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Phytic acid, inorganic phosphorus, antioxidants in bread and durum wheat and their associations with agronomic traits

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The phytic acid (PA), inorganic phosphorus (P_i), yellow pigment (YP), water soluble phenolics (WSPH) and free protein sulfhydryl groups (PSH) of 15 *Triticum aestivum* and 15 *Triticum durum* wheats grown at six different environments were evaluated for variability, intra- and inter- relations with agronomic traits. The most variable properties in bread wheat were YP and WSPH with coefficients of variation (CV) amounting to 14.59% and 14.10%, respectively, while in durum wheat those were PSH and YP with CV of 17.59% and 15.02%, respectively. PA in durum wheat showed the least variability (CV 3.61%). Among others, positive associations were obtained between WSPH and YP in bread and durum wheat, YP and PSH, WSPH and PSH, yield (YLD) and WSPH, YLD and PSH in durum wheat, while negative associations were recorded for PA and YP, PA and WSPH, YLD and antioxidants (YP and WSPH) in bread wheat, PA and antioxidants, PA and YLD in durum wheat.

Key words: *Triticum aestivum*, *Triticum durum*, phytochemicals, multi-environment trial, variability, selection

Introduction

Wheat is eurytopic plant species with prominent polymorphism, due to wide area of cultivation, from 67°N in Norway, Finland and Russia to 45°S in Argentina (Peng et al. 2011). Approximately, 95% of wheat species cultivated worldwide are represented by hexaploid common wheat (*Triticum aestivum* L.), exploited for making bread, cookies and pastries, whereas the biggest part of the remaining 5% is tetraploid durum wheat (*Triticum durum* Desf.), used for making pasta, couscous, unleavened bread, bulgur and mote (Shewry 2009).

The increasing request of more nutritious and functional wheat products with increased health beneficial effects for disease prevention will direct future breeding programmes (Shewry and Ward 2012). Phytic acid (myo-inositol [1,2,3,4,5,6]-hexakisphosphate [InsP6]) (PA) represents an antinutritional factor, since as a polyvalent anion chelates important micronutrients such as magnesium, calcium, zinc, iron, manganese and copper, in the form of mixed salts phytates. Such a form is excreted by humans and monogastric animals leading to their deficiency in the diet (Lonnerdal 2002). Furthermore, the excessive phosphorus excreted in animal waste causes eutrophication of waters, representing a significant problem in Europe and North America (Lott et al. 2000). PA binds 65–85% of the total phosphorus of cereal seed and acts as a bottle-neck in the flux of phosphorus in agro-ecosystems (Raboy 2001). The PA content in plants varies dependently on the stage of maturity, genotype, climatic factors and location (Singh, 2008). Branković et al. (2015) found that a major source of variation for the PA content was a genotype × environment interaction (GEI), followed by genotype (G) and then environment (E) for bread wheat, and the E, GEI, and G, in descending order, for durum wheat.

Wheat naturally contains various classes of antioxidants with health-beneficial effects (Zhou and Yu 2004, Dykes and Rooney 2007). Yellow pigment (YP) is an essential factor for the quality of durum wheat grains for processing into pasta. It is defined as the content of carotenoids extracted from endosperm and is expressed as β-carotene content (mg) per 100g of dry matter. Their photo-protective role enables correct assembly of the light-harvesting complexes (Graham and Rosser 2000). As antioxidants they limit oxidative damage (Cuttriss et al. 2006), β-carotene acts as a precursor of vitamin A (Yeum and Russell 2002), and lutein and zeaxanthin have been associated with the prevention of age-related macular degeneration and cataracts (Landrum and Bone 2004). According to Clarke et al. (2006) and Blanco et al. (2011) the estimate of broad-sense heritability of yellow pigment concentration was high for durum wheat (0.78–0.96). Branković et al. (2015) observed the same hierarchy of importance of the sources of variation influencing total yellow pigment: G > GEI > E for both bread and durum wheat.

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Phenolic compounds act as free radical scavengers and exhibit antioxidant, antimutagenic, antimicrobial and anti-inflammatory effects (Drankhan et al. 2003, Žilić et al. 2009). Phenolics reduce oxidation of low density lipoproteins and prevent atherosclerosis (Meyer et al. 1998), the risk of chronic diseases (Kim et al. 2003) and cancer by decreasing oxidative stress (Drankhan et al. 2003). Free sulfhydryl groups of proteins (PSH) play an important role in plants as antioxidants (Chernikova et al. 2000), quenching free radicals and preventing the effects of stress (Vieira Dos Santos and Rey 2006), reducing the trypsin inhibitors such as the Kunitz trypsin inhibitor (Kobrehel et al. 1991), taking part in the detoxification, signal transduction and apoptosis (Prakash et al. 2009), and acting as integral parts of the cell regulatory redox system (Leustek et al. 2000). Decreased levels of thiols are noted in various medical disorders (Adams et al. 1991, Prakash et al. 2009). Several authors found environment to be more important than genotype and their interaction in variation of WSPH and PSH contents in both bread and durum wheat (Moore et al. 2006, Mpofu et al. 2006, Hadži Tašković Šukalović et al. 2013, Branković et al. 2015)

Correlations between antinutrients and antioxidants are of a particular importance, but there is lack of literature data, and their relationship is to a large extent unexplored. A response to the breeding for quality traits depends on genetic variance and heritability of the trait of interest, but also on the possible undesirable correlations with important agronomic properties such as grain yield and yield components. The aim of the present study was to assess variability of the content of phytic acid as antinutrient, inorganic phosphorus and antioxidants in different bread and durum wheat genotypes,as well as to point out the correlations among them and with agronomic traits . This will contribute to the purpose of developing wheat cultivars with improved nutritional and functional quality, and will be useful for the evaluation of the possibility of indirect selection.

