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Original scientific article

PHENOTYPIC AND MOLECULAR DIVERSITY OF WHEAT SPECIES (*Triticum* spp.) IN RELATION TO PLANT HEIGHT AND HEADING TIME

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Plant height and heading time are important agronomic traits that significantly contribute to the improvement of wheat adaptability and yield. The aim of this study was to evaluate the level of phenotypic variability of these two traits in a collection of wheat species originating from 20 countries, to analyse its molecular diversity based on the microsatellite loci associated with the previously mapped quantitative trait loci, and to estimate potential of microsatellites to detect polymorphism in different wheat species and reveal allelic patterns in relation to the geographical origin. The significant differences in plant height and heading time among different wheat groups were observed, while the differences in means among three different growing seasons were significant only for heading time. The principal coordinate analysis distinguished wheat genotypes by their origin and ploidy level. Wheat varieties from America, South and Southeast Europe, and West and Central Europe had the highest molecular diversity, as was evidenced by the higher number of alleles, number of group-specific alleles, Shannon's information index and gene diversity. The Nei's genetic identity indicated genetic similarity of geographically distinct groups, such as South and Southeast Europe and Russian (0.901) and South and Southeast Europe and American genotypes (0.638). The studied collection with high observed level of both phenotypic and molecular diversity for plant height and heading time may be a valuable source of variation for wheat breeders to fine adjust these traits to achieve better agronomic performance in certain local environments.

Keywords: diversity, plant height, heading time, microsatellites, wheat

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INTRODUCTION

Ex situ collections and gene banks worldwide safeguard a vast amount of genetic resources with potentially beneficial traits, which value needs to be evaluated on phenotypic and molecular level to provide plant breeders with the useful information. Genetic improvement of wheat (*Triticum* spp.) depends on a degree of utilizing the diversity present in genetic resources. While persistent exploitation of almost exclusively elite germplasm has led to genetic bottlenecks, narrowing the genetic background of modern wheat (VAN DE WOUW *et al.*, 2010), the use of diverse genetic material from various geographic origin combined with divergent breeding outlooks and objectives has caused a considerable qualitative variation of allelic combinations among developed wheat varieties over decades (ROUSSEL *et al.*, 2005). Therefore, continuous estimation of genetic diversity of different wheat collections using both molecular tools and phenotypic assessments are necessary to identify the substantial genetic variation of many important agronomic traits and include in modern wheat breeding programs (KOBILJSKI *et al.*, 2002; CHEN *et al.*, 2012; BRBAKLIĆ *et al.*, 2015; TRKULJA *et al.*, 2019).

Plant height and heading time are important agronomic traits that give an invaluable contribution to the enhancement of wheat adaptability and yield. The introduction of *Reduce height* (*Rht*) semi-dwarf gene alleles, *Rht-B1b* and *Rht-D1b*, from Japanese germplasm during the Green Revolution in the 1960s and 1970s and their subsequent worldwide distribution markedly decreased plant height, improved lodging stability, favoured dry matter partitioning to the spike and, ultimately, increased harvest index and grain yield (HEDDEN, 2003). These mutant forms of *Rht-B1* and *Rht-D1* genes, located on group 4 of homeologous chromosomes, encode transcription factors that repress plant growth and because of their insensitivity to the exogenous treatment of gibberellic acid (GA) are denoted as GA-insensitive (THOMAS, 2017). Among numerous discovered GA-sensitive dwarfing genes, *Rht8* gene on chromosome 2D is one of the best characterised, while the other mapped GA-sensitive genes were far less investigated and validated (ELLIS *et al.*, 2005). Moreover, various types of quantitative trait loci (QTLs) mapping, genome-wide association studies and genomic selection evince that the plant height is a complex trait governed not only by major *Rht* loci, but also by many mapped minor QTLs (GRIFFITHS *et al.*, 2012; ZANKE *et al.*, 2014; ZHAO *et al.*, 2014; WÜRSCHUM *et al.*, 2015).

