

## GENOTYPIC AND ENVIRONMENTAL VARIATION OF BREAD AND DURUM WHEAT PROTEINS AND ANTIOXIDANT COMPOUNDS

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### ABSTRACT

The objective of this study was to determine effects of the genotype, environment and genotype by environment interaction on the several quality and antioxidant-related traits of six bread and durum wheat genotypes bred at the Maize Research Institute, Serbia. Trials were conducted in two successive years under contrasting temperature and moisture conditions during spring growing season, thus, the environmental effects consider the influence of “hot/dry” and “cool/wet” season. On average, 1000-seed weight, protein, wet gluten and yellow pigment contents were higher in durum than in bread wheat in both years. On the other hand, the average content of  $\alpha$ -,  $\beta$ + $\gamma$ - and total tocopherols was higher in bread than in durum wheat. The content of total phenolics and antioxidant capacity was similar for both species. Higher temperatures and lower precipitations resulted in larger kernels, higher total protein content, as well as gluten, but negatively influenced antioxidant properties, total phenolics and lipid soluble antioxidants content. Based on ANOVA (analysis of variance), all sources of variation for each of the nine quality- and antioxidant-related traits were highly significant ( $P < 0.01$ ). Average variances of studied traits associated with environmental factors were generally larger (41.6%) than those for genetic factors (23.9%) and genotype by environmental interaction effects (33.6%). Especially high environment variance was recorded for antioxidant capacity. Both, genotype and genotype by environment interaction had significant effects on wet gluten and lipid soluble antioxidants.

**Key words:** antioxidant capacity, environment, genotype, protein, tocopherols, total phenolics, wheat.

### INTRODUCTION

Wheat is the most widely grown crop and traditionally has been selected for its technological functionality resulting in the selection of bread (*Triticum aestivum* L.) and durum (*Triticum durum* Desf.) wheat varieties. The flour made from the bread wheat exhibits all the characteristics and properties required for making bread. The preeminence of bread wheat in baking industry is mainly due to the presence of a unique viscoelastic gluten protein complex that makes it the best cereal grain suitable for the manufacture of leavened bread. Fractions of gluten, glutenins and gliadins, are significantly associated with bread-making quality (Shewry et al., 1992; Zhu and Khan, 2004). Durum wheat is not ideal for use in the

bread making industry due to its gluten characteristics. However, its kernel size, hardness and golden amber colour make it most suitable for manufacturing a unique and diverse range of food products such as pasta and couscous (Elias, 1995; Flagella, 2006).

In addition to baking and pasta quality, attention has been paid to the phytonutrients of wheat as potential antioxidants acting on the health benefits (Fardet et al., 2008). Health-beneficial properties of whole wheat grains have been ascribed mainly to the levels of phenolics, tocopherols and carotenoids (Adom et al., 2003; Moore et al., 2005; Mpofu et al., 2006) that are concentrated mostly in the aleurone layer with some in the pericarp, nucellar envelope and germ (Fulcher and Duke, 2002). Epidemiological evidence has supported the role of dietary antioxidants in

the prevention of several chronic diseases including cardiovascular disease, cancer, and diabetes (Willcox et al., 2004).

Previous studies have indicated that wheat quality was affected significantly by genotype, environment, and genotype by environment interaction (Lukow and McVetty 1991; Rao et al., 1993; Luo et al., 2001; Mladenov et al., 2001; Barić et al., 2004). Study on bread wheat in Serbia showed that variances of quality traits associated with genetic factors (cultivar type) were generally higher than those for cultivar by environmental interaction effects (Denčić et al., 2011). However, Taghouti et al. (2010) reported that variation due to environmental factors was higher than that of genotype and genotype by environment interaction for protein content in durum wheat grown in Morocco. Also, genotype, environment, as well as genotype by environment interactions can likely strongly influence the levels of grain antioxidants (Mpofu et al., 2006). Industry grain sourcing could be substantially improved through integrating knowledge of cultivar distributions with key environmental measures that relate to end-use quality. A basic understanding of variation among cultivars in their response to environmental stress would further improve probability of

predicting and sourcing superior quality grain for baking and other food products.

