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Differences in proline accumulation between wheat varieties in response to heat stress

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ABSTRACT:
Proline is one of the organic osmolytes that accumulates in plants in response to stressful environmental conditions. The aim of this study was to determine the concentration of proline in the grain filling phase in ten winter wheat cultivars under conditions of prolonged periods of air temperatures above 30°C and to assess the variability of wheat variety responses. The correlation between proline accumulation and photosynthetic pigments, accumulated starch and yield was also determined. In the experimental field, flag leaves were sampled for each variety in the grain filling phase. Statistical data analysis and parameter correlation were performed using the SPSS program. The results showed that there is a statistically significant difference between proline values in moderate and high air temperature conditions when proline accumulation occurs. The significant correlation of accumulated starch content and yield with proline concentration showed that in addition to higher proline values under heat stress, wheat varieties Apač, Talas and Futura also have higher yield and starch content. A correlation was also found between proline concentration and photosynthetic pigment contents, where the Apač wheat variety showed the best adaptive response to the investigated traits and was characterized by a smaller reduction in photosynthetic pigment content under heat stress. The interrelation of proline with the photosynthetic pigment content and wheat quality parameters may be important in breeding technologies aimed at improving wheat stress tolerance.

Keywords:
Triticum aestivum, amino acid, photosynthetic pigments, adaptation, starch

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INTRODUCTION

Heat stress, as a complex function of intensity, duration and increase in air temperature, causes various and often adverse changes in plant growth and development, physiological processes, and yield (Hasanuzzaman *et al.* 2013; Lipiec *et al.* 2013; Siddique *et al.* 2018). Wheat (*Triticum aestivum* L.), the grain culture of the continental climate, is cultivated all over the world today and over 70% of the world's population is fed wheat bread (Sutar-Kapashikar *et al.* 2018). Annual global wheat production today stands at over 720 million tons and in order to provide food for the world's rapidly growing population, wheat production needs to be increased (Farooq *et al.* 2014; Thammana *et al.* 2016; Zhao *et al.* 2017). However, increased temperature is one of the most important environmental factors that reduce crop yield around the world (Siddiqu *et al.* 2018). Meteorological records show that the average annual temperature has risen by 1°C in those areas where wheat has been grown for centuries and is expected to rise even further. The annual global temperature is rising and an increase of 1.8 - 4.0°C is predicted by the end of the 21st century (Bita & Gerats 2013; Farooq *et al.* 2017). According to Zhao *et al.* (2017), with each degree Celsius increase in temperature the estimated average loss for wheat is 6.0 ± 2.9% of global yield. Heat stress adversely affects the entire life cycle of a wheat plant, including the vegetative and reproductive period, and in addition, also affects the plant height, leaf and stem mass, number of grains, their weight and size (Hasan *et al.* 2007; Ahamed *et al.* 2010). Heat stress in the early stages of reproductive development (meiosis in pollen stem cells) causes pollen sterility, leading to fewer grains (Fábián *et al.* 2019). High temperature, apart from morphological, also causes physiological, biochemical, and molecular changes in plants (Hasanuzzaman *et al.* 2013; Stikić *et al.* 2014; Djukić *et al.* 2019). Daily temperatures above 30°C can cause damage to plants at the molecular level, denaturation and aggregation of many proteins, cell membrane injuries (Fu *et al.* 2008), degrade chlorophyll and reduce photosynthetic capacity (Momčilović *et al.* 2016; Rehman *et al.* 2016). Thylakoid membranes are particularly sensitive to high temperatures. Heat stress causes major changes in chloroplasts such as altered thylakoid structural organization and an imbalance in photosystem activity. It also disrupts the photosynthetic reactions of electron transport and leads to increased oxidative stress, thus creating an excess of reactive oxygen species (ROS) (Tewari & Tripathy 1998; Phaniendra *et al.* 2015). The main role of chloroplast pigments is the absorption of light and its transformation into chemical energy, which in part accumulates in the form of transient starch within the chloroplast. Wheat grains can contain up to 70% starch. In the experiment carried out by Zanella *et al.* (2016), the connection between starch content and proline synthesis under conditions of osmotic stress was investigated. Their conclusion was that the carbon released during starch degradation supports the biosynthesis of proline which is involved in plant response to osmotic stress. Other authors have also examined the association between starch content and proline accumulation under stress conditions (Mohammadkhani & Heidari 2008; Bahaji *et al.* 2014; Anjorin *et al.* 2016). Research has confirmed that under stress conditions, plants need a supply of sugar as well as NADPH and ATP for the accumulation of free proline (Meena *et al.* 2019). Research has shown that the accumulation of proline is one of the best strategies for maintaining the productivity of cereals, because it acts as an osmoregulatory compound (Hayat *et al.* 2012). Proline is a protein stabilizer and antioxidant that controls free radical levels by stabilizing enzymes to remove them (Hoque *et al.* 2008). By increasing proline content to 80% of the total amino acid composition, the plant tries to maintain homeostasis for as long as possible under stress conditions, that is, the stable physiological conditions necessary for metabolic processes (Matysik *et al.* 2002). Proline synthesis contributes to the osmotic regulation of the cytoplasm as it increases acidity and positively influences the corresponding $NADP^+$/NADPH ratio (Szekely *et al.* 2008; Szabados & Savoure 2010). The proline metabolic cycle has been found to increase NADPH oxidation in many plants, thereby enhancing the oxidative pentose phosphate pathway that would support purine nucleotide biosynthesis during stress recovery (Liang *et al.* 2013). What is also important is the role of proline as a reservoir of carbon and nitrogen, and one of the key amino acids involved in pollen vitality and fertility (Biancucci *et al.* 2015; Siddique *et al.* 2018).

