ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 106, No. 3 (2019), p. 257–264
DOI 10.13080/z-a.2019.106.033

Genetic structure and allelic richness of the wheat core collection for association mapping of yield

Dragana TRKULJA[1], Ankica KONDIĆ-ŠPIKA[1], Ljiljana BRBAKLIĆ[1], Borislav KOBILJSKI[2], Sanja MIKIĆ[1], Milan MIROSAVLJEVIĆ[1], Svetlana GLOGOVAC[1], Gordana ŠURLAN-MOMIROVIĆ[3]

[1]Institute of Field and Vegetable Crops
Maksima Gorkog 30, 21000 Novi Sad, Serbia
E-mail: dragana.trkulja@nsseme.com

[2]Biogranum Co.
Toplice Milana 6, 21000 Novi Sad, Serbia

[3]University of Belgrade, Faculty of Agriculture
Nemanjina 6, 11080 Belgrade-Zemun, Serbia

Abstract

Knowledge about genetic structure and allelic diversity of bread wheat (*Triticum aestivum* L.) collections is a key to developing modern wheat cultivars, able to cope with various abiotic and biotic stress factors, through purposeful selection of parents. Further, information about population structure of a material is the first prerequisite of association mapping, which prevents declaring of spurious marker-trait associations. The aim of this study was to assess genetic diversity and presence of population structure within the panel of wheat accessions chosen for association mapping of yield and yield related traits in wheat. The material consisting of 282 wheat accessions, highly variable for 10 agronomically important traits, was evaluated using microsatellite markers that were distributed all over three wheat genomes. The 397 alleles were detected at 31 SSR markers, with an average number of 12.4. The highest diversity was detected at microsatellite loci from B genome, while the lowest number of alleles was observed for D genome. Structure analysis indicated the existence of three subpopulations, where genotypes were grouped according to their origin as well as pedigree data. In each subpopulation, private alleles were detected, indicating informativeness of analysed microsatellite loci for the elucidation of population structure. Statistically significant differences among groups were observed for 8 out of 10 wheat agronomically important traits, while PCA did not show a clear separation of genotypes. The analysed wheat accessions demonstrated a sufficiently high level of genetic diversity, considering both molecular and phenotypic data, which makes them suitable for the association study of breeding traits in wheat.

Key words: agronomically important traits, diversity, microsatellite loci, *Triticum aestivum*.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the staple foods and together with rice and maize accounts for 60 percent of the world's food energy intake. According to the Organisation for Economic Co-operation and Development (OECD, 2018), in 2017 in the world, wheat was harvested from around 220 million ha, while maize and rice occupied smaller areas, about 183 and 163 million ha, respectively. In the past 20 years, a stagnation of the wheat yield in the world has been observed. At the same time, the demand for food is increasing both due to the growing world population, as well as the dietary changes in countries with the rapidly growing economies (Spiertz, Ewert, 2009).

Breeding of wheat cultivars with a high and stable yield can be hastened by detection of genes and quantitative trait loci (QTLs) associated with agronomically important traits. Hitherto, a number of different approaches have been developed in order to elucidate complex nature of bread wheat genome. Among such approaches, association mapping (AM) or linkage disequilibrium (LD) mapping is increasingly being adopted as a method complementary to already traditional bi-parental linkage mapping to identify marker-trait associations (Sorrells, Yu, 2009). Relying on the use of variability that is present within germplasm of different geographic origin (natural populations, landraces, breeding material and cultivars), association mapping provides higher mapping resolution and allele number, and enables detection and mapping of multiple traits in a single set of genotypes (Neumann et al., 2011). However,

Please use the following format when citing the article:
Trkulja D., Kondić-Špika A., Brbaklić L., Kobiljski B., Mikić S., Mirosavljević M., Glogovac S., Šurlan-Momirović G. 2019. Genetic structure and allelic richness of the wheat core collection for association mapping of yield. Zemdirbyste-Agriculture, 106 (3): 257–264. DOI 10.13080/z-a.2019.106.033

in order to avoid declaring spurious associations between markers and traits, it is necessary to evaluate presence of population structure within analysed material. Namely, population structure occurs as a result of unequal distribution of alleles among subpopulations of different ancestries. During creation of a set of lines for association mapping, mixing of individuals that originate from different subpopulations, where different alleles are fixed at the same loci, false polymorphism may occur and consequently significant but spurious linkage disequilibrium can be observed. Statistically significant linkage disequilibrium detected between unlinked loci, results in false-positive marker-trait associations (Rode et al., 2012; Soto-Cerda, Cloutier, 2012).