Heading and flowering time had a crucial role in the determination of variety adaptation to particular specific environments (REYNOLDS *et al.*, 2009), hence its remarkable adaptability to a wide range of diverse environments, resulting in a broad area of its cultivation (ZHENG *et al.*, 2013). Strong selection pressure of contrasting climates forced the crop to adjust its heading time either to avoid early frosts at the higher latitudes or to escape high temperatures and drought stress during the critical reproductive developmental stages in the southern regions. In temperate environments, such as the Pannonian Plain, heading time should occur between late spring frosts and early drought and heat stress during grain filling. Moreover, appropriate wheat varieties choice, according to heading time and plant height, could enable a mismatch between the critical wheat developmental stages and the epidemiological requirements of the pathogen, resulting in avoidance of disease occurrence (SIMÓN *et al.*, 2004). This could be an admissible coping strategy in the light of the anticipated challenges of climatic change. The regulation of heading time is conveyed through signalling pathways that depend on temperature and day length (ROUSSET *et al.*, 2011). The major wheat genes involved in these processes are *Vernalization*

response (*Vrn*), *Photoperiod* (*Ppd*) and *Earliness per se* (*Eps*) genes (DISTELFELD *et al.*, 2009).

Similarly to genetic architecture of wheat height, in addition to the well-characterized genes, several small-effect QTLs controlling heading time were detected from linkage mapping, association mapping and genomic prediction studies (SHINDO *et al.*, 2003; GRIFFITHS *et al.*, 2009; ROUSSET *et al.*, 2011; ZANKE *et al.*, 2014). In a number of these mapping studies, microsatellites markers were successively employed and their extensive use could be attributed to their polymorphism, reproducibility, high genome coverage and high informative nature (WÜRSCHUM *et al.*, 2013). The presence of the small-effect QTLs could account for remaining unexplained phenotypic variation (WÜRSCHUM *et al.*, 2018) and thus be a valuable source of variability for variety improvement when the major gene alleles controlling traits of interest are fixed (KIRIGWI *et al.*, 2007). Moreover, as the major genes affecting wheat plant height and heading time have already been well studied and exploited in breeding, combining more small-effect alleles of the several QTLs may attain a fine adjustment of these traits for improved adaptability to the local environments and further advances in wheat yield improvement.

Considering the potential significance of small-effect QTLs, this study aimed 1) to evaluate the level of phenotypic diversity for height and heading time of a Serbian wheat collection that could be useful for wheat improvement in breeding programs, 2) to analyse genetic diversity based on the microsatellite loci linked to the previously mapped small-effect QTLs of plant height and heading time, and 3) to estimate the potential of microsatellites to detect polymorphism in different wheat species and reveal allelic patterns in relation to the geographical origin.

MATERIAL AND METHODS

A panel of 191 wheat genotypes was selected from the wheat collections maintained at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The selected panel contained bread wheat (*Triticum aestivum* L. subsp. *aestivum*, $2n = 6x = 42$, genome AABBDD), spelt wheat (*Triticum aestivum* L. subsp. *spelta* (L.) Thell., $2n = 6x = 42$, genome AABBDD), durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) van Slageren, $2n = 6x = 28$, genome AABB), Polish wheat (*Triticum turgidum* L. subsp. *polonicum* (L.) Thell., $2n = 6x = 28$, genome AABB) and rivet wheat (*Triticum turgidum* L. subsp. *turgidum*, $2n = 6x = 28$, genome AABB), originating from 20 countries, namely Argentina, Austria, Belgium, Canada, China, Croatia, Czech Republic, France, Germany, Hungary, Italy, Japan, Mexico, Northern Macedonia, Romania, Russia with other ex-USSR countries (in further text referred to simply as *Russia*), Serbia, Switzerland, UK and USA. The bread wheat varieties were grouped by their origin into four groups, namely America (19), Asia (12), Russia (12), South and Southeast European - SSE (61) and West and Central European group - WCE (67). The greater representation of the latter two groups was due to their economic significance and larger area under their cultivation in the Pannonian Plain in comparison to the others. Spelt wheat varieties (9) were assigned to a separate group, whilst the tetraploid wheat species, being fewer in number, were grouped together (11 in total) and designated as tetraploid wheat. Apart from the geographical distribution criteria, the varieties were selected to span more than a century-wide historical interval of their release, from 1907 until 2014.