Material and methods

Plant material and experimental design

The plant material used for multi-environment trial consisted of 15 bread wheat (*Triticum aestivum* L. ssp. *aestivum*) and of 15 durum wheat (*Triticum durum* Desf.) genotypes. All wheat genotypes were drawn from the gene banks of the Institute of Field and Vegetable Crops in Novi Sad and Maize Research Institute, Zemun Polje, both in Serbia. The names, codes, countries of origin, growth type and pedigree of used genotypes are listed in Supplementary Table 1. The field trials were carried out in the Institute of Field and Vegetable Crops at the Rimski Šančevi (RS) (45°19′51′′N; 19°50′59′′E), Maize Research Institute, Zemun Polje, in Zemun Polje (ZP) (44°52′N; 20°19′E) and PKB —Agricultural Corporation Belgrade, Agroeconomik Institute in Padinska Skela (PS) (44°57′N 20°26′E), all in Serbia, during two growing seasons 2010–2011 (11) and 2011–2012 (12). These field trials were set as randomised complete block design with four replications. The experimental plot consisted of 5 rows of 1 m in length with the inter-row spacing of 0.2 m. The basic plot consisted of 3 inner rows of 0.6 m^2 (3 × 0.2 × 1 m) and plant material within it was used for the analyses. According to IUSS Working Group WRB (2006), haplic chernozem (CHha) is the type of the soil at the RS and ZP locations, while humic gleysol (GLhu) is predominant in PS. A total of 45 kg nitrogen (N) ha^{-1}, 45 kg phosphorus (P) ha^{-1} and 45 kg potassium (K) ha^{-1} were applied in PS11 and PS12, while 18 kg N ha^{-1} and 78 kg P ha^{-1} were applied prior to sowing in the ZP11, ZP12, RS11 and RS12. These rates were recommended on the basis of analyses of soil chemical properties and available amounts of P, K and N in the soil. Sowing in both years was done mechanically in RS in mid-October and by hand in PS and ZP in late October and early November, respectively. In spring, with top dressing 46 kg N ha^{-1} were applied in PS11 and PS12, 92 kg N ha^{-1} in ZP12, 54 kg N ha^{-1} in ZP11, and 51 kg N ha^{-1} in RS11 and RS12. Integral protection against pests and weeds was accomplished by the proper use of adequate pesticides, its efficacy was monitored, and crop damages were avoided.

Agronomic traits

Measurements of agronomic traits were performed across all four replications and the obtained values are given in Supplementary table 2. Plots were hand-harvested at maturity, and grain yield (YLD) was determined per basic plot and expressed as kg ha^{-1}. Plant height (PH) and spike length (SL) were measured on 20 representative plants per basic plot and expressed in cm. Number of grains per spike (GNS) was determined on the 20 representative spikes per basic plot. Thousand grain weight (g) (TGW) was determined from the harvested grain as three samples of 1000 grains per replication per basic plot. The grain length (GL), grain width (GW) and grain thickness (GT) were measured by digital electronic micrometer from the samples of twenty grains per replication and expressed in mm. The productive tillering coefficient (PTC) was determined as a ratio of the number of spikes in the stage of maturation and the number of overwintered plants counted in the spring at the elementary plot per each repetition.

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Analyses of chemical properties

For the purpose of analysing the chemical properties, grains were ground in the Laboratory Mill 120 Perten (Perten, Sweden) and flour with particles size < 500 μm was produced. Chemical properties were determined spectrophotometrically with the Shimadzu UV-1601 spectrophotometer (Shimadzu Corporation, Japan) as detailed in the following paragraphs. All analysed chemical characteristics were determined in four replications and were expressed on the dry mass (DM) basis.

Determination of phytic acid (PA) and inorganic phosphorus (P_i) content

The contents of PA and P_i were determined by the method given by Dragičević et al. (2011). Flour was extracted with 5% trichloroacetic acid and then centrifuged at 12.000 rpm for 15 min at 4 °C. Wade reagent (prepared from $FeCl_3 \times 6H_2O$ and 5-sulphosalicylic acid) was added to 1.5 mL of supernatant and then absorbance was read at λ = 500 nm. P_i was determined from the same extract by adding the reagent prepared from ammonium heptamolybdate and ammonium metavanadate, and absorbance was then read at λ = 400 nm. The phytate phosphorus (P_p) content was obtained by dividing the value of PA by a factor of 3.55 (Barac et al. 2006) and consequently phytic phosphorus/inorganic phosphorus (P_p/P_i) relation was estimated.

Quantification of water soluble phenolics (WSPH)

The water soluble phenolics content was determined by the method of Simić et al. (2004) and expressed in μg of ferulic acid equivalent (FAE). Flour was extracted with double-distilled water and then centrifuged at 12000 rpm for 15 min at 4 °C. Furthermore, 0.05M $FeCl_3$ in 0.1M HCl and 0.008 M $K_3Fe(CN)_6$ were added to the sample solution, and after 25 min the absorbance was measured at λ = 722 nm.

Quantification of yellow pigment (YP)

Total YP was determined by the AACC (American Association of Cereal Chemists [1995]) method. Ground samples of 8 g were extracted with 40 ml of water-saturated 1-butanol. The sample was homogenised for 60 s, allowing to stand for 20 min at room temperature, and further homogenised for 30 s. The mixture was centrifuged at 5000 rpm for 10 min, and the absorbance of supernatant was recorded at 435 nm. The total YP content was calculated using the conversion factor of 1.6632 and expressed as μg of β-carotene equivalent (βCE) per g.

Quantification of free protein sulfhydryl groups (PSH)

The free protein sulfhydryl groups (PSH) content was determined by the method of de Kok et al. (1981) from the same extract used for phenolics determination. Then, 0.2 M potassium phosphate buffer (pH = 8.0) and 10 mM DTNB (5.5'-dithio-bis-(2-nitrobenzoic acid)) were added to the extract, and the absorbance was measured at λ = 415 nm.