Considering the established role of proline in plant adaptation to conditions of abiotic stress, the aim of our research was to evaluate the variability of the reaction of the analysed wheat cultivars in conditions of prolonged air temperature above 30°C and to mutually correlate the parameters of adaptive responses: the accumulation of proline with photosynthetic pigments, starch and grain yield.

MATERIAL AND METHODS

Experimental conditions and plant material. Ten winter wheat cultivars (*Triticum aestivum* L.): Talas, Carica, Futura, Renesansa, Ratarica, Vlajna, Mila, Apač, Salasar and Hyfi, were used in the experiment which was conducted in the experimental field of the Agricultural Advisory Extension Service in Kraljevo (N 43° 47′23.2″, E 20° 32′04.6″) during the 2017/18 growing season. The varieties originate from the gene bank collection of Field and Vegetable Crops in Novi Sad, and from AS Hybrids in Belgrade. The wheat varieties were sown in a randomized block design. Within the block, each variety was sown on an experimental plot of 10 m^2. The sowing density was 500 kernels per m^2. The soil type was meadow black and the preceding crop was corn. An agrochemical analysis of the soil was done and based on the chemical composition and available content of phosphorus, potassium and nitrogen, an NPK mineral fertilizer was applied (15:15:15). The proline accumulation study of the wheat leaves was performed in three replications. Three wheat leaves per sample were collected in the experimental field. The sampling was done at one week intervals. Samples were collected on days with moderate midday air temperatures of 24-26°C (after a few days with air temperatures below 27°C). After several days of high air temperatures in the experimental field (above 30°C - heat stress conditions), samples were then collected on days with high midday air temperatures of 34-35°C. Sampling was carried out in the grain filling stage, when the cereal is the most sensitive in the field conditions. The collected leaves were immediately frozen in liquid nitrogen and preserved at -80°C. Mixed homogeneous leaf samples were made in the laboratory for the analysis of each species separately.

Climatic conditions. The meteorological parameters for the key stages of the experiment for the 2018 crop cycle and the data on minimum and maximum daily temperatures and daily precipitation amounts were obtained from the Republic Hydrometeorological Service of Serbia (RHMZS) for the Kraljevo. The climatic conditions during the growing season are shown in Table 1. During the January to June period, the average temperature was 11.3°C.

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

Period	January	February	March	April	May	June	Average	Sum
Temperature (°C)								
2018	2.7	1.9	6.5	16.6	19	20.8	11.3	67.5
Precipitation (mm)								
2018	51	80.9	111.2	40.6	84.4	169.3	89.6	537.4

Based on RHMZS data, 2018 was the warmest year since 1951. The total precipitation for 2018 was 537.4 mm.

Proline concentration analysis. The determination of proline content in the wheat leaves was done by means of the spectrophotometric method, as proposed by Bates *et al.* (1973), based on the reaction of ninhydrin with the extracted proline to produce a color whose intensity is proportional to the concentration of proline in the sample. Based on the prepared standard proline solutions, a calibration curve for proline determination was drawn (the range of concentrations from 1 to 10 μmol of proline). The proline concentration in the treated wheat varieties under conditions of moderate air temperature (control conditions) and high air temperature (heat stress) is expressed in μmol/g fresh weight (fw).

Analysis of photosynthetic pigment content. Photosynthetic pigment extraction was performed using acetone. During homogenization, a small amount of $MgCO_3$ was added to prevent acidification of the solution. The absorbance of the photosynthetic pigments was determined spectrophotometrically, at wavelengths of 662 nm, 644 nm and 440 nm (Metash UV/VIS 5100B; Metash instrument Co., Ltd). The content of chlorophyll a, chlorophyll b and carotenoids was calculated using the formula of Holm (1954) and Wetstein (1957) and expressed in mg/g fw.

Wheat quality components. The wheat in the experimental field was harvested at the full maturity stage in late June and early July of 2018. After harvest, the yield and starch concentration were assessed. Yield (t/ha) was determined on a plant sample collected from an area of 1 m^2, in three repetitions with standard methods. The determination of starch concentration in the wheat samples was performed by the spectrophotometric method according to Hansen & Moller (1975). Based on the prepared standard starch solutions, a calibration curve was prepared to determine the starch concentration. The absorbance was read at a wavelength of 630 nm. The starch concentration is expressed as a percentage of the dry weight of the plant material.