The plant height and the heading time for all 191 genotypes were measured in a field trial during three consecutive growing seasons (2015/2016, 2016/2017 and 2017/2018) at Rimski

šančevi experimental field (45°20′ N, 19°51′ E). The varieties were sown in 1 m wide and 2 m long plots with 0.15 m between rows, in a randomized complete block design with three replications. In each growing season, a fertilizer was applied before sowing at the average dose of ca. 50 kg N ha^{-1}, 60 kg P ha^{-1} and 60 kg K ha^{-1}. In early February, additional 50 kg N $ha^{-}1$ of ammonium-nitrate (33% N) was top dressed according to N-min analysis. When required, an appropriate chemical control of pests and diseases were applied in spring and weeds were periodically hand-removed.

Plant height was measured from the ground level to the tip of the ear excluding awns or scurs at normally developed plants at the end of May after full elongation of the plants. Heading time was recorded as the number of days from the January 1st until the date when 50% of the ears emerged from the flag leaf sheath. Meteorological conditions for the three trial seasons from January until May were showed in Supplementary Table S1. Analysis of variance (ANOVA) and Tukey's honest significant difference test were applied to check for significance and compare different wheat group means.

The genomic DNA was isolated from young seedlings using the modified CTAB method (DREISIGACKER *et al.*, 2016). The polymerase chain reaction (PCR) was performed in a 10 µl solution containing 30 ng of template DNA, 1 × PCR buffer, 2 mM $MgCl_2$, 0.2 mM of dNTPs, 5 pmol of fluorescently labelled primer and 1 unit of Taq polymerase. The PCR was optimised according to the following steps: an initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at (52 °C, 55 °C, 60 °C or 62 °C) for 45 s and elongation at 72 °C for 45 s, completing with the final extension at 72 °C for 10 min. The 10 µL reaction volume containing 2 µL of labelled PCR products, 0.2 µL GeneScan500 LIZ size standard and 7.8 µL Hi-Di formamide (highly deionized formamide, Applied Biosystems) were separated by capillary electrophoresis using Applied Biosystems Genetic Analyzer 3130. The fragment size was obtained with the software GeneMapper, version 4.0 (Applied Biosystems). A set of microsatellite markers, encompassing 16 loci on three genomes, was chosen based on previous studies showing their associations with *Rht* genes, QTLs for plant height, heading or flowering time and their primer sequences were shown in Supplementary Table S2.

The principal coordinate analysis (PCoA) based on molecular data was used to graphically depict genetic relationship among wheat genotypes via covariance matrix with data standardization. The following molecular diversity parameters were calculated based on the molecular data: number of different alleles, number of effective alleles, number of alleles with frequency more than 5%, number of private alleles, polymorphism information content (PIC), Shannon's information index, observed heterozygosity, unbiased expected heterozygosity, Hedrick's standardized coefficient of gene differentiation (G'stH) and pairwise population matrix of Nei's genetic identity. All molecular diversity analyses were performed in GenAlEx 6.5 programme (PEAKALL and SMOUSE, 2012).

RESULTS AND DISCUSSION

The ANOVA revealed significant differences in plant height among different wheat groups (Table 1), while the differences in means among three different growing were not significant. The Tukey's honest significance test showed that the spelt wheat groups had highest values for plant height (129.5 cm), followed by Russian (102.7 cm), tetraploid wheat (99.5 cm)

and the American group (98.9 cm), without significant differences among the latter three. The SSE and WCE wheat did not significantly vary from each other, while the genotypes from Asia had the significantly shortest plants (71.6 cm), which significantly differed from all the groups except SSE. Among the hexaploid wheat, the Russian and American genotypes were the tallest, as could be corroborated with the findings in the study of KAYA *et al.* (2015), who observed that genotypes originated from Russia, Ukraine and Mexico were approximately 10 cm taller than wheat varieties from other geographic regions. The spelt wheat genotypes exhibited much narrower range of plant height and taller plants in comparison to a diversity panel of 150 spelt old and new varieties from Western Europe (LONGIN and WÜRSCHUM, 2014), which could be explained by a smaller number of the spelt wheat varieties used in this study. The coefficient of variation (CV) for plant height varied among the wheat groups from 8.6%, for WCE, to 26.7%, for the Asian group. The CV, considering all analysed genotypes, was 22.8%, indicating a high level of phenotypic diversity for plant height in the wheat collection. The results were similar to findings of BORDES *et al*. (2008) who reported a three-fold range of plant height difference and CV of 22.7% in 372 hexaploid bread accessions from 70 countries. The large variability in plant height could be contributed to the presence or absence of different dwarfing genes and their combinations in the analyzed wheat varieties and species.

