety and temperature as factors, followed by a Fisher's LSD multiple-range test at the significance level $p \leq 0.05$. Significant difference wasn't found between wheat varieties Simonida, Zvezdana and Pobeda. Significant difference ($p \leq 0.05$) was found between wheat varieties Simonida, Zvezdana and Pobeda compared to wheat varieties NS40S, Nicole, Hystar and oat variety Jadar. Wheat varieties Avenu, Ortegus and triticale variety Odisej was significantly different ($p \leq 0.05$) only from oat variety Jadar. Significant difference wasn't found between wheat varieties NS40S, Nicole and Hystar. Proline content in conditions of MT in oat variety Jadar was significantly differed to all analyzed cereal varieties (Table 2).

Table 2. Analyze of variance for contents of proline under moderate air temperature conditions

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.096	9	.122	15.650	.0051
Within Groups	.078	10	.008	-	-
Total	1.173	19	-	-	-

The obtained values of proline concentration were subjected to a two-factor analysis of variance (ANOVA test) with variety and temperature as factors, followed by a Fisher's LSD multiple-range test at the significance level $p \leq 0.05$.

In conditions of HT the obtained values of proline concentration were subjected to a two-factor analysis of variance (ANOVA test) with variety and temperature as factors, followed by a Fisher's LSD multiple-range test at the significance level $p \leq 0.05$ (Table 3). Significant difference wasn't found between wheat varieties Zvezdana, Pobeda and Avenu. A significant difference ($p < 0.05$) was found between wheat varieties Zvezdana, Pobeda and Avenu compared to wheat varieties NS40S, Nikol, Hystar, Ortegus, triticale variety Odisej and oat variety Jadar. Wheat variety Simonida was significantly different ($p \leq 0.05$) compared to wheat variety Ortegus, triticale variety Odisej and oat variety Jadar. Significant difference wasn't found between wheat varieties NS40S, Nicole and Hystar. Significant difference wasn't found between wheat variety Ortegus and triticale variety Odisej. Significant difference ($p \leq 0.05$) was found between varieties Ortegus and Odisej compared to all analyzed cereal varieties. Proline content under conditions of HT in oat variety Jadar was significantly different ($p \leq 0.05$) compared to all analyzed cereal varieties (Figure 3).

Table 3. Analyze of variance for contents of proline under high air temperature conditions

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.981	9	1.665	24.860	.0052
Within Groups	.670	10	.067	-	-
Total	15.651	19	-	-	-

The obtained values of proline concentration were subjected to a two-factor analysis of variance (ANOVA test) with variety and temperature as factors, followed by a Fisher's LSD multiple-range test at the significance level $p \leq 0.05$

Proline contents were subjected to a paired t test with variety and temperature as factors, at the significance level $p \leq 0.05$. Using paired t test, proline content under conditions of MT was compared to proline content under conditions of HT. Significant difference ($p \leq 0.05$) was found between proline content under MT conditions and proline content under HT conditions (Figure 3, Table 4, Table 5).

Table 4. Paired Samples Test

		Paired Differences				t	df	Sig. (2-tailed)
		Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1	MT - HT	.75240	.23793	-2.15823	-1.08177	-6.809	9	.005

MT - proline concentration under moderate air temperature; HT - proline concentration under high air temperature

Table 5. Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	MT	.7191	10	.24670	.07801
	HT	2.3391	10	.91242	.28853

MT - proline concentration under moderate air temperature; HT - proline concentration under high air temperature

The highest accumulation of proline in both seasons, in conditions of HT compared to MT conditions, was observed in wheat variety Zvezdana (2.514 µmol g^{-1} fp), while the lowest accumulation was observed in oat variety Jadar (0.399 µmol g^{-1} fp). A higher increase in the content of proline in conditions of HT compared to MT was observed in wheat variety Pobeda (2.267 µmol g^{-1} fp), Avenu (2.250 µmol g^{-1} fp) and Simonida (1.970 µmol g^{-1} fp). Lower accumulation of proline was observed in wheat variety Hystar (1.832 µmol g^{-1} fp), NS40S (1.825 µmol g^{-1} fp) and Nicole (1.756 µmol g^{-1} fp). Low accumulation of proline was observed in triticale variety Odisej and wheat variety Ortegus (Figure 4).