Statistical analyses

Two-way fixed combined ANOVA, based on the random complete block design (RCBD), with the effects of genotype and environment as fixed ones, was used. Environment represented year × location combination. The Tukey (HSD) test was applied to find means of chemical and agronomic properties of genotypes that were significantly different from each other. Testing the difference in characteristics means between bread and durum wheat was carried out using *t*-test. The correlation analysis included Pearson's correlation coefficients. ANOVA, Tukey (HSD) test and Pearson's correlation coefficients were calculated by the use of the STATISTICA 9.0. (Statsoft 2009). The genotype-by-trait (GT) biplot (Yan and Rajcan 2002) was created within the R computing environment (R Development Core Team 2013), and was used to visualise associations of agronomic traits, PA and antioxidants, and also genotypes traits profiles.

Results and discussion

Variability of PA and P_i

The PA content ranged from 14.21 to 17.86 mg g^{-1} for bread wheat genotypes and from 14.63 to 16.78 mg g^{-1} for durum wheat genotypes (Table 1). Individually, the bread wheat genotypes with the lowest PA levels were ZP 87/Ip and ZP AU 12 (14.21 and 14.65 mg g^{-1}, respectively), which originated from Serbia and Macedonia, respectively. The lowest PA values for genotypes of durum wheat were recorded in ZP 120/I and 37ED. 7896 (14.63 and 15.26 mg g^{-1}, respectively).

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Table 1. Phytic acid, inorganic phosphorus and antioxidants of bread and durum wheat genotypes represented as mean values across six environments

Genotype	PA (mg g^{-1})	P$_i$ (mg g^{-1})	P$_p$/P$_i$	YP (µg βCE g^{-1})	WSPH (µg FAE g^{-1})	PSH (nmol g^{-1})
Triticum aestivum L. ssp. *aestivum* (bread wheat)						
Žitarka	16.23 [feg]	0.470 [b]	9.92 [j]	3.14 [f]	1095.67 [c]	87.61 [ecd]
Stephens	15.44 [h]	0.458 [c]	10.04 [ij]	4.48 [b]	1347.39 [a]	89.18 [cd]
Renan	16.04 [g]	0.430 [d]	10.73 [g]	3.44 [e]	1056.34 [d]	86.43 [ed]
Caldwell	17.86 [a]	0.475 [b]	10.74 [g]	4.00 [c]	930.68 [gf]	97.44 [ba]
Abe	17.43 [b]	0.562 [a]	8.96 [k]	3.54 [e]	1036.29 [d]	91.10 [bcd]
Auburn	16.83 [d]	0.445 [d]	10.98 [gf]	3.79 [d]	1102.53 [c]	93.94 [bc]
Frankenmuth	16.24 [fe]	0.410 [e]	11.20 [f]	4.03 [c]	1173.83 [b]	93.81 [bc]
Apache	14.88 [i]	0.415 [e]	10.19 [ih]	5.29 [a]	1156.69 [b]	87.47 [ecd]
ZP AU 12	14.65 [ji]	0.409 [f]	10.42 [h]	3.89 [dc]	987.41 [e]	80.95 [ef]
Marija	15.60 [h]	0.453 [c]	9.94 [ij]	4.58 [b]	728.02 [h]	85.72 [efd]
ZP 87/Ip	14.21 [j]	0.335 [i]	12.52 [b]	3.87 [dc]	984.52 [e]	87.13 [ecd]
Tecumseh	17.20 [c]	0.431 [d]	11.53 [e]	3.48 [e]	934.87 [f]	102.23 [a]
Pobeda	16.42 [e]	0.342 [h]	13.66 [a]	3.49 [e]	918.86 [gf]	78.95 [f]
Zemunska rosa	15.67 [h]	0.388 [g]	11.85 [d]	3.80 [d]	995.95 [e]	93.89 [bc]
Ludwig	16.04 [fg]	0.388 [g]	12.15 [c]	3.24 [f]	899.83 [g]	88.23 [cd]
Mean	16.05 [A]	0.427 [A]	10.99 [A]	3.87 [A]	1023.26 [A]	89.61 [A]
CV (%)	6.40	13.09	10.95	14.59	14.10	6.72
Min	14.21	0.335	8.96	3.14	728.02	78.95
Max	17.86	0.562	13.66	5.29	1347.39	102.23
Triticum durum Desf. (durum wheat)						
37ED.7922	15.30 [h]	0.383 [f]	11.55 [de]	5.32 [a]	884.10 [i]	87.48 [egf]
37ED.7896	15.26 [h]	0.426 [dc]	10.51 [g]	4.82 [c]	1074.18 [b]	95.56 [d]
37ED.7817	15.47 [gh]	0.476 [b]	9.57 [i]	4.24 [ed]	994.34 [fe]	49.20 [i]
Varano	16.40 [bc]	0.499 [a]	9.64 [i]	3.78 [g]	1007.21 [de]	73.18 [h]
37ED.7821	16.37 [dc]	0.467 [b]	10.19 [h]	4.05 [ef]	974.80 [fg]	86.75 [gf]
37ED.7880	16.06 [e]	0.312 [h]	14.65 [a]	4.21 [ed]	1011.59 [de]	84.80 [g]
ZP 10/I	16.20 [dce]	0.426 [dc]	11.12 [f]	4.16 [ef]	1023.21 [dc]	108.57 [b]
SOD 55	16.78 [a]	0.405 [e]	12.09 [c]	3.48 [h]	920.15 [h]	108.50 [b]
37ED./07 7803	15.71 [f]	0.392 [fe]	11.63 [d]	5.01 [b]	912.37 [h]	92.37 [ed]
DSP 66	16.11 [de]	0.371 [g]	12.39 [b]	3.49 [h]	918.18 [h]	93.13 [d]
ZP 34/I	16.61 [ba]	0.401 [e]	11.83 [dc]	3.05 [i]	949.56 [g]	91.83 [edf]
37ED.7820	15.99 [e]	0.467 [b]	9.98 [h]	4.89 [cb]	1030.31 [dc]	105.10 [cb]
37ED./07 7857	16.08 [e]	0.429 [c]	11.27 [fe]	4.39 [d]	1045.94 [c]	103.83 [cb]
37ED./07 7849	15.65 [gf]	0.410 [d]	11.13 [f]	3.98 [f]	1113.44 [a]	102.43 [c]
ZP 120/I	14.63 [i]	0.410 [d]	10.09 [h]	4.75 [c]	1124.21 [a]	114.58 [a]
Mean	15.91 [A]	0.418 [A]	11.18 [A]	4.24 [A]	998.91 [A]	93.15 [A]
CV (%)	3.61	11.23	11.75	15.02	7.32	17.59
Min	14.63	0.312	9.57	3.05	884.10	49.20
Max	16.78	0.499	14.65	5.32	1124.21	114.58