Another very important factor for achieving a high and stable yield is the existence of genetic diversity within breeding material, necessary for creation of wheat cultivars with enhanced resilience to abiotic and biotic stress factors. Therefore, a number of studies point out the significance of investigating genetic structure and allelic diversity of a population, as a key for further improvements of wheat yield related traits, by broadening the genetic base through purposeful selection of parents (Zhang et al., 2011; Nielsen et al., 2014). Further, knowledge on the levels and distribution of genetic diversity in the available wheat germplasm allows the advancement of strategies for genetic resources management and exploitation.

Molecular markers present a very powerful tool for assessing genetic diversity in crops. Microsatellites or simple sequence repeat (SSR) markers are very informative, highly reproducible, highly polymorphic and mostly genome specific, which makes them suitable for revealing underlying population structure in wheat (Hao et al., 2011; Würschum et al., 2013). In the past decade, single nucleotide polymorphism (SNP) markers have received considerable attention due to their occurrence at much higher frequency in the genome than SSRs and development of high throughput screening methods (Lai et al., 2012). However, microsatellites are still very suitable for preliminary screening of a material, because a substantially higher number of SNPs must be used as compared to the SSRs (Van Inghelandt et al., 2010; Würschum et al., 2013).

The aim of this study was to (1) characterize genetic diversity and population structure based on the molecular data in a panel of wheat cultivars and breeding lines originating from 26 countries worldwide and highly variable for agronomically important traits in wheat, and (2) assess discriminating power of molecular compared to phenotypic data for the analysed material. The results are intended to serve as a resource for association mapping of breeding traits in wheat using this collection.

Materials and methods

Plant material and field trials. The hexaploid bread wheat (*Triticum aestivum* L.) collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia comprises about 3000 accessions from around 50 countries. In order to collect data relevant for breeding and different research purposes, 710 most divergent wheat genotypes originating from 38 countries, were assembled into a core collection and phenotyped for 54 agronomic, morphological and physiological traits in the field and laboratory conditions during 15 years (1995–2009). Based on the data recorded during five growing seasons, from 2005 to 2009, a set of 282 wheat accessions highly variable for 10 phenological and yield-related traits was chosen to analyse allelic variation at loci associated with yield-related traits. The panel included cultivars and advanced breeding lines from 26 countries worldwide, where the majority of the selected cultivars originated from Serbia (72) and the USA (63). Field trials were set up at the locality Rimski Šancevi, Novi Sad, Serbia and in each growing season the genotypes were sown in plots of 1.2 m^2 in 3 to 7 replications. The analysed traits are heading and flowering time, stem height, spike length, number of spikelets per spike, number of sterile spikelets per spike, number of grains per spike, spike weight, grain weight per spike and thousand kernel weight.