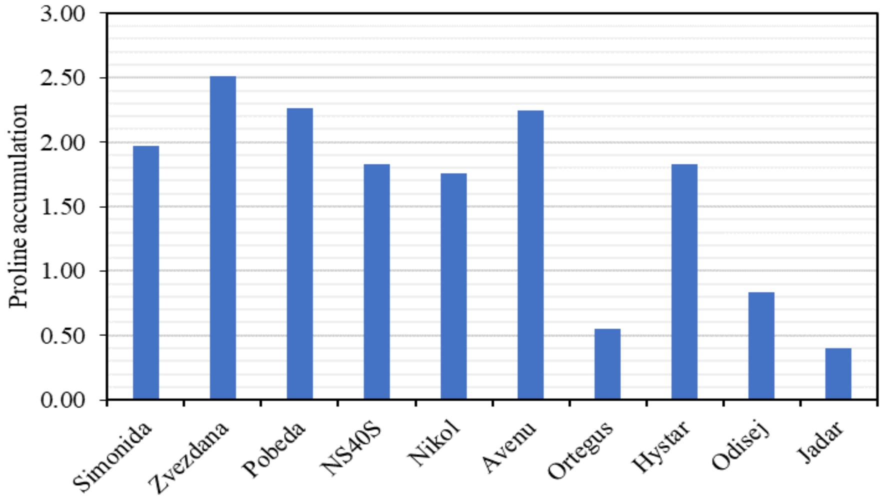

Figure 4. Average accumulation of proline in conditions of high air temperatures compared to moderate air temperatures in June 2017 and June 2018.

Yield traits

Based on the determined weight of dry grain (WDG) and grains number per spike (GNS) in 2017 and 2018, the average value of yield traits was calculated. In the analyzed cereal varieties, the average WDG ranged from 1.29 g in the Jadar variety to 2.47 g in the Zvezdana variety. Higher WDG was also observed in varieties Nikol (2.31 g) and Pobeda (2.14 g). The highest grain number per spike was observed in the Nikol variety (47.2). A larger grain number per spike was observed in the Avenu variety (45.5), Hystar (45.3) and Zvezdana variety (44.7), while the smallest grain number per spike was observed in the Ortegus variety (36.5) (Table 6).

Table 6. Average values of yield traits of ten cereal varieties from two consecutive years (2017 and 2018)

Variety	GNS	WDS
Simonida	44.2 ± 3.5	1.78 ± 0.1
Zvezdana	44.7 ± 1.3	2.47 ± 0.1
Pobeda	44.3 ± 3.7	2.14 ± 0.3
NS40S	42.5 ± 3.5	1.67 ± 0.2
Nikol	47.2 ± 2.8	2.31 ± 0.3
Avenu	45.5 ± 4.5	1.65 ± 0.2
Ortegus	36.5 ± 0.5	1.49 ± 0.1
Hystar	45.3 ± 0.7	1.90 ± 0.2
Odisej	41.0 ± 4.0	1.82 ± 0.1
Jadar	43.0 ± 2.0	1.29 ± 0.2

WDG - weight of dry grain; GNS - grain number per spike; ± standard error of mean.

Correlation between proline accumulation and yield traits

For the ten cereal varieties, average proline accumulation was correlated with average weight of dry grain and average grain number per spike under HT conditions during 2017 and 2018 (two-year average).

A significant ($p \leq 0.05$) correlation was observed between proline accumulation under HT conditions and WDG (Figure 5A). Pearson's correlation coefficient of R = 0.696 indicates a significant correlation. In varieties with a higher proline accumulation under HT conditions compared to MT conditions, a higher WDG was also observed. Correlation was also observed between proline accumulation and GNS, with statistical significance ($p \leq 0.05$) and with Pearson’s correlation coefficient of R = 686 (Figure 5B).