Data are averages from six replicates. Mean values in each column labelled with the same lowercase letter are not significantly different (*p*< 0.05) based on the Tukey (HSD) test. Means with the same uppercase letter are not significantly different between wheat species according to the t-test (*p*< 0.05). CV = coefficient of variation; PA = phytic acid; P$_i$ = inorganic phosphorus; P$_p$/P$_i$ = phytic phosphorus/inorganic phosphorus; YP = yellow pigment; WSPH = water soluble phenolics; PSH = free protein sulfhydryl groups

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In comparison to our results, higher mean PA contents for Indian bread and durum wheat of 23.9 mg g^{-1} and 19.3 mg g^{-1}, respectively, were reported by Yenagi and Basarkar (2008). Khan et al. (2007) found the PA range to vary from 9.8 to 21.7 mg g^{-1} for 66 mutants and cultivars of bread wheat, with the mean value of 15 mg g^{-1}. The P_i content was in the range of 0.335–0.562 and 0.312–0.499 mg g^{-1} for bread wheat genotypes and durum wheat genotypes, respectively (Table 1). The highest P_i level (0.562 mg g^{-1}) among bread wheat genotypes was found in Abe. Varano and 37ED. 7817 (0.499 and 0.476 mg g^{-1}, respectively) had the highest P_i content among durum wheat genotypes. According to Ficco et al. (2009) 84 durum wheat cultivars grown in the multi-environment trial showed different P_i means based on the classification to the three groups of cultivars: old (0.62 mg g^{-1}), modern (0.59 mg g^{-1}) and advanced (0.55 mg g^{-1}). The mean values of PA and P_i were higher in bread wheat assortment (16.05 and 0.427 mg g^{-1}, respectively) in comparison to durum wheat (15.91 and 0.418 mg g^{-1}), but not significantly ($p < 0.05$) as shown by t-test (Table 1). The high broad-sense heritability of the phytic acid content of 93.4% and 86% were observed in bread wheat by Gupta et al. (2015) and Ahmad et al. (2013), respectively. The lpa mutant Js-12-LPA of *Triticum aestivum* produced seed in which PA phosphorus represented 48.2% of seed total P, in contrast to 74.7% of seed total P of nonmutant control, Js-12-WT (Guttieri et al. 2004). The seed P_i was increased from 9.1% in Js-12-WT to 50.1% in Js-12-LPA. Unfortunately, these lpa mutations have pleiotropic effects and adversely affected the yield in wheat by reducing it by about 25% while the kernel size was smaller by approximately 3 mg (Guttieri et al. 2006, Zhao et al. 2008). The strategy of developing *lpa* crops using mutation breeding may seriously obstruct seed germination and plant performance, which will eventually affect yields (Shahzad et al. 2014). Vella (2007) discovered major QTLs responsible for the phytic acid level in *Triticum aestivum* located on chromosomes 7B, 1AS and 1AL, and also minor QTLs located on chromosomes 3B, 6A, 2B, 1D, 1B and 7D. Genetic engineering approaches to reduce PA in maize and soya bean showed the possibility to produce GMO cereal with low PA and with no effects on agronomic performance by tissue-specific silencing the expression of transporters involved in the biosynthesis of PA (Shi et al. 2007). The P_p/P_i ratio is significant because it shows bioavailability of phosphorus in humans and nonruminant animals, ranging from 8.96 to 13.66 for bread wheat assortment and from 9.57 to 14.65 for durum wheat (Table 1). The lowest P_p/P_i level among bread wheat genotypes was in Abe (8.96). Also the lowest P_p/P_i was found in 37ED. 7817 and Varano (9.57 and 9.64, respectively) for durum wheat assortment. A P_p/P_i mean value was found to be higher in durum wheat genotypes (11.18) than in bread wheat genotypes (10.99), but it was not significantly ($p < 0.05$) different.