Statistical data analysis. The statistical analysis of the data was carried out using the SPSS program (IBM SPSS Statistics, Version 20, Inc. 1989-2011, USA). The obtained values of proline concentration and photosynthetic pigment content were subjected to a paired t-test with a significance level of $p \leq 0.05$ and a two-factor analysis of variance (ANOVA test) with variety and temperature as factors, followed by Fisher's LSD multiple-range test at the significance level of $p \leq 0.05$. The obtained proline concentration values were correlated with the obtained values of wheat quality components and photosynthetic pigment content with a significance level of $p \leq 0.05$.

RESULTS

Proline concentration. The results of the study showed that the concentration of proline in the wheat varieties varied in the range of 0.445 μmol/g fw to 1.034 μmol/g fw at moderate air temperature conditions (MT), while at high air temperatures (HT) the proline concentration ranged from 1.991 μmol/g fw to 3.036 μmol/g fw (Fig. 1). The highest concentration of proline under HT conditions was found in the Apač variety (3.036 μmol/g fw), while the lowest proline concentration was observed in the Re-

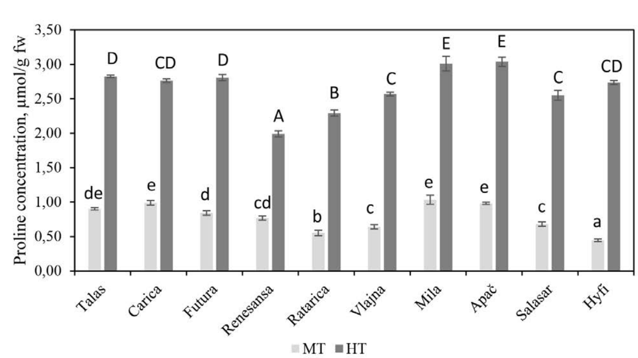

Fig 1. The average value of proline content in the leaves of 10 wheat varieties after exposure to high (35°C) or moderate (26°C) air temperatures expressed in μmol/g fw. The data were analyzed by two-factor ANOVA, followed by Fisher's LSD multiple-range test. The means labeled with different letters in 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.

Table 2. Analysis of variance for proline content under moderate air temperature conditions.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.073	9	0.119	32.977	0.0053
Within Groups	0.072	20	0.004	-	-
Total	1.146	29	-	-	-

The obtained values of proline concentration (Fig. 1) were subjected to two-factor analysis of variance (ANOVA test) with variety and temperature as factors, followed by Fisher's LSD multiple-range test at significance level $p \leq 0.05$.

Table 3. Analysis of variance for proline content under high air temperature conditions.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.785	9	0.309	34.829	0.0051
Within Groups	0.178	20	0.009	-	-
Total	2.962	29	-	-	-

The obtained values of proline concentration (Fig. 1) were subjected to two-factor analysis of variance (ANOVA test) with variety and temperature as factors, followed by Fisher's LSD multiple-range test at significance level $p \leq 0.05$.

Table 4. Mean content of photosynthetic pigments (mg/g fw) in the flag leaves of ten wheat varieties grown in the experimental field under moderate and high air temperatures.

	Moderate air temperature (26°C)			High air temperature (35°C)		
Variety	Chl *a*	Chl *b*	Caro	Chl *a*	Chl *b*	Caro
Talas	2.73 ± 0.05	1.20 ± 0.09	0.85 ± 0.05	1.46 ± 0.5	0.80 ± 0.09	0.54 ± 0.02
Carica	3.40 ± 0.13	1.36 ± 0.07	1.07 ± 0.08	1.04 ± 0.04	0.66 ± 0.06	0.67 ± 0.07
Futura	2.81 ± 0.05	1.14 ± 0.03	1.15 ± 0.07	1.25 ± 0.08	0.58 ± 0.09	0.47 ± 0.06
Renesansa	1.87 ± 0.06	0.85 ± 0.02	0.79 ± 0.03	0.10 ± 0.008	0.18 ± 0.03	0.03 ± 0.006
Ratarica	4.07 ± 0.07	1.71 ± 0.09	1.29 ± 0.10	0.15 ± 0.01	0.33 ± 0.04	0.01 ± 0.003
Vlajna	3.70 ± 0.17	1.60 ± 0.07	1.25 ± 0.03	0.17 ± 0.005	0.25 ± 0.03	0.01 ± 0.001
Mila	2.48 ± 0.12	1.23 ± 0.03	0.90 ± 0.01	0.71 ± 0.03	0.44 ± 0.04	0.32 ± 0.01
Apač	2.20 ± 0.05	0.96 ± 0.06	0.82 ± 0.03	1.95 ± 0.09	0.83 ± 0.02	1.07 ± 0.11
Salasar	3.50 ± 0.16	1.40 ± 0.09	1.17 ± 0.10	1.04 ± 0.16	0.59 ± 0.03	0.61 ± 0.01
Hyfi	1.26 ± 0.03	0.41 ± 0.008	0.61 ± 0.01	0.43 ± 0.03	0.21 ± 0.02	0.34 ± 0.01

Chl – chlorophyll; Caro – carotenoids; ± standard error of mean.