Microsatellite analysis. Ten seedlings per genotype were used for DNA extraction following the CTAB (cetyl trimethylammonium bromide) method (Borges et al., 2009). A set of 40 publicly available microsatellite markers, situated along all three wheat genomes, was used for molecular evaluation of accessions. Public primer sets were used from Beltsville Agricultural Research Station (barc), French National Institute for Agricultural Research (cfd and cfa), the Leibniz Institute of Plant Genetics and Crop Plant Research (gwm and gdm), John Innes Centre (psp) and Wheat Microsatellite Consortium (wmc) collections, and can be found on the GrainGenes website (http://www.wheat.pw.usda.gov/). The 10 µl of polymerase chain reaction (PCR) mixture contained 30 ng of template DNA, 1 × PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each deoxynucleotide (dATP, dCTP, dGTP and dTTP), 5 pmol of each primer (fluorescently labelled forward and unlabelled reverse primer) and 1 unit of Taq polymerase (Applied Biosystems, USA). The high quality PCR products were obtained applying two types of protocols. In the first protocol, the initial denaturation step, 5 min at 94°C, was followed by 35 cycles with 30 sec at 94°C, 45 sec at annealing temperature (52, 60 or 62 °C) and 45 sec at 72°C, with final extension step of 10 min at 72°C. For the simple sequence repeat (SSR) markers with annealing temperature of 50°C, the touchdown protocol was applied. Here, denaturation at 94°C for 5 min, was followed by five cycles of 45 sec at 94°C, 1 min at annealing temperature (started at 68°C and decreased for 2°C at each cycle) and 1 min at 72°C. For the next five cycles, annealing temperature started at 58°C and decreased by 2°C at each subsequent cycle. After that, PCR was conducted for additional 30 cycles with 30 sec at 94°C, 45 sec at 50°C and 45 sec at 72°C, with a final extension step at 72°C for 10 min. The PCR products were separated by capillary electrophoresis using ABI Genetic Analyzer 3130 (Applied Biosystems, USA), and size of analysed SSR fragments was determined using software *GeneMapper*, version 4.0 (Applied Biosystems). Reactions that did not show an amplified product were repeated three times. The null allele was scored if the repeat did not result in a PCR product, while DNA quality was good (which could be seen from the scores of the other analysed microsatellites), and the number of missing amplifications for that marker was lower than 5%. Otherwise, the data point was scored as missing.

Statistical analysis. The basic parameters of genetic diversity were calculated using software *GenAlEx 6.5* (Peakall, Smouse, 2012). The presence of

subpopulations within analysed wheat genotypes was determined by the software *Structure 2.3.4* (Stanford University, USA), where admixture model was applied on the genotypic data, with burn-in of 100,000 and 100,000 MCMC (Markov Chain Monte Carlo) duration to test for a K value from 1 to 10. The obtained results were visualized using software *Structure Harvester* (Earl, von Holdt, 2012). The sub-groups derived from the structure analysis were additionally evaluated by the principal coordinate analysis (PCoA) in *GenAlEx 6.5* using genetic distance matrix, while genetic distances among accessions calculated by the *PowerMarker* (North Carolina State University, USA) were used for the construction of unweighted pair group method with arithmetic mean (UPGMA) dendrogram in Dendroscope (Huson, Scornavacca, 2012). An analysis of molecular variance (*AMOVA*) was conducted by *GenAlEx 6.5*, to partition the variation among and within clusters. In order to assess power of microsatellite markers for separation of genotypes, differences between mean values for phenotypic traits for each detected subpopulation were tested using analysis of variance (*ANOVA*) and Tukey's multiple comparison of means test for 95% and 99% confidence levels. A multivariate data analysis method, principal component analysis (PCA) was used for visualizing grouping of samples based on phenotypic data implementing package *ggplot2* (Wickham, 2016).

Results and discussion

After optimization of conditions of polymerase chain reactions for amplification of 40 chosen SSR loci, 9 markers were excluded from further analysis due to monomorphic products or ambiguous scoring. At the rest of 31 microsatellite markers, the total number of 397 alleles was detected. The number of alleles varied from 2 (*Xbarc1060*) to 33 (*Xwmc3*) per locus, with the average number of 12.41. The size of detected microsatellite fragments varied in the range of 50 bp at 27 analysed loci and 100 bp at 5 loci. Only at *Xwmc3* locus, alleles showed variability in the range from 184 to 316 bp (Table 1), which is consistent with data recorded at wheat database (http://wheatssr.lab.nig.ac.jp/markerdb/markers/show/wmc003), where size of this microsatellite marker varied from 103 to 307 bp in a set of 32 wheat accessions.