The objectives of this research were i) to evaluate the effects of genotype, environment (temperature and moisture conditions during spring growing season) and their interaction on grain quality and antioxidant capacity of six wheat genotypes recently bred at Maize Research Institute, Serbia; ii) to analyze correlations between various quality- and antioxidant-related traits. The ultimate goal is to develop a better understanding of the complex link between weather and wheat quality that could facilitate the prediction of quality in advance of harvest.

## MATERIAL AND METHODS

### Plant material and environmental parameters

The experimental material consisted of three bread (*Triticum aestivum* L.) and three durum (*Triticum durum* Desf.) wheat genotypes (breeding lines and cultivars) recently developed at the Maize Research Institute Zemun Polje (MRIZP), Serbia. The genotypes were chosen on the basis of their differences in agronomic traits such as yield and its components. Their names, origin and growth type are given in table 1.

Table 1. Name, pedigree, growth type and origin of bread and durum genotypes; country code from the UN website

Genotype	Parents (Origin)	Country	Growth type
Bread wheat			
ZP 87/I	L ZA-99 (SRB) x Pobeda (SRB)	SRB	winter
ZP Zemunska rosa	Skopljanka (MKD) x Proteinka (SRB)	SRB	winter
ZP Zlatna	Jasenica (SRB) x Rodna (SRB)	SRB	winter
Durum wheat			
ZP 34/I	SOD 55 (SVK) x Korifla (ICARDA)	SRB	facultative
ZP 10/I	Windur (DEU) x SOD 64 (SVK)	SRB	winter
ZP DSP/01	Mina (SRB) x L ZP-48/2 (SRB)	SRB	winter

ICARDA = International Center for Agricultural Research in the Dry Areas (SYR)

Grain samples of bread and durum wheat were collected from plants grown in a field-trial at the same experimental farm in 2008-2009 and 2009-2010 growing seasons. The experiment was laid out in the randomized complete block design (RCBD) with two

replications. Field plots of 5 m<sup>2</sup> with 10 rows spaced 10 cm apart were planted at a rate of 550 germinated seed per m<sup>2</sup>. The genotypes were planted in late October and harvested at the beginning of July. Standard agronomic practices were used to provide adequate

nutrition and to keep the plots free of weeds and diseases. Meteorological parameters such as temperature and precipitations were measured daily from tillering to physiological

maturity (March - June). Mean monthly minimum, maximum and average temperatures, as well as sum of precipitations are presented in Figure 1.

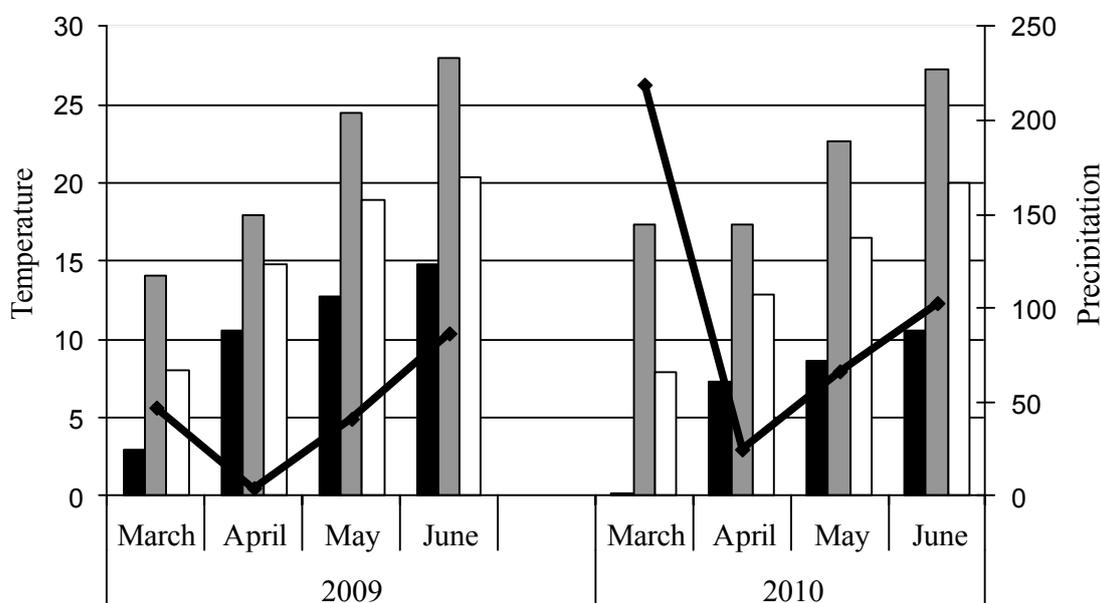


Figure 1. Sum of precipitation ( $\text{mm m}^{-2}$ ) —, minimal (■), maximal (▒) and average temperatures ( $^{\circ}\text{C}$ ) □ during the spring vegetative period (March – June) for the 2009 and 2010 years