Table 1. Tukey's pairwise comparisons of means between wheat groups and growing seasons, minimum, maximum values and coefficient of variation for plant height and heading time

Group/ Season	Plant height (cm)		Min (cm)	Max (cm)	CV (%)	Heading time (days)		Min (cm)	Max (cm)	CV (%)
America	98.9	c	43	140	21.6	124.4	c	110	138	5.0
Asia	72.1	a	32	105	26.7	114.7	a	109	128	4.4
Russia	102.7	c	70	132	14.4	128.2	d	118	141	3.8
SSE	82.0	ab	40	130	19.8	123.6	c	114	138	3.6
WCE	83.3	b	68	103	8.6	128.6	d	120	142	3.6
Tetraploid	99.5	c	80	128	15.1	119.4	b	116	126	1.9
Spelt	129.5	d	111	148	6.4	124.2	c	109	140	8.8
2015/2016	97.0	a	32	148	26.3	122.9	a	109	140	5.7
2016/2017	94.0	a	33	132	22.3	124.0	b	109	142	5.5
2017/2018	95.3	a	36	144	22.4	123.4	ab	110	136	4.7

SSE - South and Southeast Europe, WCE - West and Central Europe, Min - minimum, Max - maximum, CV - coefficient of variation

The mean values of heading time significantly differed among the wheat groups (Table 1). The earliest heading time was observed in the wheat group from Asia, followed by the tetraploid wheat species. The SSE, American and spelt wheat genotypes entered the heading stage later than the previous groups, while the latest heading was noted on genotypes originating from Russia and WCE. The observed differences in heading time between with the genotypes from the lower and higher geographic latitudes, such as between WCE and SSE group, could be related to varietal adaptation to different day lengths so that the genotypes from the lower latitudes enter heading developmental stage earlier as a result of photoperiod insensitivity

(SHCHERBAN *et al.*, 2012). The CV for heading time varied from 1.9%, for tetraploid wheat species, to 8.8%, for spelt wheat. Although the CV for heading time, including all wheat genotypes (5.4%), was much smaller than the CV for plant height, it indicated the existence of a significant source of variation in the collection, which encompassed a range of two weeks between the earliest and the latest heading group. Even though the range was considerably smaller that a one-month wide range between the first and the last-heading accessions in a 372 diverse wheat collection of BORDES *et al.* (2008), their CVs were similar, indicating that the heading time variation in this study was not negligible.

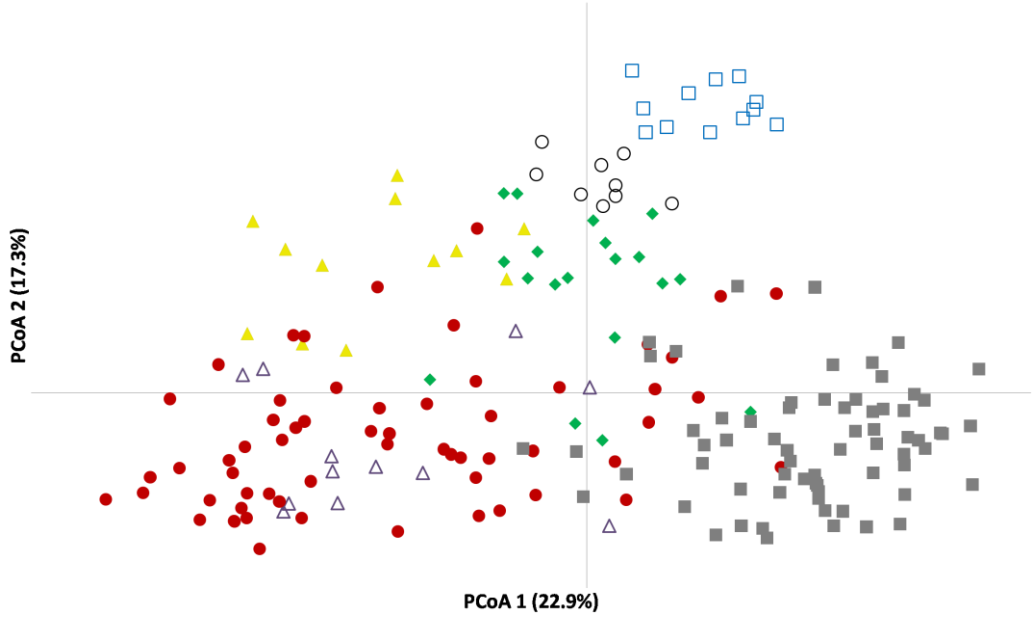

Figure 1. Principal coordinate analysis of 191 wheat genotypes based on molecular data
Red circles - South and Southeast Europe, grey squares - West and Central Europe, empty grey triangles - Russia, yellow triangles - Asia, green diamonds - America, empty black circles - tetraploid wheats, blue empty squares - spelt wheat