Variability of YP

The YP content varied from 3.14 to 5.29 µg βCE g^{-1} in bread wheat genotypes and from 3.05–5.32 mg g^{-1} in durum wheat genotypes (Table 1). The highest YP contents (5.29 and 5.32 µg βCE g^{-1}) were recorded in Apache (bread wheat genotype) and 37ED. 7922 (durum wheat genotypes), respectively. As expected, a higher mean YP value was ascertained in durum in comparison to bread wheat (3.87 vs. 4.24 µg βCE g^{-1}), but the difference between them was not significant ($p < 0.05$). Solar radiation and a precipitation sum were found to be essential climatic factors influencing the YP content in bread and durum wheat (Clarke et al. 2006, Lukow et al. 2012, Branković et al. 2015). Petrova (2007) reported the YP mean value of 6.5 µg g^{-1} for the Bulgarian durum wheat assortment, comprising of 589 durum wheat breeding lines and released varieties, tested over the 1986–2005 period. The high YP content (above 8.5 ppm) is the requirement for pasta processing industry worldwide (Abdalla et al. 1995). However, Blanco et al. (2011) reported YP values of up to 10.96 µg g^{-1} for the segregant population of durum wheat evaluated at the two environments. According to Geleta and Grausgruber (2013) the YP content was in the range of 2.0–4.8 µg g^{-1} for bread wheat and 2.6–5.5 µg g^{-1} durum wheat. Lower values than ours for the YP content for genotypes of bread and durum wheat were reported by other authors (Panfili et al. 2004, Borrelli et al. 2008, Hadži-Tašković Šukalović et al. 2013), whereas higher values were also documented (Ram and Mishra 2008, Sakin et al. 2011, Mohammed et al. 2012). The location of QTLs for the YP content was proved to be at the durum wheat chromosomes 6B and 7B (Pozniak et al. 2007), 3B, 5B and 1A (Patil et al. 2008) and 7A (Singh et al. 2009). The QTLs for the YP content in bread wheat are located at chromosomes 3A (Parker et al. 1998), 7A (Singh et al. 2009) and 7B (Pozniak et al. 2007). The content of β-carotene increase was achieved by genetic transformation of rice ("golden rice") via integration of the phytoene synthase (Psy) gene from daffodil (*Narcissus pseudonarcissus* L.**)** and the carotene desaturase (CrtI) gene from the bacterium *Pantoea ananatis* (previously known as *Erwinia uredovora*) (Ye et al. 2000).

Variability of WSPH

The WSPH content ranged from 728.02 to 1347.39 µg FAE g^{-1} for bread wheat genotypes and from 884.10 to 1124.21 mg g^{-1} for durum wheat genotypes (Table 1). Among bread wheat genotypes cultivar Stephens expressed the highest WSPH content (1347.39 µg FAE g^{-1}). The highest WSPH content among durum wheat genotypes was found in ZP 120/I and 37ED./07 7849 (1124.21 and 1113.44 µg FAE g^{-1}, respectively). The WSPH mean value was higher in bread (1023.26 µg FAE g^{-1}) than in durum wheat (998.91 µg FAE g^{-1}), but not significantly different ($p < 0.05$). According to Žilić et al. (2011) the total phenolics content for bread wheat was in the range of 1.02–1.60 mg CE g^{-1}

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with the mean value of 1.30 mg CE g^{-1}, and for durum wheat 1.52–1.65 mg CE g^{-1} with the mean value of 1.58 mg CE g^{-1} with the similar CV as in our study for both species. Žilić et al. (2013) documented 1.19-fold higher total phenolic compounds in durum wheat samples than in bread wheat samples and the average value of 1.20 CE mg g^{-1} (ranged from 0.99 to 1.60) and 1.43 CE mg g^{-1} (ranged from 1.27 to 1.65) in bread and durum wheat, respectively. A lower total phenolics content than reported in our investigation was found by Dinelli et al. (2009), whereas higher values were also reported (Abdel-Aal and Rabalski 2008, Hadži-Tašković Šukalović et al. 2013, Pasqualone et al. 2014). Vences et al. (1982) studying *Triticum aestivum* L. found QTLs for the phenolic compounds to be located on the chromosomes or chromosome arms 1 B, 2 BL, 3 BL, 5 A, 6 AL, 7 B and 7 DS.

Variability of PSH

The PSH content varied from 78.95 to 102.23 nmol g^{-1} in bread wheat genotypes and from 49.20–114.58 nmol g^{-1} in durum wheat genotypes (Table 1). The highest PSH content recorded in bread wheat genotypes (Tecumseh), i.e. durum wheat genotypes (ZP 120/I) amounted to 102.23, i.e. 114.58 nmol g^{-1}, respectively. The mean value of PSH was higher in the durum assortment (93.15 nmol g^{-1}) in comparison to bread wheat (89.61 nmol g^{-1}) although not significantly ($p < 0.05$). Purna (2010) obtained lower values for bread wheat samples ranging from 8.17 to 34.91 nmol g^{-1}. According to Antes and Wieser (2000) the highest PSH content found in commercial wheat flour was in the SDS soluble glutenins, then water soluble proteins and then in globulins. Quantitative trait loci (QTLs) controlling amino acid cysteine containing a free sulfhydril group in *Triticum aestivum* L. was reported to be on chromosomes 2 D and 3 D (Jiang et al. 2013).