Table 5. Paired t-test of photosynthetic pigments in the flag leaves of 10 wheat varieties grown in the experimental field under conditions of moderate (26°C) and high (35°C) air temperatures.

		Mean	Std. Deviation	Std. Error Mean	t	df	Sig. (2-tailed)
Pair 1	Chl *a* MT – Chl *a* HT	1.97200	1.13648	.35939	5.487	9	.000
Pair 2	Chl *b* MT – Chl *b* HT	.69900	.42038	.13294	5.258	9	.001
Pair 3	Caro MT – Caro HT	.58300	.45444	.14371	4.057	9	.003

Chl – chlorophyll; Caro – Carotenoids; MT - Moderate air temperature; HT - High air temperature.

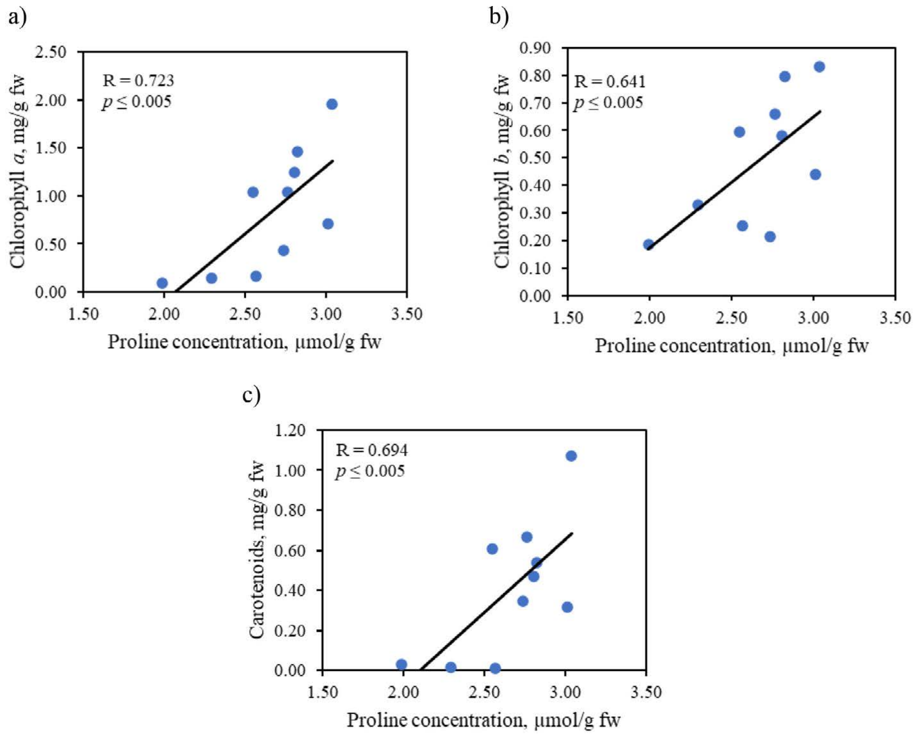

Fig. 2. Correlations between the proline concentration and the content of chlorophyll *a* (a), chlorophyll *b* (b) and carotenoids (c) under HT conditions.

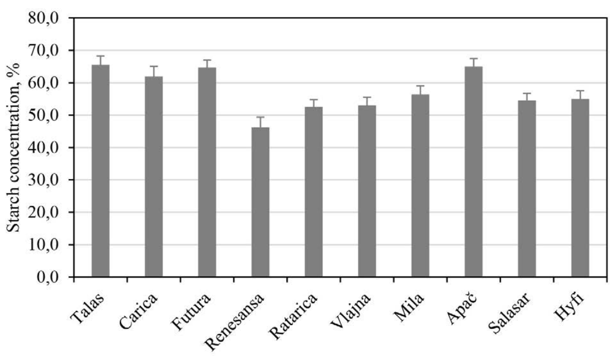

Fig. 3. Starch concentration in the analyzed wheat varieties under conditions of high air temperature expressed as a percentage of the dry weight of plant material.

naissance variety (1.991 µmol/g fw). The mean concentration of proline under MT conditions was 0.784 µmol/g fw, while the mean value of proline concentration in the samples exposed to HT was 2.657 µmol/g fw. The varieties exposed to HT had significantly higher proline concentrations in the leaves than those in MT conditions. The average proline concentration at HT compared to MT in the 10 wheat varieties increased by 1.873 µmol/g fw. The highest increase was observed in the Hyfi (2.290 µmol/g fw) and Apač (2.055 µmol/g fw) varieties.

The average values of proline content in the leaves of 10 wheat varieties, after exposure to high (35°C) or moderate (26°C) air temperatures, were analyzed by two-factor ANOVA, followed by Fisher's LSD multiple-range test (Tables 2, 3; Fig. 1). In conditions of MT a significant difference ($p \leq 0.05$) was found between the Carica, Mila and Apač wheat varieties compared to the wheat varieties Futura, Renesansa, Ratarica, Vlajna, Salasar and Hyfi. A difference was found also between Futura and Ratarica. The proline content in conditions of MT in the Hyfi variety differed from all the analyzed cereal varieties.