The significant differences were found also among the growing seasons. In 2015/2016 the heading time of the analysed genotypes was observed significantly earlier that in 2016/2017, whereas the 2017/2018 season did not differ from either of the two seasons. This could be interpreted by the meteorological conditions from January to May in 2015/2016 that were characterised by 2 °C higher mean daily temperatures, higher relative humidity and by 100 mm more precipitation compared to the long-term averages. In 2016/2017, however, the mean daily temperatures were lower than the long-term averages, except for the mean daily maximum temperatures. It could be that lower temperatures had a much larger effect on the delay in heading time that slightly drier conditions during this season, with recorded relative humidity

and precipitation little lower than the long-term average (Supplementary Table S1). This was supported by SALAZAR-GUTIERREZ *et al.* (2013), who reported the earliest heading in locations with the highest temperatures. WÜRSCHUM *et al.* (2018) confirmed their findings pointed out that the temperature was the main cause of the differences in heading time between environments.

The PCoA based on molecular data gave a visual insight into the genetic relationship among the analyzed wheat genotypes on a biplot. The first two coordinates explained 40.2% of the total variation (Figure 1). Most genotypes from SSE grouped together in the third quadrant, although not compactly. The majority of the WCE wheat grouped in the fourth quadrant, spreading to some extent into the first quadrant, and with some overlap with the SSE wheat cluster. All genotypes from Asia were clustered in the second quadrant. Spelt and tetraploid wheat were positioned in the first quadrant and could be distinguished from each other. The geographic differentiation was not observed in Russian wheat, being mixed mostly with the wheat genotypes from SSE. The genotypes from the American continent formed a separate cluster mostly between the first and the second quadrant, with some of the genotypes positioning among SSE and WCE genotypes. Despite some scattering of Russian and American wheat groups, the PCoA clearly differentiated most of the wheat genotypes by their origin and ploidy level.

The wheat genotypes from Asia, Russia and SSE, having the common most frequent 192 bp allele of the microsatellite locus *Xgwm261* (Supplementary Table S3) grouped together on the left side of the PCA biplot, opposing the WCE wheat cluster, carrying the 174 bp allele. The 192 bp allele has been widely used in Southern European for breeding varieties with reduced height without yield decreases (NIELSEN *et al.*, 2014). Moreover, NIELSEN *et al.* (2014) noticed that the 174 bp allele was the most frequent in wheat varieties from Western Europe. This finding was supported by KÖRMÖCZI *et al.* (2019) reporting that the 192 bp allele was also most frequent (55.3%), among the wheat varieties from three different Hungarian breeding programmes, followed by 174 bp (22.3%) allele present in genotypes with Western European progenitors and 198 bp (12.9%) allele. Similarly, TRKULJA *et al.* (2019) observed a high frequency of 192 bp allele among Serbian varieties and prevalence of 174 bp allele in wheat genotypes from the USA and Western Europe. TOŠOVIĆ-MARIĆ *et al.* (2008) also determined a high frequency of 192 bp allele (78%) among Serbian wheat varieties and much lower share of this allele (36%) in varieties from the other countries collectively, without a detailed distribution of this alleles by geographical regions. The common prevalent 174 bp allele in wheat genotypes from the American continent and Western Europe was also found in our study. The effect of 192 bp allele was 7 cm to 8 cm in plant height reduction comparing to the effect of 174 bp (KORZUN *et al.*, 1998). This reduction was not so pronounced in our research, which could be explained by the effects of other loci affecting plant height and also due to different environments and genotypes used in different studies. The Japanese variety Akakomugi was considered as an important source of the allele 192 bp of the *Rht8* gene (ELLIS *et al.*, 2005). The spread of this allele through their lineage has been accelerated with the growing popularity of some of their descendants. For example, due to its good quality and winter hardiness, the Russian wheat variety 'Bezostaya 1', the most notable source of *Rht*8 gene inherited from Akakomugi, had been widely used in breeding programmes in East and Central Europe (WORLAND *et al.*, 2001). Likewise, many Italian varieties carrying *Rht8* gene from Akakomugi were introduced to South