Table 1. Parameters of genetic diversity for 282 wheat accessions detected at 32 SSR loci

SSR locus	Chromo-some	Total number of alleles (rare)	Range of allele size (bp)	The most frequent allele (bp), frequency %	Shannon's information index	Number of effective alleles	Nei's diversity index (expected heterozygosity)
Xwmc24	1A	10 (6)	115–156	138 (36.27)	1.50	3.66	0.726
Xbarc212	2A	24 (17)	183–232	223 (17.09)	2.52	8.50	0.896
Xgwm425	2A	13 (7)	122–156	136 (31.29)	1.95	5.39	0.820
Xwmc177	2A	12 (5)	173–200	190 (22.11)	2.10	6.49	0.854
Xgwm388	2B	13 (8)	156–184	164 (30.18)	1.82	4.62	0.800
Xgwm261	2D	15 (12)	160–212	192 (39.75)	1.56	3.50	0.717
Xbarc1060	3A	2 (0)	242–245	245 (55.46)	0.69	1.95	0.495
Xbarc12	3A	18 (12)	142–230	156 (22.58)	2.27	5.67	0.868
Xwmc264	3A	9 (5)	120–142	128 (33.33)	1.49	3.40	0.752
Xbarc164	3B	12 (8)	169–214	175 (37.63)	1.73	3.47	0.763
Xgwm284	3B	16 (12)	84–130	114 (32.03)	1.86	4.98	0.794
Xwmc3	3B	33 (28)	184–316	262 (19.71)	2.71	10.03	0.900
Xbarc1047	4A	14 (9)	150–198	166 (49.65)	1.51	2.89	0.685
Xgpw3017	4B	14 (12)	265–329	289 (50.90)	1.41	2.79	0.646
Xgwm495	4B	17 (13)	148–194	174 (61.97)	1.47	3.44	0.593
Xwmc238	4B	9 (2)	218–234	220 (21.91)	1.93	4.61	0.840
Xwmc331	4D	7 (3)	122–134	126 (55.48)	1.29	2.74	0.632
Xwmc457	4D	7 (4)	153–165	157 (51.59)	1.12	2.43	0.612
Xcfa2155	5A	5 (1)	211–221	219 (38.38)	1.35	3.31	0.726
Xbarc243	5B	13 (8)	192–226	207 (52.99)	1.71	4.03	0.691
Xgwm499	5B	21 (18)	91–154	124 (58.10)	1.51	3.13	0.617
Xbarc320	5D	7 (4)	175–202	199 (76.60)	0.81	1.76	0.392
Xbarc3	6A	15 (10)	159–232	196 (23.67)	1.92	4.38	0.828
Xpsp3071	6A	12 (7)	147–175	163 (28.70)	1.93	5.69	0.825
Xwmc553	6A	13 (5)	337–361	359 (23.16)	2.25	7.93	0.874
Xgwm518	6B	6 (3)	119–139	121 (40.35)	1.29	3.10	0.689
Xgwm276	7A	20 (15)	55–103	65 (40.78)	1.98	5.19	0.778
Xgwm297	7B	15 (8)	133–173	151 (20.00)	2.09	5.68	0.857
Xwmc396	7B	13 (8)	142–175	152 (40.00)	1.90	4.98	0.784
Xcfd14	7D	5 (1)	112–120	120 (35.79)	1.44	3.69	0.742
Xbarc1057.1		4 (2)	182–188	188 (82.50)	0.50	1.52	0.292
Xbarc1057.2		3 (2)	191–200	191 (95.70)	0.19	1.13	0.083
Average		12.41(7.97)			1.62	4.25	0.705

All tested loci revealed presence of one product, except for marker *BARC1057*, where for each accession two products were detected, indicating existence of two loci, further designated as *Xbarc1057.1* and *Xbarc1057.2*. The average value of Nei's diversity index was 0.705, while the total number of alleles with frequency less than 5%, which were labelled as rare, was 255 (64.2%). Similarly, in a sample of 3942 wheat

cultivars originating from 73 countries worldwide and at 38 SSR loci, Balfourier et al. (2007) detected about 80% of rare alleles and observed mean value of 0.742 for expected heterozygosity. From 32 analysed SSR loci, 23 were with dinucleotide repeats, 7 with tri- and only 1 with tetranucleotide motif. The observed allelic variability was higher for microsatellites with di- than with trinucleotide repeats, with average values of 13.4 and 10.6 alleles per locus, respectively, corresponding to the paper of Kalia et al. (2011). For markers *WMC177*, *GWM388*, *WMC264*, *WMC553*, *GWM518*, *GWM297*, *WMC396* and *CFD14* products were scored within all studied accessions. At the rest of the analysed SSR loci null alleles were detected with frequency lower than 4%, which made these markers suitable for detection of variability of wheat accessions (Wingen et al., 2014).