### A) Analytical procedures

#### Determination of wet gluten and protein content

Wet gluten content was determined by washing the dough obtained from wheat flour (10 g), with 2% NaCl solution, followed by water, to remove the starch and other soluble compounds of the sample (AACC 2000). Grain protein content was determined by Kjeltac Analyzer Unit. The results are given as percentage of dry weight (DW).

#### Determination of antioxidant capacity as DPPH<sup>•</sup> scavenging activity

For the DPPH (2,2-diphenyl-1-picrylhydrazyl) test the wheat grain extract was prepared by continuous shaking 0.3 g of wholemeal in 10 mL of 70% (v/v) acetone for 30 min at room temperature. After centrifugation (10 min at 20000 g) supernatant was used for the detection of the DPPH<sup>•</sup> scavenging activity according to the Abe et al. (1998) assay. Briefly, an aliquot of extract (0.3 mL) was mixed with the DPPH reagent

(0.5 mM in ethanol, 0.25 mL) and the acetate buffer (100 mM, pH 5.5, 0.5 mL). After standing for 30 min in the dark, the absorbance was measured at 517 nm against a blank containing acetone instead of a sample. The results were expressed as an  $\text{IC}_{50}$  value that represents the amount of wholemeal (in mg of DW) providing 50% inhibition of DPPH<sup>•</sup>.

#### Determination of total phenolic content

Total phenolics were determined from the same extract as for the DPPH test by using the Folin-Ciocalteu procedure (Hagerman et al. 2000). Thus, our results are related to soluble free phenolics. Aliquots (0.2 mL) of aqueous acetone extracts were transferred into test tubes and their volumes made up to 0.5 mL with distilled water. After addition of Folin-Ciocalteu reagent (0.25 mL) and 20% aqueous sodium carbonate solution (1.25 mL), tubes were vortexed. After 40 min the absorbance was recorded at 725 nm against a blank containing only extraction solvent (0.2 mL) instead of sample. The total phenolic content

was calculated as a catechin equivalent (CE) from the calibration curve of catechin standard solutions, and expressed in mg g<sup>-1</sup> DW.

#### **Determination of total yellow pigment content**

The reference method AACC (1995) was used. Briefly, 8 g of sample was extracted with 40 ml of water-saturated 1-butanol for 30 min. After centrifugation 10 min at 13000 g the absorbance of supernatant was measured at 435 nm. The content of pigment was calculated using the conversion factor of 1.6632 and expressed as  $\beta$ -carotene (1 mg of this pigment in 100 mL water-saturated 1-butanol has optical density of 1.6632 in 1 cm cuvette at 435 nm wavelength). The total yellow pigment content is expressed as  $\mu$ g of  $\beta$ -carotene equivalent ( $\beta$ CE) g<sup>-1</sup> DW.

#### **Determination of tocopherol content**

The tocopherol content was determined by the HPLC method. Extraction was done according to Panfili et al. (2003). The separation of tocopherols was performed on a column (LichroCARTE 250 mm  $\times$  4 mm Lichrosphere 100, 5  $\mu$ m particle size). The mobile phase consisted of methanol and water in a ratio of 95:5 (v/v). Flow rate was 1.0 ml min<sup>-1</sup> at 20 °C. Peaks were detected with a Shimadzu RF-535 fluorescence detector (Shimadzu Scientific Instruments, Columbia, MD) using an excitation wavelength of 295 nm and emission wavelength of 330 nm. Amount of detected compounds were estimated from calibration curves obtained by injecting mixtures of tocopherol standard (Sigma Co. St. Louis, MO). Identified peaks were confirmed and quantified by data acquisition and spectral evaluation using "Clarify" chromatographic software. The content of tocopherols is expressed as  $\mu$ g g<sup>-1</sup> DW.