and Central Europe after the Second World War (BOROJEVIC and BOROJEVIC, 2005). The presence of this historically important Japanese variety and their descendants in pedigrees of many SSE, Russian and Asian wheat varieties could explain the prevalence of the 192 bp allele in these groups in our study. Although the association of the microsatellite *gwm261* with plant height has been well documented, this marker in our study did not contribute to the differentiation of the wheat groups as much as some other loci.

The molecular analysis showed that the selected microsatellites exhibited a relatively high level polymorphism with the number of detected alleles ranging from 3, for locus *Xgwm639-5AL*, to 11, for loci *Xgwm296-7DS* and *Xwmc617-4BS* (Table 2), and an average of 6.5. The average PIC was 0.69, correlating with the number of alleles. Besides, other molecular diversity parameters, such as the average of 4.09 effective alleles per locus, 4.06 alleles per locus with a frequency equal or more than 5%, the Shannon's Information Index of 1.48, observed heterozygosity of 0.02 and unbiased expected heterozygosity of 0.71 (Table 3), suggested the presence of a substantial molecular diversity in the whole wheat collection. The most frequent alleles, taking into account all genotypes, were determined for each locus and their frequencies varied from 0.225, for *Xwmc617-4DS*, to 0.571, for *Xgwm495-4BS*, with an average of 0.401. For each locus, the wheat groups were identified in which the most frequent alleles prevailed. The most frequent alleles considering all genotypes were the most numerous in the Russian and WCE groups, being detected in five loci. The largest proportion of the most frequent alleles was determined in three loci for the Asian group, two loci for the SSE group and one locus for the group of tetraploid wheat. In addition to the high number of alleles and high values of PIC, the majority of the microsatellites demonstrated high differentiation power above 0.700. The highest values of G'stH were observed in two loci, namely *Xgwm291-5AL* and *Xwmc25-2DS*, which contributed most to the separation of genotypes into geographic groups. These loci showed high diversity in terms of the average number of alleles, PIC and gene diversity in previous studies (SHARMA *et al.*, 2002; GANEVA and KORZUN, 2012; LANDJEVA *et al.*, 2014).

A comparison of molecular diversity parameters among the wheat groups revealed the largest values of molecular diversity parameters in wheat varieties originating from the American continent and SSE (Table 3). The wheat varieties from WCE also showed a considerable molecular diversity reflected in high number of alleles per loci, a number of group-specific alleles, Shannon's information index and gene diversity. TRKULJA *et al.* (2019) also observed high values of parameters of genetic diversity in a cluster with genotypes from the American continent, somewhat less diverse cluster with genotypes from Southeast and East Europe, while the cluster for Western Europe showed least genetic diversity. In a study by HUANG *et al.* (2002) encompassing wheat genotypes from all continents, SSE wheat groups seemed to be more diverse than the American and West European groups. Relatively large genetic diversity of these groups suggests their wide genetic bases established on comprehensive breeding programmes comprising germplasm from other geographical regions. Spelt wheat and tetraploid wheat species had the lowest values of molecular diversity parameters, which could be due to smaller sample sizes of these two groups, and in case of durum wheat also due to the level of polyploidy, which was manifested in a less variation in plant height for spelt wheat, and in heading time for both groups.

Table 2. Microsatellite loci, allele range, the most frequent alleles and coefficient of gene differentiation of the analysed wheat genotypes