Correlation analysis

Correlations between PA, antioxidants and agronomic traits of bread and durum wheat, are of particular importance for breeding varieties for functional food purpose, but their relationships remain to a large extent unexplored. Highly significant, positive, medium high to high, consistent correlations across four environments were observed for PA and P$_i$ in bread wheat (Supplementary Table 3). Israel et al. (2006) also detected a positive correlation between PA and P$_i$ (0.72, $p < 0.01$) among wild-type soya bean lines of the maturity group V, which was in our reported interval. Significant, positive, medium high to high, consistent correlations across two environments were found between PSH and YP in durum wheat (Supplementary Table 4). Antioxidants in wheat and among them YP and PSH are concentrated mostly in the aleurone layer of bran with some in the pericarp, nucellar envelope, and germ (Moore et al. 2005). The same climatic factors as increased precipitation in later phases of vegetation season and also less solar radiation contribute to the higher levels of PSH and YP in durum wheat as reported by Branković et al. (2015). Significant, negative, medium correlations were revealed for PA and YP in two environments, P$_p$/P$_i$ and WSPH in single environment in bread wheat (Supplementary Table 3), and also for PA and WSPH in two environments in durum wheat (Supplementary Table 4). As reported by Branković et al. (2015) genotype was the most important source of variation for YP, and GEI was for PA in bread wheat. At the two environments RS11 and RS12 the correlation between YP and PA was negative, probably due to inverse effects of the highest observed sunshine hours duration and the lowest precipitations during May and June at this location in both growing seasons in comparison to the other two locations. The same climatic factors probably influenced P$_i$ and WSPH, but in the same direction leading to negative correlation between P$_p$/P$_i$ and WSPH, as it was shown previously by Branković et al. (2015). The negative correlations observed between PA and WSPH can be explained with the effects of decreased precipitation and sunshine hours at the ZP11 and ZP12 environments in comparison to the other environments, which contributed to the higher PA and smaller WSPH levels in durum wheat in these environments (Branković et al. 2015). It is of special interest to determine the relation between PA as antinutrient and WSPH because according to Dykes and Rooney (2007) this antioxidant contributes significantly to the total antioxidant wheat grain activity. Mladenović Drinić et al. (2009) reported a low to medium positive correlation between PA and WSPH, as well as between PA and PSH in maize. Liu et al. (2006) found a significant correlation between PA and P$_i$ (r = 0.43, $p < 0.01$). The pairs of significantly positively correlated agronomic and chemical characteristics with a medium correlation were: PH and PA in bread wheat in two environments (Supplementary Table 3) and GW and PSH in durum wheat in two environments (Supplementary Table 4). The pairs of significantly and highly significantly, negatively medium to medium high correlated agronomic and chemical characteristics in two different environments were: GL and PA, SL and PA, SL and P$_i$ in bread wheat (Supplementary Table 3); and TGW and YP, GT and YP, GW and YP for durum wheat (Supplementary Table 4). Ficco et al. (2009) using the principal component analysis and the Pearson's correlation found a negative association of P$_i$ and TGW (–0.31 $p < 0.01$)

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and of P_i and YLD (–0.27 p < 0.05) in durum wheat, while similar findings were confirmed by other studies (Monasterio and Graham 2000, Liu et al. 2006). According to Lee at al. (2014) phytase activity affecting the PA content in wheat appeared to be determined by the grain length to width ratio. The shape and size of wheat grains affect the test weight, protein content, the activity of hydrolytic enzymes, which affect the baking quality and the quality of the final product (Evers 2000). Clarke et al. (2006) found a variable, weak, and a negative correlation between the kernel weight and YP (–0.45, p < 0.05) in durum wheat while in our study the TGW and YP correlation was stronger (–0.614, p < 0.05 and –0.666, p < 0.05).

Genotype by trait analyses of interrelationship between chemical and agronomic characteristics from multi-environment trial

Based on the GT analysis for the six environments used in our investigation for bread and durum wheat (Figs. 1 and 2), the possibilities were deduced that could be used for breeding and improving the properties of PA and antioxidants content in wheat. The favourable direction is reducing PA and increasing the antioxidant content of bread and durum wheat genotypes. A GT biplot can help comprehend the relationships among breeding objectives and identify traits that can be used in indirect selection for another trait (Yan and Tinker 2006). We took into account associations of characteristics only if they were recorded in four and more test-environments studied. A positive association was determined between WSPH and YP and PA and P_i in bread wheat and durum wheat (Figs. 1 and 2), while correlation coefficients confirmed this only for durum wheat (Supplementary Table 4). PA and PSH and P_i and PSH in bread wheat were positively associated (Fig. 1). Also, positive associations were found for WSPH and PSH and WSPH and P_i in durum wheat (Fig. 2), which is in accordance with the observed correlation coefficients (Supplementary Table 4). A negative association was determined between PA and YP and PA and WSPH in bread wheat (Fig. 1), and also between PA and each of examined antioxidants (YP, WSPH, PSH) in durum wheat (Fig. 2). YP and PSH were negatively associated in bread wheat (Fig. 1) and positively in durum wheat (Fig. 2), which corresponded with the observed correlation coefficients for durum wheat (Supplementary Table 4). The relation between PSH and WSPH was less pronounced, while a positive relationship existed in three environments, but also a negative one existed in three environments for bread wheat (Fig. 1). YLD had a negative association with each of the examined antioxidants PSH, WSPH (confirmed also by the correlation coefficient) (Supplementary Table 3), YP in bread wheat (Fig. 1). YLD showed a negative association with YP and PA (Fig. 2) (confirmed also with the correlation coefficient) (Supplementary Table 4) in durum wheat. YLD was positively associated with WSPH and PSH in durum wheat (Fig. 2). Effects of climatic factors associated to drought stress and high temperature during grain filling may lead to high phenolic content (Menga et al. 2010) and PSH content (Branković et al. 2014), but to decreased yield in bread wheat, whereas durum wheat is capable of enduring these conditions without significant yield reductions. In bread wheat, the association between YLD and PA was not consistent and was positive in three environments (Fig. 1), but also negative in three environments. The correlation observed between these traits was significant but negative. Such correlation indicates that the development of high-yielding genotypes with a lower PA should be an attainable goal in maize according to Mladenović Drinić et al. (2009).