#### **B) Statistical analysis**

All chemical analyses were performed in two replicates per plot and collected data were subjected to the analysis of variance (ANOVA) set up according to the RCBD. Significant differences between genotype means

were determined by the Fisher's least significant difference (LSD) test, while a t-test was performed to test the significance of differences between the species means. Differences with  $P < 0.05$  were considered significant in both tests. The coefficient of variation (CV) was determined for each trait and Pearson's correlation coefficient was calculated between each pair of them. Relative magnitude of year, genotype and their interaction attributed to total sum of squares were calculated as percentage according to Sváb (1973).

## **RESULTS**

Average daily air temperature from tillering to physiological maturity (March - June) was higher in 2009 (15.5°C) than in 2010 (13.3°C) (Figure 1). On the other hand, total precipitation was much higher in 2010 (413.7 mm m<sup>-2</sup>) than in 2009 (179.9 mm m<sup>-2</sup>). It could be considered that plants were grown under two regimes corresponding to typical "hot/dry" (harvest 2009, common for Serbia) and "cool/wet" (harvest 2010) season.

1000-Seed weight, total protein and wet gluten content of bread and durum wheat wholemeal are shown in table 2. Generally, durum wheat had higher mass of 1000-seed weight, total protein and wet gluten contents than bread wheat in both years. The highest coefficient of variation amongst these three traits in both species was found for wet gluten content. Thus, in bread wheat samples the content of wet gluten ranged from 21.12% to 25.20% (2009) and from 17.35% to 29.25% (2010), while in durum wheat it ranked from 23.35% to 35.10% (2009) and from 20.0% to 26.85% (2010). All the parameters presented in Table 2, except wet gluten content of ZP Zlatna, were higher in samples collected in 2009 than in 2010.

Genotypic differences of antioxidant capacity, detected as DPPH<sup>•</sup> scavenging activity, were not significant, irrespective of growing season (Table 3). However "cool/wet" conditions during 2010 increased bread and durum wheat mean values of antioxidant capacity for about 30% and 23%, respectively.

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The content of extractable total phenolics was higher in durum than bread wheat, but non significant difference among durum and bread genotypes were detected (Table 3). Environmental conditions influenced total

phenolic content, thus it was significantly higher in samples collected in year 2010 than those in 2009 (in average for about 30 and 22%, in bread and durum wheat, respectively).

Table 2. Effect of “hot/dry” (2009) and “cool/wet” (2010) growing seasons on 1000-seed weight, total protein and wet gluten contents of bread and durum wheat genotypes

Genotype	1000-Seed weight (g)		Protein (% DW)		Wet gluten (% DW)	
	2009	2010	2009	2010	2009	2010
Bread wheat						
ZP 87/I	43.5 <sup>bA</sup>	39.0 <sup>bB</sup>	12.09 <sup>aA</sup>	9.02 <sup>cB</sup>	21.12 <sup>cA</sup>	17.35 <sup>cB</sup>
ZP Zlatna	41.6 <sup>cA</sup>	37.7 <sup>cB</sup>	12.12 <sup>aA</sup>	11.22 <sup>aB</sup>	22.90 <sup>bB</sup>	29.25 <sup>aA</sup>
ZP Zemunska rosa	51.0 <sup>aA</sup>	40.7 <sup>aB</sup>	11.40 <sup>bA</sup>	10.04 <sup>bB</sup>	25.20 <sup>aA</sup>	22.37 <sup>bB</sup>
CV (%)	9.86	3.47	3.34	9.86	7.99	23.28
Durum wheat						
ZP 34/I	50.1 <sup>cA</sup>	41.4 <sup>cB</sup>	14.47 <sup>aA</sup>	11.14 <sup>aB</sup>	37.10 <sup>aA</sup>	25.16 <sup>bB</sup>
ZP DSP/01	53.1 <sup>aA</sup>	40.8 <sup>bB</sup>	13.79 <sup>bA</sup>	10.04 <sup>bB</sup>	30.45 <sup>bA</sup>	26.85 <sup>aB</sup>
ZP 10/I	52.8 <sup>bA</sup>	44.4 <sup>aB</sup>	11.46 <sup>cA</sup>	9.12 <sup>cB</sup>	23.35 <sup>cA</sup>	20.00 <sup>cB</sup>
CV (%)	2.93	4.78	10.81	9.03	20.33	13.32
Mean bread wheat	45.37 <sup>bA</sup>	39.13 <sup>bB</sup>	11.87 <sup>bA</sup>	10.09 <sup>aB</sup>	23.07 <sup>bA</sup>	22.99 <sup>abA</sup>
Mean durum wheat	52.00 <sup>aA</sup>	41.96 <sup>aB</sup>	13.24 <sup>aA</sup>	10.10 <sup>aB</sup>	30.30 <sup>aA</sup>	24.00 <sup>aB</sup>

Mean values in a column (lower case) and in a row (upper case) followed by the same letter are not significantly different ( $P>0.05$ ).