Locus	Range	Na	PIC	MFA (bp)	MFA freq.	MFA group	G'stH	P (G'stH)
Xgpw3017-4BS	283-319	5	0.66	289	0.461	Russia	0.471	0.001
Xgwm261-2DS	165-205	4	0.60	192	0.445	SSE	0.651	0.001
Xgwm291-5AL	103-129	5	0.65	103	0.518	Asia	0.835	0.001
Xgwm296-2AS	124-138	5	0.63	134	0.387	Asia	0.795	0.001
Xgwm296-2DS	138-168	7	0.80	168	0.288	Asia	0.439	0.001
Xgwm296-7DS	146-186	11	0.87	172	0.298	WCE	0.588	0.001
Xgwm495-4BS	158-176	7	0.63	174	0.571	Russia	0.705	0.001
Xgwm639-5AL	133-137	3	0.23	135	0.545	Russia	0.760	0.001
Xgwm639-5BL	141-149	6	0.70	145	0.419	Russia	0.790	0.001
Xgwm639-5DL	151-175	6	0.66	155	0.492	WCE	0.679	0.001
Xwmc25-2BS	148-176	4	0.57	166	0.455	Tetraploid	0.488	0.001
Xwmc25-2DS	190-218	5	0.75	190	0.361	Russia	0.819	0.001
Xwmc125-4BS	182-252	8	0.74	246	0.346	WCE	0.530	0.001
Xwmc617-4AS	188-210	7	0.76	190	0.288	WCE	0.686	0.001
Xwmc617-4BS	202-266	11	0.87	204	0.314	WCE	0.728	0.001
Xwmc617-4DS	222-283	10	0.88	238	0.225	SSE	0.718	0.001

Na - number of alleles, PIC - polymorphism information content, MFA - the most frequent allele, MFA freq - frequency of the most frequent allele, G'stH - Hedrick's standardized coefficient of gene differentiation, P (G'stH) - probabilities for G'stH for 95% confidence level

Table 3. Means and standard errors of parameters of molecular diversity and pairwise group matrix of Nei's unbiased genetic identity

	America	Asia	Russia	SSE	WCE	Spelt	TP	Total
Na	4.93±0.42	3.00±0.35	3.47±0.38	5.73±0.55	5.27±0.49	2.13±0.19	2.20±0.24	6.50±0.68
Ne	3.35±0.37	1.99±0.20	2.22±0.27	2.88±0.30	2.78±0.40	1.62±0.12	1.84±0.20	4.09±0.51
Na5%	4.73±0.41	2.93±0.33	3.33±0.40	3.93±0.28	3.67±0.47	2.13±0.19	2.20±0.24	5.06±0.41
Np	0.13±0.09	0.00±0.00	0.00±0.00	0.07±0.07	0.07±0.07	0.07±0.07	0.00±0.00	-
I	1.29±0.09	0.76±0.12	0.87±0.11	1.24±0.09	1.14±0.11	0.53±0.08	0.60±0.11	1.48±0.11
Ho	0.02±0.01	0.01±0.01	0.02±0.01	0.01±0.01	0.02±0.02	0.04±0.03	0.00±0.00	0.02±0.01
He	0.68±0.03	0.44±0.06	0.49±0.05	0.61±0.03	0.57±0.04	0.36±0.06	0.40±0.07	0.71±0.03
AM	1.000							
Asia	0.533	1.000						
Russia	0.611	0.514	1.000					
SSE	0.638	0.574	0.901	1.000				
WCE	0.582	0.341	0.608	0.605	1.000			
Spelt	0.482	0.292	0.211	0.252	0.295	1.000		
TP	0.521	0.227	0.471	0.361	0.393	0.471	1.000	

AM -America, SSE - South and Southeast Europe, WCE - West and Central Europe, TP - tetraploid wheats, Na - number of alleles, Ne - number of effective alleles, Na5% - number of alleles with frequency ≥ 5%, Np - number of private alleles, I - Shannon's Information Index, Ho - observed heterozygosity, uHe - unbiased expected heterozygosity (gene diversity)

The genetic similarity among the wheat groups was assessed based on Nei's unbiased genetic identity, which ranged from 0.221, between the Russian wheat group and spelt wheat, to 0.901, between Russian and SSE genotypes, implying that the Russian genotypes shared most of the alleles with SSE, and the least common alleles with spelt wheat (Table 3). The value Nei's unbiased genetic identity above 0.600 was observed between four wheat group pairs, namely American and Russian, American and SSE, Russia and WCE, and WCE and SSE, implying high genetic similarity.

This is in line with the finding that a relative contribution of the former USSR, USA and Canada germplasm, along with the Japanese variety Akakomugi, was the most important to Yugoslav winter wheat breeding (JOŠT and COX, 1990). Despite fewer shared most frequent alleles between SSE and WCE groups, and between Russian and WCE groups, the high values of their Nei's unbiased genetic identity suggest a high proportion of other less frequent common alleles. Certainly, the widespread exchange and use of diverse germplasm in distant breeding programmes had a great impact on the complexity and structure of genetic diversity, as it was also reflected in wheat groups overlapping in the PCoA biplot (Figure 1).