In conditions of HT a significant difference ($p \leq 0.05$) was found between the Vlajna and Salasar wheat varieties and the Mila, Apač, Renesansa, Futura and Talas varieties. A difference was also found between the wheat varieties Mila and Apač when compared to all the analyzed wheat varieties. The proline content under conditions of HT in the Renesansa variety differed from all the other analyzed wheat varieties. Proline concentrations were significantly different ($p \leq 0.05$) under MT conditions compared to HT conditions.

Photosynthetic pigment contents. The content of photosynthetic pigments was analysed in the flag leaves of 10 wheat varieties under HT (35°C) and MT (26°C) conditions. The wheat variety with the highest chlorophyll *a* content under MT was the Ratarica variety (4.07 mg/g fw), while the lowest chlorophyll *a* content under MT conditions was found in Hyfi (1.26 mg/g fw) (Table 4). Under MT conditions, the chlorophyll *b* content ranged from 0.41 mg/g fw (Hyfi) to 1.71 mg/g fw (Ratarica). The carotenoid content ranged from 0.61 mg/g fw (Hyfi) to 1.29 mg/g fw (Ratarica) under MT conditions (Table 4).

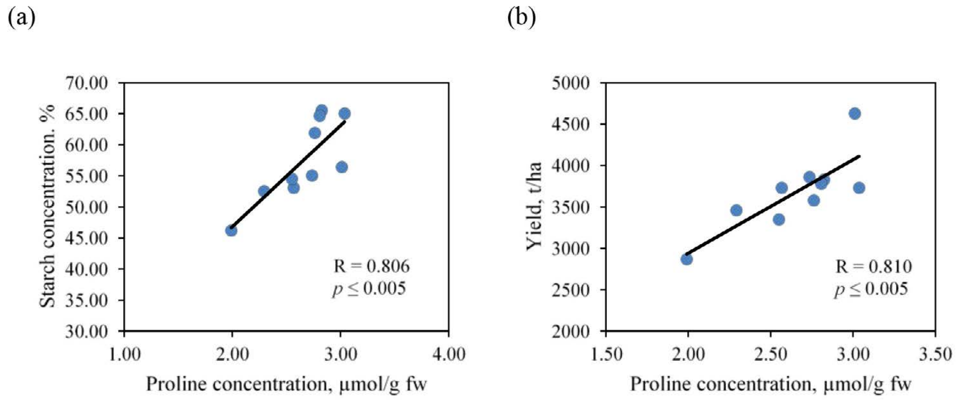

Fig. 4. Correlations between proline and starch concentration (a) and yield (b) under conditions of high air temperature.

Under HT conditions, the highest values of chlorophyll *a* (1.95 mg/g fw), chlorophyll *b* (0.83 mg/g fw) and carotenoids (1.07 mg/g fw) were observed in the Apač variety (Table 4), which indicates that Apač was characterized by higher photosynthetic activity than the other analysed varieties. In conditions of HT, the lowest content of chlorophyll *a* (0.10 mg/g fw), chlorophyll *b* (0.18 mg/g fw) and carotenoids (0.03 mg/g fw) was observed in the Renesansa variety. High photosynthetic activity under conditions of MT and HT was also observed in the Talas variety, while the Renesansa and Hyfi varieties recorded the lowest photosynthetic activity. Using a paired t-test, the photosynthetic pigment content under conditions of MT was compared to the photosynthetic pigment content under conditions of HT (Table 5). A significant difference ($p \leq 0.05$) was found between photosynthetic pigment content under MT and HT conditions.

Correlations between proline and photosynthetic pigments. For the analyzed wheat varieties, the proline concentrations were correlated with the values of photosynthetic pigment contents. A statistically significant correlation ($p \leq 0.05$) was found between the proline concentration and the content of chlorophyll *a* (Fig. 2a) under HT conditions. Pearson's correlation coefficient was 0.723. A statistically significant correlation ($p \leq 0.05$) was also found between the proline concentration and the content of chlorophyll *b* and carotenoids (Fig. 2b, c) under HT conditions. Pearson's correlation coefficients of 0.641 and 0.694 indicated that the correlation between the proline concentration and the content of chlorophyll *b* and carotenoids is high. These findings indicate that varieties with higher proline content under HT conditions also have higher photosynthetic pigment contents, thus showing that these varieties have lower stress values under HT conditions.

Wheat quality components. The obtained values of proline concentration under HT conditions were correlated with the obtained values of wheat quality components (Figs. 3, 4). There is a significant correlation ($p \leq 0.05$) between proline concentration under high temperature conditions and starch concentration (Fig. 4a). A significant correlation was also found between proline concentration under high temperature conditions and grain yield (Fig. 4b). Those varieties with higher proline concentrations under high temperatures also had batter wheat quality components.