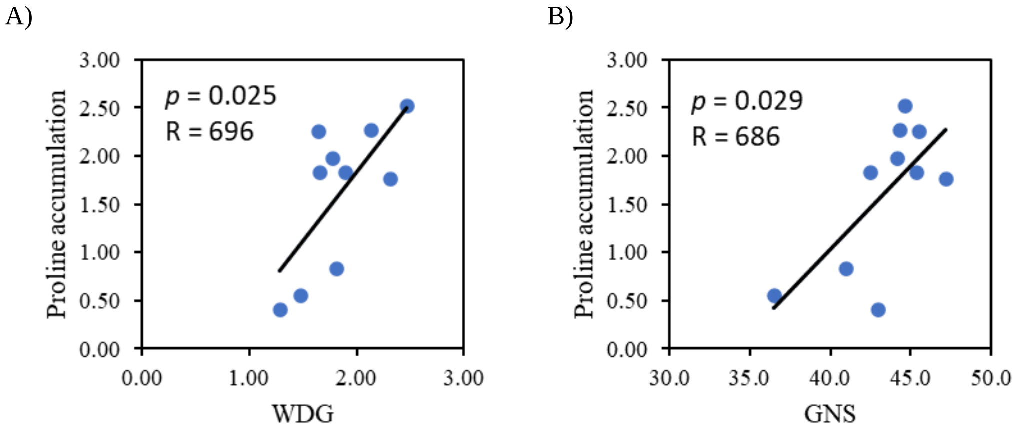

Figure 5. Correlation between average proline accumulation and average weight of dry grain (A), and grain number per spike (B) under high temperature conditions in 2017 and 2018. For the given values, statistical analysis was performed in the SPSS program. Pearson's correlation coefficient (R) was determined and significance tested by *p*-value.

DISCUSSION

In this study the effect of high air temperature on the content of proline in the different wheat, triticale, and oat varieties during the two vegetation seasons (2017 and 2018) was investigated in field experiment. In conditions of HT compared to MT conditions, accumulation of proline occurred in analyzed cereal varieties. Obtained results shown statistically significant ($p \leq 0.05$) increase in proline content under HT conditions compared to MT conditions. KHAN *et al*. (2013) also shown that proline content under heat stress conditions was increased by 84.7% compared to control. AHMED and HASAN (2011) have shown that heat tolerant wheat varieties have higher content of proline, compared to heat sensitive wheat varieties. The average value of proline in heat tolerant wheat in conditions of stress was 1.953 μmol g^{-1} fp, while the average value of proline in heat sensitive wheat under stress conditions was 1.535 μmol g^{-1} fp. Compared to research of AHMED and HASAN (2011), results from current study shown that in investigated cereal varieties content of proline was higher under HT conditions. Average proline content during the first experimental year under conditions of MT was 0.661 μmol g^{-1} fp, while in the conditions of HT the average proline content increased to 2.169 μmol g^{-1} fp, which is an increase of 1.507 μmol g^{-1} fp. Average proline content during the second experimental year under conditions of MT was 0.777 μmol g^{-1} fp, while in conditions of HT average proline content increased to 2.510 μmol g^{-1} fp, which is an increase of 1.732 μmol g^{-1} fp. The obtained results indicate that high temperature leads to an increase in proline accumulation in cereals. Higher proline values during the second year of the experiment can be associated with higher air temperatures during the vegetation season compared to the first experimental year (Table 1). Varieties that were characterized with higher proline accumulation in HT conditions was also characterized with higher WDG and GNS. Among the analyzed cereal varieties, results showed that wheat varieties are characterized by higher content of proline in moderate air temperature conditions as well as under conditions of high air temperature, compared with the content of proline in triticale and oat varieties. Many studies have shown positive effect of proline on plant metabolism and its protective role from abiotic stress in different plant spaces (VERBRUGGEN and HERMANS, 2008). Proline proved to be a good protein stabilizer, osmolyte (BOZORGMEHR and MONHEMI, 2015), participates in an antioxidant response as one of the non-enzymatic antioxidants (KUMAR *et al.*, 2002). Proline also represents a lipid peroxidation inhibitor (JAIN *et al.*, 2001). WAHID *et al.* (2007) has shown that as a result of heat stress, proline accumulation occurs as a protective mechanism in plants. To maintain a higher yield in conditions abiotic stress is becoming an increasing challenge for modern agriculture (GILL and TUTEJA, 2010). Considering the important role of proline, it is important to find varieties of cereals with higher proline content which are potentially more resistant to stressful conditions.