A closer inspection into the most frequent alleles of each locus revealed common allelic patterns among wheat groups (Supplementary Table S3). The Russian and SSE groups shared the most frequent alleles for all loci but two (*Xgwm296-7DS* and *Xwmc617-4BS*). More than 40% of the most frequent alleles in Asian and American groups were also present among either the Russian or the SSE group, which was in accordance with the genetically similar among these groups from the Nei's genetic identity matrix. The proportions of the shared most frequent alleles were much less for the other group pairs. The spelt wheat group seemed to be with the least common most frequent alleles. For the locus *gwm296-7DS* none of the wheat group had the same most frequent allele. Interestingly enough, *gwm296-7DS* did not have a very high coefficient of gene differentiation (0.588) but was one of the loci with the highest number of detected alleles.

The observed phenotypic and molecular diversity among investigated genotypes of these two important plant height and heading time indicated the presence of sufficient variability, which is indispensable for wheat breeders to address the forthcoming challenges related to climate change. Optimising plant height to achieve a balance between lodging stability, photosynthetic active area, harvest index and nutrient use efficiency could further improve wheat production in target environments (ZANKE *et al.*, 2014). Correspondingly, subtle adjustments of heading time to specific local conditions to avoid heat and drought stress during the summer (LOPES *et al.*, 2012) using available germplasm and molecular tools could contribute to maximising yield potential.

CONCLUSION

The selected microsatellite markers linked to the previously mapped small-effect QTLs for plant height and heading time proved to be useful in detecting polymorphism and revealing allelic patterns in relation to the geographical origin and different wheat species in a Serbian wheat collection based on the microsatellite loci, distinguished wheat genotypes by their origin and ploidy level. The analysed collection showed a high degree of genetic diversity, especially in the wheat groups from the American continent, South and Southeast Europe, and West and Central Europe. The selected microsatellite markers proved to be useful in detecting

polymorphism and revealing allelic patterns in relation to the geographical origin and different wheat species. The evaluated collection with the high observed level of both phenotypic and molecular diversity for plant height and heading time may be a valuable source of variation for wheat breeders to fine adjust these traits to achieve better agronomic performance in certain local environments.

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FENOTIPSKI I MOLEKULARNI DIVERZITET VRSTA PŠENICA (*Triticum* SPP.) POVEZANIH SA VISINOM I VREMENOM KLASANJA

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Izvod

Visina i vreme klasanja su važne agromonske osobine koje značajno doprinose poboljšanju sposobnosti pšenice da se prilagodi na uslove spoljašnje sredine i povećanju prinosa. Cilj ovog rada je da se oceni nivo fenotipske varijabilnosti ove dve osobine u kolekciji pšenica koje potiču iz 20 zemalja sveta, da se oceni njen molekularni diverzitet pomoću mikrosatelitskih markera povezanih sa prethodno mapiranim lokusima za kvantitativne osobine, i da se proceni potencijal mikrosatelita da utvrde polimorfizam različitih vrsta pšenice i alelne kombinacije u vezi sa geografskim poreklom. Značajne razlike između grupa pšenice utvrđene su za visinu biljke i vreme klasanja, dok su razlike između tri vegetacione sezone ustanovljene samo za vreme klasanja. Analizom glavnih kordinata razdvojeni su genotipovi pšenice prema poreklu i ploidnosti. Najveće vrednosti pokazatelja molekularnog diverziteta utvrđene su kod tri grupe pšenice: iz Amerike, južne i jugoistočne Evrope, i zapadne i centralne Evrope. Indeks genetičkog identiteta prema Nei-u ukazao je na genetičku sličnost između geografski udaljenih grupa pšenice južne i jugoistočne Evrope i Rusije (0.901) i grupa južne i jugoistočne Evrope i Amerike (0.638). Visok stepen diverziteta visine i vremena klasanja u ocenjenoj kolekciji pšenice predstavlja vredan izvor varijabilnosti za fino podešavanje ovih osobina u oplemenjivanju pšenice u cilju postizanja boljih prinosa u određenim agroekonomskim uslovima.

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