DISCUSSION

Heat stress can impact significantly on photosynthetic pigments chlorophyll *a*, chlorophyll *b* and carotenoids. Photosynthetic pigment content may decrease under high temperature stress in wheat due to disorders in chloroplast structure and function, leading to reduced photosynthesis (Xu *et al.* 1995). Gupta *et al.* (2013) found that chlorophyll content and membrane stability index decrease under heat stress conditions. A significant correlation was found between thylakoid membrane damage, photosystem II and chlorophyll content (Ristic *et al.* 2007). In this study we found that photosynthetic pigment content was significantly lower in the investigated wheat varieties under HT conditions compared to MT conditions. Based on the obtained results, we can conclude that the Apač variety exhibited the most intensive photosynthetic activity when compared to the other analysed varieties under HT conditions. The Apač, Talas and Futura varieties, with higher photosynthetic pigment content, were also characterized by higher proline content. The positive linear correlation between photosynthetic pigment content and proline was significant. Ahmed & Hasan (2011) showed that at 35°C heat-tolerant genotypes produced more than twice (> 200%) the amount of proline than at 25°C. Proline concentration in numerous varieties was closely related to the proline concentrations in some of the ten tested samples collected in the Kraljevo area. Studies conducted to mitigate the negative effects of heat stress on photosynthesis in wheat (*Triticum aestivum*) with salicylic acid treatment showed that proline metabolism was markedly increased.

Proline biosynthesis induced by heat stress increased proline content by 84.7% compared to control, whereas proline accumulation under heat stress together with salicylic acid (0.5 mM) increased proline accumulation by 168.6% in comparison to control (Khan *et al.* 2013). In this study the statistical analysis of the obtained proline concentration in the investigated wheat varieties showed a statistically significant increase in proline content under HT conditions compared to MT conditions. Hasan *et al.* (2007) tested wheat varieties under moderate and high temperature conditions. The proline content in leaves under normal growing conditions was significantly higher in heat-tolerant varieties than in heat-sensitive varieties. Relative changes due to heat stress indicated that there was a significant increase in proline in wheat leaves relative to normal conditions (Hasan *et al.* 2007). It has been established that proline accumulation in plants under high air temperature conditions plays a significant role in the development of stress tolerance capacity as it acts as an osmoregulatory compound. The association between the accumulation of proline in the cells of different plants under heat stress conditions suggests that proline may have a protective function (Kavi-Kishor *et al.* 2005). In order to develop varieties resistant to heat stress, the determination and comparative analysis of proline concentrations with photosynthetic pigment content, starch and yield were performed on the wheat varieties. The quantitative analysis of the proline content in the leaves of ten genetically divergent wheat varieties found variability between the varieties according to proline content. In this study, the Apač variety (3.036 μmol/g fw) exhibited the highest concentration of proline under conditions of heat stress, while the smallest value was recorded for the Renaissance variety (1.991 μmol/g fw). Given that it was shown that high temperatures can affect the quality and quantity of yield elements in cereals (Viswanathan & Khanna-Chopra 2001), we wanted to determine whether varieties with a higher concentration of proline under heat stress conditions had wheat batter quality components. The Apač, Talas and Futura varieties with higher proline concentrations under HT conditions also had wheat batter quality components. Stewart *et al.* (1996) showed that the amount of sugar and starch is also important for proline metabolism. These findings are closely related to the results of this study, where it was shown that those varieties characterized by higher proline content also had higher values of starch.

CONCLUSION

Variation in the values of the proline and photosynthetic pigments of the analyzed varieties showed differences in the adaptive response to high air temperatures. The statistically significant correlation of wheat quality parameters (starch content and yield) with proline concentration showed that the wheat varieties Apač, Talas and Futura with higher proline concentrations have also higher yield and starch. A significant positive linear correlation was also found between proline concentration and photosynthetic pigment contents (chlorophyll *a*, chlorophyll *b* and carotenoids), where the wheat varieties Apač, Talas and Futura were characterized by a lower reduction in photosynthetic pigment contents. Among the analyzed wheat varieties, the Apač wheat variety showed the best adaptive response to the investigated traits.

The accumulation of proline and its interrelation with the content of photosynthetic pigments, starch and grain yield may be important in breeding technologies aimed at improving crop stress tolerance.

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REFERENCES

Ahamed KU, Nahar K, Fujita M & Hasanuzzaman M. 2010. Variation in plant growth, tiller dynamics and yield components of wheat (*Triticum aestivum* L.) due to high temperature stress. *Advances in Agriculture & Botanics* **2**: 213-224.

Ahmed JU & Hasan MA. 2011. Evaluation of seedling proline content of wheat genotypes in relation to heat tolerance. *Bangladesh Journal of Botany* **40**: 17-22.

Anjorin FB, Adejumo SA, Agboola L & Samuel YD. 2016. Proline, soluble sugar, leaf starch and relative water contents of four maize varieties in response to different watering regimes. *Agronomic Research in Moldavia* **3**: 51-62.