Table 3. Effect of “hot/dry” (2009) and “cool/wet” (2010) growing seasons on total phenolic content and DPPH<sup>\*</sup> scavenging activity of bread and durum wheat genotypes

Genotype	DPPH <sup>*</sup> scavenging activity (IC <sub>50</sub> , mg DW)		Total phenolics (CE mg g <sup>-1</sup> DW)	
	2009	2010	2009	2010
Bread wheat				
ZP 87/I	13.41 <sup>bB</sup>	9.04 <sup>aA</sup>	1.02 <sup>aB</sup>	1.25 <sup>bA</sup>
ZP Zlatna	12.48 <sup>aB</sup>	8.82 <sup>aA</sup>	1.13 <sup>aB</sup>	1.32 <sup>bA</sup>
ZP Zemunska rosa	12.03 <sup>aB</sup>	8.82 <sup>aA</sup>	1.07 <sup>aB</sup>	1.60 <sup>aA</sup>
CV (%)	5.24	2.11	7.23	14.28
Durum wheat				
ZP 34/I	12.39 <sup>abB</sup>	10.36 <sup>bA</sup>	1.21 <sup>abB</sup>	1.58 <sup>aA</sup>
ZP DSP/01	12.12 <sup>aB</sup>	8.88 <sup>aA</sup>	1.40 <sup>aB</sup>	1.60 <sup>aA</sup>
ZP 10/I	12.16 <sup>aB</sup>	8.91 <sup>aA</sup>	1.25 <sup>abB</sup>	1.52 <sup>aA</sup>
CV (%)	1.48	8.31	10.39	8.12
Mean bread wheat	12.66 <sup>aB</sup>	8.89 <sup>aA</sup>	1.07 <sup>bB</sup>	1.39 <sup>abA</sup>
Mean durum wheat	12.22 <sup>aB</sup>	9.38 <sup>aA</sup>	1.29 <sup>aB</sup>	1.57 <sup>aA</sup>

Mean values in a column (lower case) and in a row (upper case) followed by the same letter are not significantly different ( $P>0.05$ ).

Yellow pigment content was higher in durum wheat compared to bread wheat (Table 4). Mean values for durum wheat were 4.45 and 4.57  $\mu\text{g g}^{-1}$  DW, and for bread wheat 3.01 and 3.93  $\mu\text{g g}^{-1}$  DW in 2009 and 2010, respectively. The content of  $\alpha$ - and  $\beta$ + $\gamma$ -tocopherol was presented in Table 4 (including total tocopherol content, calculated as the sum of  $\alpha$ - and  $\beta$ + $\gamma$ -tocopherol). In the year 2009, bread and durum wheat genotypes contained  $\alpha$ -tocopherol within the range of 4.9

to 7.82 and 4.46 to 6.9  $\mu\text{g g}^{-1}$  DW, respectively. In average,  $\alpha$ -tocopherol was higher in 2010 for about 63% and 14% for bread and durum wheat samples, respectively. The content of  $\beta$ + $\gamma$ -tocopherols was lower than that of  $\alpha$ -tocopherol; it was ranged from 1.18 to 2.98  $\mu\text{g g}^{-1}$  DW or 1.71 to 2.22  $\mu\text{g g}^{-1}$  DW in year 2009, in bread and durum samples, respectively. Except of ZP 10/1, higher content of  $\beta$ + $\gamma$ -tocopherols was detected in samples collected in 2010.