CONCLUSIONS

This study shows that high air temperatures led to the accumulation of proline in statistically significant concentrations in analyzed cereal varieties and that the proline could represent one of the mechanisms of cereal protection from stress caused by high air temperatures. Variation in the values of proline and yield associated traits among analyzed varieties showed differences in the adaptive response to heat stress. The results from June 2017 and June 2018 showed that among the analyzed cereal varieties, wheat varieties Zvezdana, Pobeda, Simonida and Avenu are characterized by higher contents of proline in conditions of moderate air temperature and in conditions of high air temperature, compared with the content of proline in other analyzed cereals. The highest increase in proline in both seasons, under conditions of high air temperature compared to moderate air temperature, was observed in the wheat variety Zvezdana. Statistically significant correlation between yield associated traits (WDG and GNS) and proline content shown that varieties with higher proline content have also higher yield. Varieties with higher proline content are considered as varieties that are potentially more resistant to heat stress.

ACKNOWLEDGEMENT

This work was supported by the Serbian Ministry of Education, Science and Technological Development (Agreement No. 451-03-68/2020-14/200122).

Received, April 26zh, 2020.
Accepted October 22nd 2020

REFERENCES

ADOM, K.K., R.H., LIU (2002): Antioxidant activity of grains. Journal of Agricultural and Food Chemistry, *50:* 6182-6187.

AHMED, J.U., M.A., HASAN (2011): Evaluation of seedling proline content of wheat genotypes in relation to heat tolerance. Bangladesh Journal of Botany, *40:* 17-22.

ASHRAF, M., M.R., FOOLAD (2007): Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, *59:* 206-216.

BARNABÁS, B., K., JÄGER, A., FEHÉR (2008): The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell & Environment, *31:* 11-38.

BATES, L.S., R.P., WALDREN, I.D., TEARE (1973): Rapid determination of free proline for water-stress studies. Plant Soil, *39:* 205-207.

BOZORGMEHR, M.R., H., MONHEMI (2015): How Can a Free Amino Acid Stabilize a Protein? Insights from Molecular Dynamics Simulation. Journal of Solution Chemistry, *44:* 45-53.

BRAY, E.A., J., BAILEY-SERRES, E., WERETILNYK (2000): Responses to abiotic stresses. In Buchanan B, Gruissem W and Jones R (eds) Biochemistry and Molecular Biology of Plants, pp. 1158-1203. ASPB, Rockville, MD, USA.

CIAIS, P.H., M., REICHSTEIN, N., VIOVY, A., GRANIER, J., OGÉE, V., ALLARD, M., AUBINET, C., BERNHOFER, A., CARRARA, F., CHEVALLIR, N., DE NOBLET, A.D., FRIEND, P., FRIEDLINGSTEIN, T., GRÜNWALD, B., HEINESCH, P., KERONEN, A., KNOHL, G., KRINNER, D., LOUSTAU, G., MANCA, G., MATTEUCCI, F., MIGLIETTA, J.M., OURCIVAL, D., PAPALE, K., PILEGAARD, S., RAMBAL, G., SEUFER, J.F., SOUSSANA, M.J., SANZ, E.D., SCHULZE, T., VESALA, R., VALENTINI (2005): Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature, *437:* 529-533.

GILL, S.S, N., TUTEJA (2010): Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, *48:* 909-930.