The highest number of alleles was detected at SSR loci from B genome (Table 2). However, analysed loci from A genome had the highest average polymorphism information content (PIC) value, due to high frequency of rare alleles observed at B genome (70.3%). The lowest genetic diversity of the D wheat genome was also reported using different marker techniques, SNP (Würschum et al., 2013; Kobayashi et al., 2016) and DArT (Nielsen et al., 2014). These results could be explained by the evolutionary history of hexaploid wheat, where bread wheat assembled a greater proportion of genetic diversity from its tetraploid ancestor (BBAA) than from *Aegilops tauschii* (DD), resulting in a higher frequency of effective recombinations in the A and B genomes, compared to D (Chao et al., 2009).

Table 2. The basic parameters of genetic diversity within wheat genomes

Parameter	Genome		
	A	B	D
Number of analysed SSR loci	13	12	5
Total number of alleles (rare alleles %)	167 (59.3)	182 (70.3)	41 (58.5)
Average allele number	12.85	15.17	8.20
Average polymorphism information content (PIC) value	0.744	0.719	0.573

Structure analysis showed presence of three subpopulations (Fig. 1), assigned as Q1, Q2 and Q3.

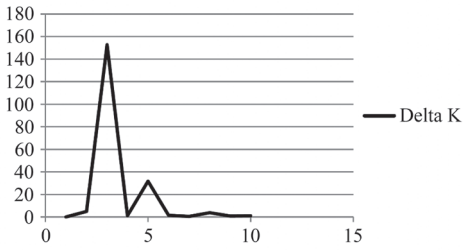
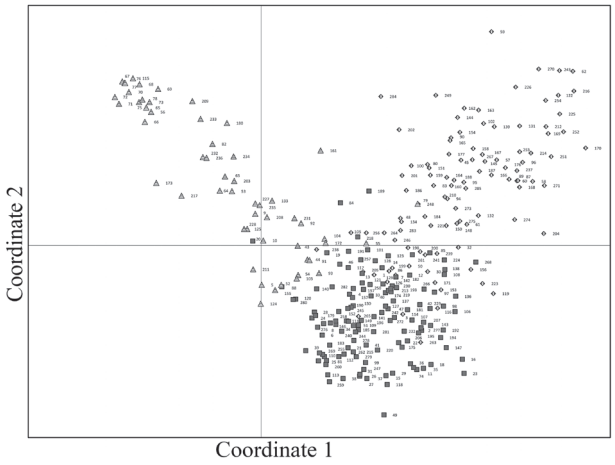


Figure 1. Detected subpopulations within 282 wheat accessions for K ranged from 1 to 10

The largest number of accessions, 127, belonged to subpopulation Q2. The subpopulation Q1 counted for 101, while Q3 included 54 accessions. Principal coordinate analysis (PCoA) confirmed obtained results (Fig. 2). The first coordinate separated subpopulation Q2 from the rest two groups, while the second coordinate partially separated Q3 from Q1 and Q2.

Both PCoA (Fig. 2) and UPGMA (Fig. 3) dendrograms demonstrated certain overlaps among groups, as a result of the frequent use of particular genotypes (e.g., 'Bezostaya 1', 'Mara', 'Marquis' and 'Norin10') in different breeding programs worldwide.