Table 4. Effect of "hot/dry" (2009) and "cool/wet" (2010) growing seasons on yellow pigment and tocopherols contents of bread and durum wheat genotypes

Genotype	$\alpha$ -Tocopherol ( $\mu\text{g g}^{-1}$ DW)		$\beta$ + $\gamma$ -Tocopherol ( $\mu\text{g g}^{-1}$ DW)		Total tocopherols ( $\mu\text{g g}^{-1}$ DW)		Yellow pigment ( $\mu\text{g } \beta\text{CE g}^{-1}$ DW)	
	2009	2010	2009	2010	2009	2010	2009	2010
Bread wheat								
ZP 87/I	7.82 <sup>ab</sup>	10.90 <sup>ba</sup>	2.98 <sup>aA</sup>	3.12 <sup>cA</sup>	10.80 <sup>aB</sup>	14.02 <sup>ba</sup>	3.40 <sup>aA</sup>	3.66 <sup>ba</sup>
ZP Zlatna	7.60 <sup>ab</sup>	12.40 <sup>aA</sup>	2.72 <sup>bb</sup>	16.50 <sup>aA</sup>	10.32 <sup>bb</sup>	28.32 <sup>aA</sup>	3.43 <sup>ab</sup>	4.40 <sup>aA</sup>
ZP Zemunska rosa	4.90 <sup>bb</sup>	9.80 <sup>cA</sup>	1.18 <sup>cb</sup>	4.02 <sup>ba</sup>	6.08 <sup>cb</sup>	13.82 <sup>ba</sup>	2.19 <sup>bb</sup>	3.72 <sup>ba</sup>
CV (%)	21.75	10.73	38.25	84.90	25.73	39.74	22.36	10.21
Durum wheat								
ZP 34/I	6.04 <sup>bb</sup>	7.88 <sup>ba</sup>	1.90 <sup>abA</sup>	2.15 <sup>abA</sup>	7.94 <sup>2Bd</sup>	10.03 <sup>ba</sup>	4.25 <sup>ba</sup>	4.52 <sup>ba</sup>
ZP DSP/01	6.90 <sup>ab</sup>	9.20 <sup>aA</sup>	2.22 <sup>aA</sup>	2.52 <sup>aA</sup>	9.12 <sup>1Bc</sup>	12.42 <sup>aA</sup>	4.88 <sup>aA</sup>	4.83 <sup>aA</sup>
ZP 10/I	4.46 <sup>cA</sup>	2.70 <sup>cb</sup>	1.71 <sup>ba</sup>	0.98 <sup>bb</sup>	6.17 <sup>3Ac</sup>	3.68 <sup>cb</sup>	4.25 <sup>ba</sup>	4.37 <sup>bcA</sup>
CV (%)	19.32	46.66	12.87	36.34	17.27	46.43	8.38	6.12
Mean bread wheat	6.77 <sup>ab</sup>	11.03 <sup>aA</sup>	2.29 <sup>ab</sup>	7.88 <sup>aA</sup>	9.06 <sup>ab</sup>	18.72 <sup>aA</sup>	3.01 <sup>bb</sup>	3.93 <sup>ba</sup>
Mean durum wheat	5.80 <sup>bb</sup>	6.59 <sup>ba</sup>	1.94 <sup>aA</sup>	1.89 <sup>ba</sup>	7.74 <sup>bb</sup>	8.71 <sup>ba</sup>	4.45 <sup>aA</sup>	4.57 <sup>aA</sup>

Mean values in a column (lower case) and in a row (upper case) followed by the same letter are not significantly different ( $P>0.05$ ).

Analysis of variance showed that all sources of variation were highly significant in all nine quality- and antioxidant-related traits under study (Table 5). Variance components in percentage for genotypes (G), environment (E) and their interaction (G×E) illustrate the relative contribution of each source of variation to total variance (Table 5). Generally, variance component due to environment explained most of the total variation, ranging from 7.1% ( $\beta$ + $\gamma$ -tocopherol) to 94.9% (DPPH<sup>\*</sup> scavenging activity). Variance component due to genotype explained most of the total variation for yellow pigment (55.2%) and

$\alpha$ -tocopherol (38.9%). The effect of G×E ranged from 4.8% of the total variance for DPPH<sup>\*</sup> scavenging activity to a very high 83.9% of the total variance for  $\beta$ + $\gamma$ -tocopherol.

Correlations among the different traits are shown in Table 6. Protein content had significant and positive correlation with wet gluten content ( $r=0.78^*$ ), but negative with antioxidant capacity ( $r=-0.77^*$ ).

On the other hand, antioxidant capacity had significant and positive correlation with total phenolic content ( $r=0.73^*$ ), but negative with 1000-seed weight ( $r=-0.65$ ).