SL was positively associated with YP in bread and durum wheat (Figs. 1 and 2) what was confirmed with the correlation coefficient for durum wheat (Supplementary Table 4), and also with WSPH and P_i (Fig. 2), but only in durum wheat. PH had a positive relation with PSH and PA (Fig. 1) (also confirmed by the correlation coefficients, Supplementary Table 3), in bread wheat, and with each of the examined antioxidants YP, WSPH (also confirmed by the correlation coefficients, Supplementary Table 4) and PSH (Fig. 2) (also confirmed by the correlation coefficient, Supplementary Table 4) in durum wheat. PTC was positively associated with PA (Fig. 1) in bread wheat and with WSPH and PSH (Fig. 2) in durum wheat. GL showed a positive association with P_i and with each of the examined antioxidants PSH, YP and WSPH (Fig. 2) in durum wheat. Geleta and Grausgruber (2013) reported a negative association of YP and TGW based on the GT analysis for both, bread and durum wheat. GNS was positively associated with YP and with PSH in durum wheat (Fig. 2). GW, GT, and TGW, each, were positively associated with PSH (Fig. 2) in durum wheat, but the correlation coefficient confirmed only the association between TGW and PSH (Supplementary Table 4). GW and PA and also GT and PA (Fig. 2) were positively associated in durum wheat. In contrast to conventional breeding, biofortification aims to positively affect human micronutrient status, merging breeding with nutrition and socioeconomics research, to improve health characteristics (Cakmak et al. 2010) while maintaining favourable agronomic characteristics.
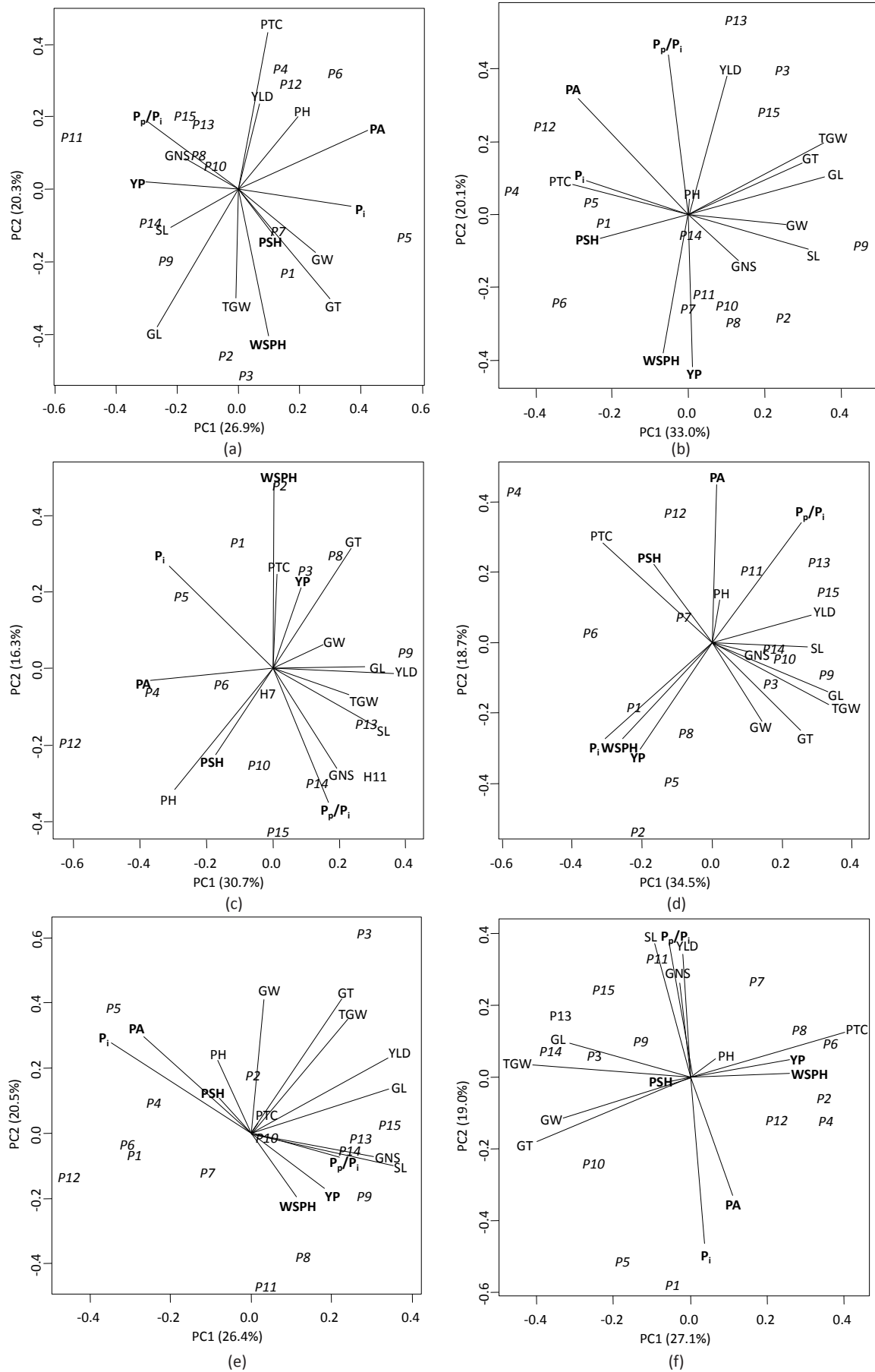
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Fig. 1. Genotype by trait (GT) biplot of 15 bread wheat genotypes (*P1-P*15) by nine agronomic and six chemical characteristics measured across six environments (Rimski Šančevi for 2010–2011 (a) and 2011–2012 (b) vegetation season, Zemun Polje for 2010–2011 (c) and 2011–2012 (d) vegetation season and Padinska Skela for 2010–2011 (e) and 2011–2012 (f) vegetation season). YLD = grain yield; TGW = thousand grain weight; PH = plant height; SL = spike length; GNS = grain number per spike; GL = grain length; GW = grain width; GT = grain thickness; PTC = productive tillering coefficient; PA = phytic acid; P_i = inorganic phosphorus; P_p/P_i = phytic phosphorus/inorganic phosphorus; YP = yellow pigment; WSPH = water soluble phenolics; PSH = free protein sulfhydryl groups