In previous studies, it was reported that grouping of wheat genotypes using molecular data and software *Structure* is in accordance with their geographic origin and pedigree (Neumann et al., 2011; Nielsen et al., 2014). In order to define trend for separation of genotypes into subpopulations in the present paper, the origin and pedigree data (http://www.wheatpedigree.net/) of analysed accessions were studied. With regard to geographic origin, it was observed that most of the cultivars created in Serbia (56 out of 72), Russia and Romania were assembled in Q1, as well as a few USA genotypes. The USA accessions (52 out of 63), were predominantly grouped in Q2, together with a half of

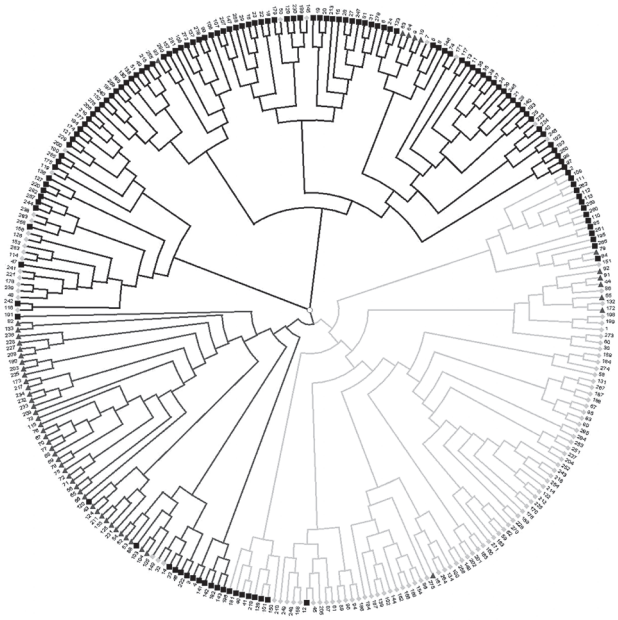


Note. Sub-groups derived from structure analysis are marked with different colours and patterns: Q1 – the cluster with genotypes from Southeast and East Europe (light grey rhombs), Q2 – the cluster with genotypes from the American continent and Central Europe (black squares), Q3 – the cluster with genotypes from Western Europe (dark grey triangles).

Figure 2. Principal coordinate analysis of 282 wheat accessions revealed by 32 microsatellite loci

Australian, and almost all Croatian and Hungarian cultivars. Finally, the subpopulation Q3 mostly consisted of accessions from Western Europe, namely United Kingdom, France and Germany.

Analysis of pedigree data indicated that in each group, specific cultivars dominated in family trees. Thus, 'Bezostaya 1' and cultivars that have 'Bezostaya 1' in their pedigree: 'Kavkaz', 'Avrora', 'Balkan', 'Zitnica', NS 14-33 and 'Novosadska Rana 2', dominated in the cluster with genotypes from the Southeast and East Europe (Q1). Further, a great proportion of accessions in this group were derived from crossing with the cultivars from the breeding program of Nazareno Strampelli ('Mara', 'Fortunato', 'San Pastore' and 'Ardito'). Finally, about 20% of studied accessions had 'Heines-VII' (DEU)



Note. Sub-groups derived from structure analysis are marked with different colours and patterns: Q1 – the cluster with genotypes from Southeast and East Europe (light grey rhombs), Q2 – the cluster with genotypes from the American continent and Central Europe (black squares), Q3 – the cluster with genotypes from Western Europe (dark grey triangles).

Figure 3. Radial dendrogram of 282 wheat accessions based on Roger's allele frequency distance of 32 microsatellite loci

in family tree. Further, within the pedigrees of genotypes from the American continent and Central Europe (Q2), the most frequent was Canadian cultivar 'Marquis', as well as accessions derived from 'Norin10' × 'Brevor' cross from CIMMYT breeding program ('Lerma Rojo 64', 'Sonora 64', 'Pitic 62' and 'Penjamo 62'). Although less frequent, Chinese cultivar 'Chinese Spring' and Brazilian cultivar 'Frontana' were also present in the family tree of Q2. Finally, in the pedigrees of the genotypes from Western Europe (Q3), the most present was French cultivar 'Cappelle Desprez' and its offspring ('Caribo', 'Perseus', 'Maris-Huntsman', 'Maris-Ploughman' and 'Hobbit'), as well as British accession 'Squarehead'. These results confirmed the impact of plant breeding on the wheat germplasm structure (Le Couviour et al., 2011).