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Table 5. Mean squares (MS) and variance components (VC) for genotype (G), environment (E), and interaction effects ( $G \times E$ ) for quality- and antioxidant-related traits (combined over bread and durum wheat genotypes)

Source of variation	G		E		G x E	
Degree of freedom	5		1		5	
Parameter	MS	VC	MS	VC	MS	VC
1000-Seed weight	217.63**	17.4	1576.26**	62.7	79.11**	19.9
Protein	3.70**	14.5	36.26**	69.9	1.29**	15.3
Wet gluten	78.26**	37.0	61.38**	7.7	33.46**	55.1
DPPH <sup>•</sup> scavenging activity	0.65**	0.3	65.41**	94.9	0.58**	4.8
Total phenolics	0.07**	19.4	0.53**	65.4	0.02**	10.6
Yellow pigment	1.83**	55.2	1.63**	15.9	0.37**	26.3
$\alpha$ -Tocopherol	20.54**	38.9	38.30**	28.8	6.00**	32.0
$\beta$ + $\gamma$ -Tocopherol	37.09**	9.0	45.95**	7.1	30.53**	83.9
Total tocopherols	90.65**	23.0	169.18**	22.2	49.31**	54.8

\*\* significant at  $P < 0.01$

Table 6. Pearson's correlation coefficients between quality- and antioxidant-related traits (combined over bread and durum wheat genotypes)

Trait	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1000-Seed weight (1)	0.60	0.39	-0.65	-0.30	-0.75*	-0.50	-0.65	-0.06
Protein (2)		0.78*	-0.77*	-0.41	-0.18	-0.04	-0.12	0.08
Wet gluten (3)			-0.26	0.01	-0.01	0.19	0.12	0.35
DPPH <sup>•</sup> scavenging activity (4)				0.73*	0.43	0.38	0.44	0.33
Total phenolics (5)					0.14	0.01	0.08	0.66
$\alpha$ -Tocopherol (6)						0.68	0.89**	0.12
$\beta$ + $\gamma$ -Tocopherol (7)							0.94**	0.16
Total tocopherols (8)								0.17
Yellow pigment (9)								

\* significant at  $P < 0.05$ ; \*\* significant at  $P < 0.01$

## DISCUSSION

It is now clearly established that grain quality is a function of grain composition principally in proteins, which influence the bread- and pasta-making quality traits, and phytochemicals important in a healthy diet to reduce the risk of many chronic diseases. Although the qualitative composition of the grain is genetically determined, the quantitative composition could be significantly modified by the growing conditions (Mpofu et al., 2006). Understanding the changes in flour and semolina quality due to different environments would be useful for improving

bread- and pasta-making quality. This work reports relative contributions of genotype, environment and their interaction ( $G \times E$ ) to the variation in grain quality and antioxidant capacity of six bread and durum wheat genotypes tested across two successive years under contrasting temperature and moisture conditions during spring growing season. The meteorological conditions during March - June period in 2009 were relatively common for Serbia, with moderately high average daily temperatures between 18 and 22°C during grain development (May - June). However, in 2010 plants matured at cooler and rather wet conditions.

It was previously reported that environmental conditions after anthesis primarily affect kernel size and composition (DuPont and Altenbach 2003). Our results, like other (Miezan et al., 1977; Zhu and Khan, 2001), provide the evidence that 1000-seed weight and protein content are much more influenced by environmental conditions than by genotype (Table 5). As presented in our findings, the protein content was positively associated with moderately high temperatures during grain filling what was in accordance with results of Rao et al. (1993); Uhlen et al. (1998), and negatively with relatively high amount of precipitation that also confirmed by Mpofu et al. (2006). A positive correlation between grain size and protein content in this study ( $r = 0.60$ ) has not been widely reported in the literature. However, similar to our results, Gulmezoglu et al. (2010) found an increase of protein content in bread and durum wheat grains with increase of 1000 grain weight, dependent on location and environmental conditions. Hot and dry growing conditions in the first year caused a smaller number of grains in spikelets to be formed than in the second year, which probably contributed to the larger grains due to compensation effect. Although there was positive correlation with grain size, protein content was in negative correlation with yield because of dilution effects (more starch accumulated).