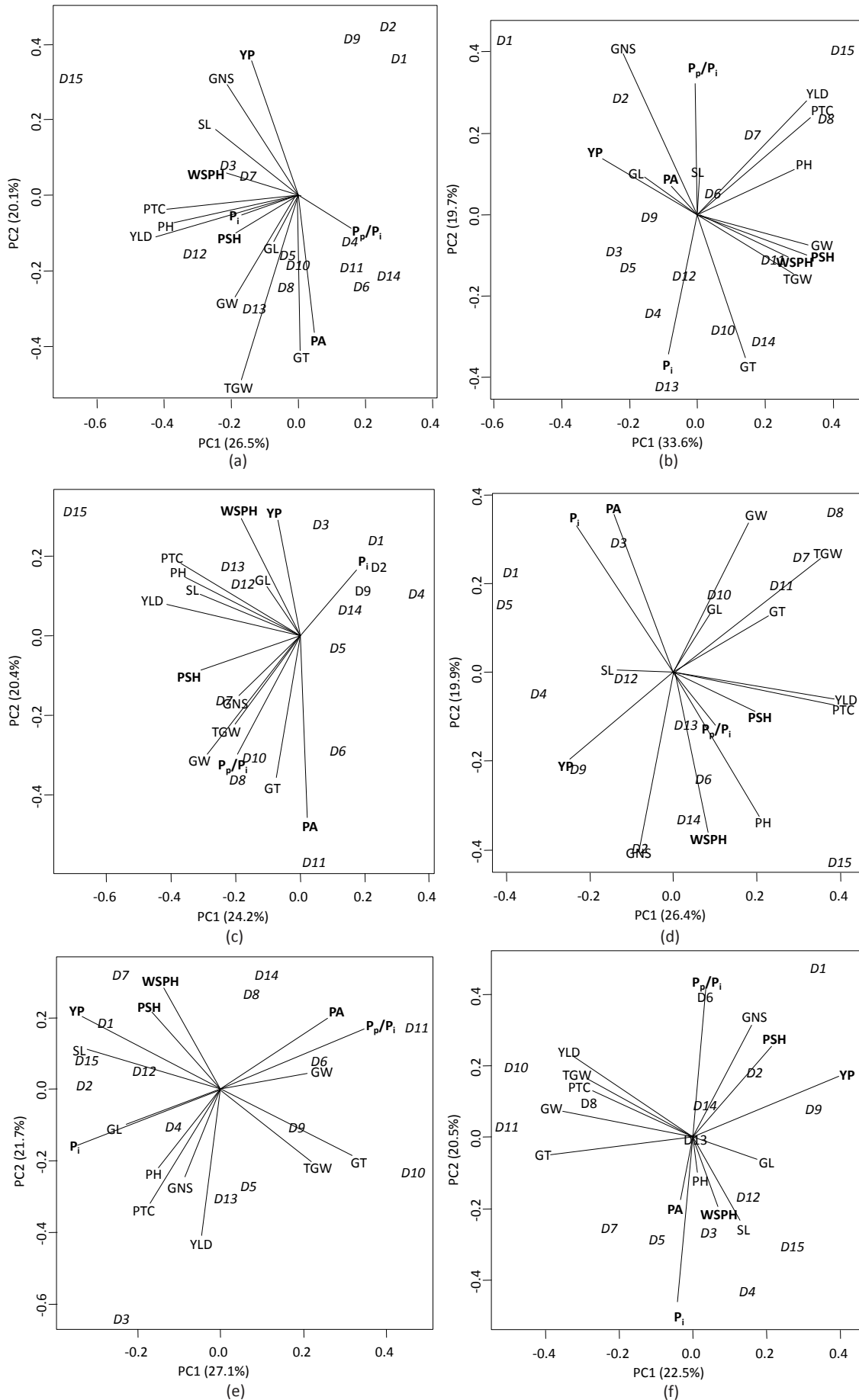
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Fig. 2. Genotype by trait (GT) biplot of 15 durum wheat genotypes (*D*1-*D*15) by nine agronomic and six chemical characteristics, measured across six environments (Rimski Šančevi for 2010-2011 (a) and 2011–2012 (b) vegetation season, Zemun Polje for 2010–2011 (c) and 2011–2012 (d) vegetation season and Padinska Skela for 2010–2011 (e) and 2011–2012 (f) vegetation season). YLD = grain yield; TGW = thousand grain weight; PH = plant height; SL = spike length; GNS = grain number per spike; GL = grain length; GW = grain width; GT = grain thickness; PTC = productive tillering coefficient; PA = phytic acid; P_i = inorganic phosphorus; P_p/P_i = phytic phosphorus/inorganic phosphorus; YP = yellow pigment; WSPH = water soluble phenolics; PSH = free protein sulfhydryl groups

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Conclusions

Associations obtained in the multi-environment trail showed possibility for simultaneous breeding for high WSPH and YP in bread and durum wheat, YP and PSH, and WSPH and PSH in durum wheat. The negative association was found between PA and YP, PA and WSPH in bread wheat, and also between PA and each of examined antioxidants in durum wheat, which can lead to the development of cultivars with better bioavailability of micronutrient and higher antioxidants contents for functional food purposes. The negative association of YLD and each of examined antioxidants unfortunately indicates difficulties for breeders to select at the same time for high YLD and high antioxidants levels in bread wheat, although the relation with PA was not consistent across environments. Suitable agronomic traits for indirect selection for PA proved to be PH and PTC in bread wheat, whereas GW and GT in durum wheat. On the contrary, different genetic background of tetraploid durum wheat led to the positive associations of YLD with WSPH, and PSH, and the negative one with PA, indicating ability for deriving productive, nutritious and health beneficial durum wheat cultivars at the same time.

Acknowledgement

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