The determined subpopulations differed in variability, and in each group private alleles were detected. The highest genetic diversity was observed in the group Q2 (Table 3). Only at 6 out of 31 SSR loci (*Xbarc1047*, *Xgwm495*, *Xgwm499*, *Xbarc243*, *Xbarc320* and *Xbarc1057*) the same allele was the most frequent in all three subpopulations. However, AMOVA revealed the highest percentage of molecular variance among individuals, 88% (Table 4), which is in accordance with the results of Zhang et al. (2011) and Nielsen et al. (2014). According to Nei's parameter, the subpopulation Q1 and Q3 were the most distant (Table 3).

Analysing population structure of the European hexaploid bread wheat cultivars, Nielsen et al. (2014) observed that a large proportion of the markers contributing to the genetic separation of the subpopulations were located on the chromosome 2DS, near the reduced height 8 (*Rht8*) locus. Further, they reported that the allele of 192 bp at the *Xgwm261* locus, associated with the *Rht8* gene, dominated within cultivars from Southeast Europe, while allele of 174 bp was the most frequent in the group of cultivars from

Table 3. Parameters of genetic diversity for detected wheat subpopulations

Parameter	Group		
	Q1	Q2	Q3
Total number of alleles	271	327	183
Average number of alleles per locus	8.47	10.22	5.72
Number of effective alleles	4.49	4.89	2.71
Total number of private alleles	49	90	18
Average number of private alleles per locus	1.53	2.81	0.56
Average frequency of private alleles per group	0.013	0.021	0.025
Expected heterozygosity	0.710	0.740	0.540
Shannon's information index	1.62	1.72	1.12
Group / Nei's genetic distance	Q1	Q2	Q3
Q1	0.000		
Q2	0.220	0.000	
Q3	0.383	0.264	0.000

Table 4. Analysis of molecular variance among and within three wheat sub-groups

Source	Degrees of freedom	Sum of squares	Mean square	Estimated variance	Estimated variance %	*Fst* value	*P* value
Among groups	2	515.582	257.791	1.328	11	0.111	0.001
Among individuals	279	5888.031	21.104	10.525	88		
Within individuals	282	15.000	0.053	0.053	1		
Total	563	6418.613		11.906	100		

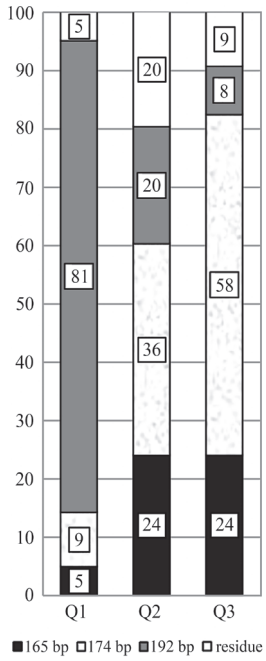
Western Europe. In this study, 192 bp allele was the most frequent within subpopulation Q1 with 81% (Fig. 4), mostly consisted of Serbian cultivars, while allele of 174 bp was the most common in the Q2 (36%) and Q3 (58%), where the majority of cultivars originated from the USA and Western Europe, respectively, which corroborate the results of Nielsen et al. (2014).

Based on the results of the phenotypic data, statistically significant differences were observed among subpopulations for all analysed traits except for number of spikelets per spike and grain number per spike. The flowering time was the most discriminative trait for grouping of genotypes (Table 5).

The flowering time is a critical developmental switch and a key adaptive trait in both crop and wild cereal species, and selection of optimal anthesis made possible cultivation of hexaploid wheat in a wide range of environments. The three different signalling pathways are included in the control of flowering time: the vernalisation (*Vrn*), photoperiod (*Ppd*) and earliness *per se* (*Eps*). For example, at high northern latitudes vernalisation genes delay ear initiation in winter wheat to protect this organ from damage caused by extreme low temperatures, while in the parts of the world with hot and dry summers, photoperiod insensitive genes accelerate anthesis to ensure the completion of reproductive phase before the onset of high temperatures (Kamran et al., 2014; Langer et al., 2014). Consequently, the mean value for flowering time for subpopulation Q1 (136.8), consisting of cultivars bred for cultivation in Southeast Europe, was significantly lower from the mean value for flowering time observed for subpopulation Q3 (143.1), where cultivars originating from Western Europe were grouped (Table 5). In order to compare discriminating power of molecular and phenotypic data for separation of wheat accessions, PCA was applied using only traits that showed statistically significant differences among groups for $P < 0.01$ (Table 5).