Although grain protein composition depends primarily on genotype (Žilić et al., 2011), it is significantly affected by environment factors and their interactions (Triboi et al., 2000; Zhu and Khan, 2001). According to Jamieson et al. (2001), the accumulation of the different protein fractions is highly asynchronous, inferring that the protein composition of the grain changes during grain development. One of the consequences is that conditions that influence grain filling affect the balance of protein fractions. As storage proteins accumulate from approximately 6 days after anthesis to the end of grain filling (Gupta et al., 1996; Panozzo et al., 2001), lower minimal temperatures and higher precipitations during the period May - June 2010 negatively

influenced gluten synthesis. Variance components showed that the influence of  $G \times E$  for wet gluten content was dominant (55.1%) although the effect of genotype was also expressed (37.0%). Many studies have indicated that protein quality of wheat was affected significantly by temperature, but effects of temperature on storage protein composition are unclear, and may vary with genotype (DuPont and Altenbach, 2003). Field studies by Daniel and Triboi (2002) reported that environmental conditions, particularly fertilizer and temperature, affect the amount, composition and/or polymerization of the gluten proteins. They found that moderately high temperatures of 25 to 32°C lead to variation of the composition of the gliadin fraction and therefore had a positive effect on dough properties. However, higher temperatures with heat shock effect increase the ratio of gliadin to glutenin and decrease the proportion of high molecular mass glutenin polymers, resulting in weaker breadmaking quality (Zhu and Khan, 2001).

The nutritive value of grain being influenced by quality and quantities of the constituents, any changes of their proportion, influence the grain quality. Different concentrations of proteins and phytochemicals, were found in anatomic parts of the grain (Fulcher and Duke, 2002; Zhou et al., 2004; Li et al., 2005; Lampi et al., 2008). Given that storage proteins are synthesized and deposited in the starchy endosperm cells, reduction in protein content of wheat grown during “cool/wet” season probably caused decreases the amount of endosperm and consequently increases the proportions of constituents concentrated in other parts of grain. Thus, the amount of yellow pigments and phenolics which are mainly concentrated in outer part of grain and in bran layers (Li et al., 2005), respectively, was increased, as well as the content of tocopherols, found mostly in the germ. These observations are in agreement with those of Digesù et al. (2009) for carotenoids or Lampi et al. (2008) for tocopherols who found negative correlations of these lipid soluble antioxidants and grain weight. In general,  $G \times E$  effects had a strong impact on lipid soluble antioxidants.

Genotype effect was also evident, especially for yellow pigment and  $\alpha$ -tocopherol. Therefore the genotypes relatively stable to environmental factors could be potential candidates for the breeding of stable and high lipid soluble antioxidants wheat cultivars.

Phenolic compounds significantly contribute to overall antioxidant capacity of wheat grains. That has been reported in many other papers (Li et al., 2005; Žilić et al., 2010) and confirmed by the significant correlations between total phenolic content and DPPH $\cdot$  scavenging activity presented in table 6. The variations of total phenolic content among wheat varieties reported previously (Adom et al., 2003; Moore et al., 2005; Serpen et al., 2008) may be explained by genotype and environmental effects. The results presented in this work point that the effect of the environmental factors on the total phenolic content and antioxidant capacity was much higher than the effect of genotype, suggesting the potential to modulate wheat antioxidant capacity through agricultural practice. However, the contrasting effect on grain weight, protein and gluten content must also be considered. The impact of cooler and wetter conditions, although increased the content of total phenolics, as well as antioxidant capacity, resulted in crops with lower protein and gluten content that negatively influenced the technological properties for bread- and pasta-making.

## CONCLUSIONS

This study showed that all sources of variation for each of the nine quality- and antioxidant-related traits were highly significant. The influence of environment was predominant in expression of properties such as 1000-seed weight, protein and phenolic contents and DPPH $\cdot$  scavenging capacity. Thus, during cooler and wetter conditions wheat grain with higher concentration and better composition of health-beneficial phytochemicals could be expected, but with decreased protein and gluten content that negatively influence the technological properties for bakery and pasta industry. From genetic aspect, the variation attributed to the genotype was predominant for yellow

pigment and  $\alpha$ -tocopherol. Interaction component ( $G \times E$ ) was especially significant for  $\beta$ + $\gamma$ -tocopherol, wet gluten and total tocopherols. There were no significant correlations of protein and gluten contents with lipid soluble antioxidants which imply that it should be still possible to selectively breed for lines with high nutrition capacities, as well as improved diet requirements.

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