GRIFFIN, J.J., T.G., RANNEY, D.M., PHARR (2004): Heat and drought influence photosynthesis and water relations, and soluble carbohydrates of two ecotypes of redbud (*Cercis canadensis*). Journal of the American Society for Horticultural Science, *129:* 497-502.

GUY, C. (1999): The influence of temperature extremes on gene expression, genomic structure, and the evolution of induced tolerance in plants. In Lerner HR (ed). Plant Responses to Environmental Stresses, pp. 497-548. Marcel Dekker, New York, NY, USA.

JAIN, M., G., MATHUR, S., KOUL, N., SARIN (2001): Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). Plant Cell Report, *20:* 463-468.

KAVI-KISHOR, P.B., S., SANGAM, R.N., AMRUTHA, P., SRI-LAXMI, K.R., NAIDU, K.R.S.S., RAO, S., RAO, K.J., REDDY, P., THERIAPPAN, N., SREENIVASULU (2005): Regulation of Proline Biosynthesis, Degradation, Uptake and Transport in Higher Plants: Its Implications in Plant Growth and Abiotic Stress Tolerance. Current Science, *88:* 424-438.

KALUĐERSKI, G., N., FILIPOVIĆ (1998): Metode ispitivanja kvaliteta žita, brašna i gotovih proizvoda, Novi Sad, Zavod za tehnologiju žita i brašna.

KHAN, M.I., N., IQBAL, A., MASOOD, T., PER, N.A., KHAN (2013): Salicylic acid alleviates adverse effect of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signaling & Behavior, *8:* e26374.

KUMAR, R., S., VEERABHADRA, K., RAO, G.C., SRIVASTAVA (2002): Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science, *163:* 1037-1046.

LIU, X., B., HUANG (2000): Heat Stress Injury in Relation to Membrane Lipid Peroxidation in Creeping Bentgrass. Crop Science, *40:* 1-2.

MAESTRI, E., N., KLUEVA, C., PERROTTA, M., GULLI, T., NGUYEN, N., MARMIROLI (2002): Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Molecular Biology, *48:* 667-681.

MALENČIĆ, DJ., B., KIPROVSKI, V., BURSIĆ, G., VUKOVIĆ, N., HRISTOV, A., KONDIĆ-ŠPIKA (2016): Whole grain phenolics and antioxidant activity of Triticum cultivars and wild accessions. Journal of the Serbian Chemical Society, *81:* 499-508.

NELSON, G.C., M.W., ROSEGRANT, A., PALAZZO, I., GRAY, C., INGERSOLL, R., ROBERTSON, S., TOKGOZ, T., ZHU, T.B., SULSER, C., RINGLER, S.Y., MSANGI (2010): Food security, farming, and climate change to 2050: challenges to 2050 and beyond. IFPRI Issue Brief No. 66. Washington DC, USA: International Food Policy Research Institute (IFPRI).

OZDEN, M., U., DEMIREL, A., KAHRAMAN (2009): Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. Scientia Horticulturae, *119:* 163-168.

PORTER, J.R., D.J., MOOT (1998): Research beyond the means: climatic variability and plant growth. In Dalezios NR (ed). International Symposium on Applied Agrometeorology and Agroclimatology, pp. 13-23. Office for Official Publication of the European Commission, Luxembourg.

RAĐEN S. (2012): Nutrition - a role in health promotion and disease prevention. Media Center "Defense" Belgrade, pp. 103-113.

ROY, S.J., E.J., TUCKER, M., TESTER (2011): Genetic analysis of abiotic stress tolerance in crops. Current Opinion in Plant Biology, *14:* 232-239.

SIMON-SARKADI, L., G., KOCSY, A., VÁRHEGYI, G., GALIBA, J.A., DE RONDE (2006): Stress-induced changes in the free amino acid composition in transgenic soybean plants having increased proline content. Biologia plantarum, *50:* 793-796.

SINGH, N., P., VERMA, B.R., PandEY (2012): Therapeutic Potential of Organic *Triticum aestivum* L. (Wheat Grass) in Prevention and Treatment of Chronic Diseases: An Overview. International Journal of Pharmaceutical Sciences and Drug Research, *4:* 10-14.