Bahaji A, Li J, Sánchez-López ÁM, Baroja-Fernández E, Muñoz FJ, Ovecka M, Almagro G, Montero M, Ezquer I, Etxeberria E & Pozueta-Romero J. 2014. Starch biosynthesis, its regulation and biotechnological approaches to improve crop yields. *Biotechnology Advances* **32**: 87-106.

Bates LS, Waldren RP & Teare ID. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**: 205-207.

Biancucci M, Mattioli R, Dietmar Funck G, Costantino P & Trovato M. 2015. Role of proline and GABA in sexual reproduction of angiosperms. *Frontiers in Plant Science* **6**: 680-695.

Bita CE & Gerats T. 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science* **4**: 273.

Djukić N, Knežević D, Pantelić D, Živančev D, Torbica A & Marković S. 2019. Expression of protein synthesis elongation factors in winter wheat and oat in response to heat stress. *Journal of Plant Physiology* **240**: 153015.

Fábián A, Sáfrán E, Szabó-Eitel G, Barnabás B & Jäger K. 2019. Stigma functionality and fertility are reduced by heat and drought co-stress in wheat. *Frontiers in Plant Science* **10**: 244.

Farooq M, Hussain M & Siddique KHM. 2014. Drought stress in wheat during flowering and grain-filling periods. *Critical Reviews in Plant Sciences* **33**: 331-349.

Farooq M, Nadeem F, Gogoi N, Ullah A, Alghamdi SS, Nayyar H & Siddique KHM. 2017. Heat stress in grain legumes during reproductive and grain-filling phases. *Crop and Pasture Science* **68**: 985-1005.

Fu J, Momčilović I, Clemente TE, Nersesian N, Trick HN & Ristic Z. 2008. Heterologous expression of a plastid EF-Tu reduces protein thermal aggregation and enhances CO_2 fixation in wheat (*Triticum aestivum*) following heat stress. *Plant Molecular Biology* **68**: 277-288.

Gupta NK, Agarwal S, Agarwal VP, Nathawat NS, Gupta S & Singh G. 2013. Effect of short-term heat stress on growth, physiology and antioxidative defense system in wheat seedlings. *Acta Physiologiae Plantarum* **35**: 1837-1842.

Hansen J & Moller I. 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. *Analytical Biochemistry* **68**: 87-94.

Hasan MA, Ahmed JU, Bahadur M, Haque MM & Sikder S. 2007. Effect of late planting heat stress on membrane thermostability, proline content and heat susceptibility index of different wheat cultivars. *Journal of the National Science Foundation of Sri Lanka* **35**: 109-117.

Hasanuzzaman M, Nahar K, Mahabub Alam M, Roychowdhury R & Fujita M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences* **14**: 9643-9684.

Hayat S, Hayat Q, Alyemeni MN, Wani A, Pichtel J & Ahmad A. 2012. Role of proline under changing environments. A review. *Plant Signaling & Behavior* **7**: 1456-1466.

Holm G. 1954. Chlorophyll mutations in barley. *Acta Agriculturae Scandinavica* **4**(1): 457-471.

Hoque MA, Banu MNA, Nakamura Y, Shimoishi Y & Murata Y. 2008. Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *Journal of Plant Physiology* **165**: 813-824.

Kavi-Kishor PB, Sangam S, Amrutha RN, Sri-laxmi P, Naidu KR, Rao S, Rao Krss, Reddy KJ, Theriappan P & Sreenivasulu N. 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.

Khan MI, Iqbal N, Masood A, Per T & Khan NA. 2013. Salicylic acid alleviates adverse effect of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signaling & Behavior* **8**: e26374.

Liang X, Zhang L, Kumar Natarajan S & Becker DF. 2013. Proline mechanisms of stress survival. *Antioxidants & Redox Signaling* **19**: 998-1011.

Lipiec J, Doussan C, Nosalewicz A & Kondracka K. 2013. Effect of drought and heat stresses on plant growth and yield: a review. *International Agrophysics* **27**: 463-477.

Matysik J, Alia Bhalu B & Mohanty P. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Current Science* **82**: 525-532.

Meena M, Divyanshu K, Kumar S, Swapnil P, Zehra A, Shukla V, Yadav M & Upadhyay RS. 2019. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon* **5**: e02998.

Mohammadkhani N. & Heidari R. 2008. Drought-induced accumulation of soluble sugars and proline in two maize varieties. *World Applied Sciences Journal* **3**: 448-453.

Momčilović I, Pantelić D, Zdravković-Korać S, Oljača J, Rudić J & Fu J. 2016. Heat-induced accumulation of protein synthesis elongation factor 1A implies an important role in heat tolerance in potato. *Planta* **244**: 671-679.

Phaniendra A, Jestadi DB & Periyasamy L. 2015. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry* **30**: 11-26.

Rehman US, Bilal M, Rana MR, Tahir NM, Nawaz Shah KM, Ayalew H & Yan G. 2016. Cell membrane stability and chlorophyll content variation in wheat (*Triticum aestivum*) genotypes under conditions of heat and drought. *Crop and Pasture Science* **67**: 712-718.