SZABADOS, L., A., SAVOURÉ (2010): Proline: a multifunctional amino acid. Trends Plant Science, *15:* 89-97.

THOMPSON, L.U. (1994): Antioxidants and hormone-mediated health benefits of whole grains. Critical Reviews in Food Science and Nutrition, *34:* 1-473.

VERBRUGGEN, N., C., HERMANS (2008): Proline accumulation in plants: a review. Journal of Amino Acids, *35:* 753-759.

WAHID, A., S., GELANI, M., ASHRAF, M.R., FOOLAD (2007): Heat tolerance in plants: An overview. Environmental and Experimental Botany, *61:* 199-223.

WEIS, E., J.A., BERRY (1988): Plants and high temperature stress. In Long SP and Woodward FI (eds) Plants and Temperature, 1988; pp. 329-346. Company of Biologists Ltd, Cambridge, UK.

WHEELER T., J., BRAUN (2013): Climate change impacts on global food security. Science, *341:* 508-513.

TOPLOTNO-INDUKOVANA AKUMULACIJA PROLINA I KOMPONENTE PRINOSA U GENETIČKI DIVERGENTNIM SORTAMA ŽITARICA

Stefan M. MARKOVIĆ[1 *], Desimir KNEŽEVIĆ[2], Nenad M. NEŠOVIĆ[3], Nevena H. ĐUKIĆ[1]

[1] Univerzitet u Kragujevcu, Prirodno-matematički fakultet, Departman za biologiju i ekologiju, Kragujevac, Srbija
[2] Univerzitet u Prištini, Privredni fakultet, Kosovska Mitrovica, Lešak, Srbija
[3] Poljoprivredna savetodavna i stručna služba Kraljevo, Kraljevo, Srbija

.

Izvod

Na rast i razvoj žitarica utiču abiotički faktori, naročito visoka temperatura. Bitan faktor u biljnom prilagođavanju na abiotički stres može predstavljati akumulacija prolina. Prolin je aminokiselina uključena u niz metaboličkih procesa i važna je kao stabilizator proteina, osmolit i antioksidans. Cilj ovog rada bio je da se utvrdi uticaj visoke temperature na sadržaj prolina i elemente prinosa u različitim sortama žitarica tokom dve vegetacione sezone. Za istraživanje je korišćen biljni materijal 8 genetički divergentnih sorti ozime pšenice, 1 sorta pšenoraži i 1 sorta ovsa. Uzorci su prikupljeni u danima sa umerenom podnevnom temperaturom vazduha od 24-26°C u fazi mlečnog sazrevanja i nakon nekoliko dana u istoj fenološkoj fazi, u uslovima visoke podnevne temperature vazduha od 34-36°C. Akumulacija prolina određena je spektrofotometrijski. Statistička analiza podataka je urađena u SPSS programu. Rezultati u toku prve eksperimentalne godine pokazali su da je pri umerenoj temperaturi vazduha prosečni sadržaj prolina iznosio 0,661 μmol g^{-1} sveže biljke (sb), a druge eksperimentalne godine 0,777 μmol g^{-1} sb. U uslovima toplotnog stresa u toku prve godine prosečni sadržaj prolina porastao je na 2,169 μmol g^{-1} sb, a u drugoj eksperimentalnoj godini prosečan sadržaj prolina je iznosio 2,510 μmol g^{-1} sb, što potvrđuje porast akumulacije prolina u uslovima toplotnog stresa. U poređenju sa ostalim sortama žitarica, sorte pšenice Zvezdana, Pobeda, Simonida i Avenu karakterisale su se većim sadržajem prolina u uslovima toplotnog stresa u obe vegetacione sezone. Statistička analiza i korelacija rezultata akumulacije prolina i elemenata prinosa pokazala je da otpornije sorte na uslove toplotnog stresa imaju veći prinos.

Primljeno 26. IV.2020.
Odobreno 22 X 2020.