Ristic Z, Bukovnik U & Prasad PVV. 2007. Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. *Crop Science* **47**: 2067-2073.

Siddique A, Kandpal G & Kumar P. 2018. Proline accumulation and its defensive role under diverse stress condition in plants: an overview. *Journal of Pure and Applied Microbiology* **12**: 1655-1659.

Stewart CR, Morris CJ & Thompson JF. 1966. Changes in amino acid content of excised leaves during incubation. III. Role of sugar in the accumulation of proline in wilted leaves. *Plant Physiology* **41**: 1585-1590.

Stikić R, Jovanović Z & Prokić Lj. 2014. Mitigation of plant drought stress in a changing climate. *Botanica Serbica* **38**: 35-42.

Sutar-Kapashikar PS, Gawali TR, Koli SR, Khot AS, Dehankar SP & Patil PD. 2018. Phenolic content in *Triticum aestivum*: a review. *International Journal of New Technology and Research* **4**: 1-2.
Szabados L & Savouré A. 2010. Proline: a multifunctional amino acid. *Trends in Plant Science* **15**: 89-97.
Székely G, Ábrahám E, Cséplő A, Rigó G, Zsigmond L, Csiszár J, Ayaydin F, Strizhov N, Jásik J, Schmelzer E, Koncz C & Szabados L. 2008. Duplicated P5CS genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. *The Plant Journal* **53**: 11-28.
Tewari AK & Tripathy BC. 1998. Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. *Plant Physiology* **117**: 851-858.
Thammana M, Sreerangam S & Nambaaru S. 2016. A mini review on wheatgrass. *Journal of Pharmacognosy and Phytochemistry* **68**: 423-432.
Viswanathan C & Khanna-Chopra R. 2001. Effect of heat stress on grain growth, starch synthesis and protein synthesis in grains of wheat (*Triticum aestivum* L.) varieties differing in grain weight stability. *Journal Agronomy & Crop Science* **186**: 1-7.
Wettstein D. 1957. Chlorophyll letale und der submikroskopishe formweschsel der plastiden. *Experimental Cell Research* **12**: 427
Xu Q, Paulsen AQ, Guikema JA & Paulsen GM. 1995. Functional and ultrastructural injury to photosynthesis in wheat by high temperature during maturation. *Environmental and Experimental Botany* **35**: 43-54.
Zanella M, Borghi GL, Pirone C, Thalmann M, Pazmino D, Costa A, Santelia D, Trost P & Sparla F. 2016. β-amylase 1 (BAM1) degrades transitory starch to sustain proline biosynthesis during drought stress. *Journal of Experimental Botany* **67**: 1819-1826.
Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand JL, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, Peng S, Peñuelas J, Ruane AC, Wallach D, Wang T, Wu D, Liu Z, Zhu Y, Zhu Z & Asseng S. 2017. Temperature increase reduces global yields of major crops in four independent estimates. *Agricultural Sciences, Sustainability Science* **114**: 9326-9331.

REZIME

Botanica SERBICA

Razlike u akumulaciji prolina između sorti pšenice kao odgovor na toplotni stres

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Prolin je jedan od organskih osmolita koji se akumulira u različitim biljnim vrstama kao odgovor na stresne uslove sredine. Cilj ovog rada bio je da se odredi koncentracija prolina kod deset sorti ozime pšenice u uslovima produženog trajanja temperature vazduha iznad 30°C i da se oceni varijabilnost reakcije sorti pšenice. Ispitivana je, takođe, korelacija akumulacije prolina sa fotosintetičkim pigmentima, akumulacijom skroba i prinosom. Na eksperimentalnom polju, uzorkovani su listovi zastavičari za svaku sortu u fazi nalivanja zrna. Statistička analiza podataka i korelacija parametara izvedeni su korišćenjem SPSS programa. Rezultati su pokazali da postoji statistički značajna razlika između vrednosti prolina u uslovima umerene i uslovima povišene temperature vazduha, kada dolazi do povećane akumulacije prolina. Značajna korelacija između akumuliranog sadržaja skroba i prinosa sa koncentracijom prolina pokazala je da Apač, Talas i Futura, sorte pšenice sa većim vrednostima prolina pod toplotnim stresom imaju i veći prinos i sadržaj skroba. Utvrđena je, takođe, korelacija između koncentracije prolina i sadržaja fotosintetičkih pigmenta, gde je sorta pšenice Apač pokazala najbolji adaptivni odgovor u vezi sa ispitivanim osobinama i odlikovala se manjim smanjenjem sadržaja fotosintetičkih pigmenta u uslovima toplotnog stresa. Međusobna veza prolina sa sadržajem fotosintetičkih pigmenata i parametrima kvaliteta pšenice mogu biti važni u tehnologijama oplemenjivanja čiji je cilj poboljšanje tolerancije pšenice na stres.

Ključne reči: *Triticum aestivum*, aminokiselina, fotosintetički pigmenti, adaptacija, skrob