However, PCA did not show clear separation among subpopulations derived from structure analysis (Fig. 5). The first coordinate explained 40.7%, while the second interpreted 28.9%, accounting together for the 69.6% of the total variation. The traits flowering time and thousand kernel weight mostly contributed to the PC1, whereas stem height contributes the most to the PC2.

The obtained results confirmed advantages of molecular markers for elucidation of genetic diversity in wheat (Pagnotta et al., 2009; Najaphy et al., 2012).



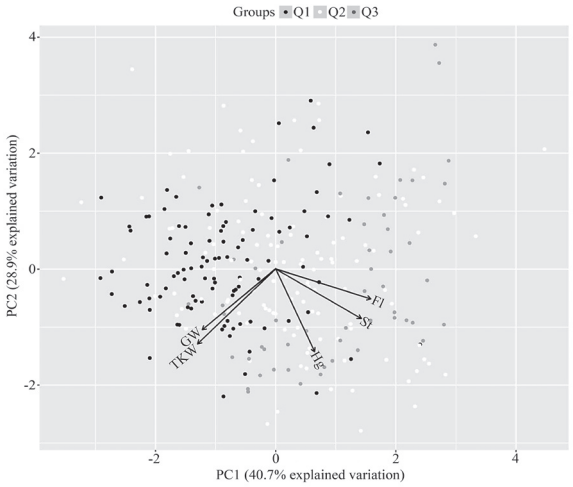
Residue – frequency of the rest of recorded alleles

Figure 4. Distribution of *Xgwm261-165*, *Xgwm261-174* and *Xgwm261-192* alleles within three detected wheat subpopulations Q1, Q2 and Q3

Table 5. Analysis of variance and comparison of means for three wheat sub-groups for 10 traits

Group	Hg	Hd	Fl	SL	SPS	St	GN	SW	GW	TKW
Q1	79.9 a	131.9 a	136.8 a	9.8 a	20.1 a	1.7 a	43.3 a	2.2 a	1.69 a	39.82 a
Q2	89.0 b	133.3 b	138.4 b	9.8 a	21.8 a	2.5 b	43.2 a	2.0 b	1.53 b	36.30 b
Q3	85.4 ab	138.7 c	143.1 c	10.4 b	20.8 a	2.6 b	42.2 a	2.0 b	1.51 b	36.84 b
	**	*	**	*	ns	**	ns	*	**	**

Hg – stem height, Hd – heading time, Fl – flowering time, SL – spike length, SPS – number of spikelets per spike, St – number of sterile spikelets per spike, GN – number of grains per spike, SW – spike weight, GW – grain weight per spike, TKW – thousand kernel weight; a-c – $P < 0.05$; **, * – significant at 0.01 and 0.05 probability level, ns – non significant



Note. Hg – stem height, Fl – flowering time, St – number of sterile spikelets per spike, GW – grain weight per spike, TKW – thousand kernel weight; Q1 – the cluster with genotypes from Southeast and East Europe (black dots), Q2 – the cluster with genotypes from the American continent and Central Europe (white dots), Q3 – the cluster with genotypes from the Western Europe (grey dots).

Figure 5. Principal component analysis of 282 wheat accessions based on phenotypic data for five traits

Conclusions

1. Microsatellite markers revealed a substantially high level of genetic diversity in a set of 282 hexaploid bread wheat accessions originated from 26 countries worldwide, indicating its potential for the creation of new modern cultivars.

2. According to structure analysis, examined accessions were separated into three subpopulations consistent with their origin and pedigree, and principal coordinate analysis (PCoA) and unweighted pair group method with arithmetic mean (UPGMA) dendrogram indicated certain overlaps between groups, illustrating the significance of plant breeding for the wheat germplasm structure.

3. Marker-based method demonstrated better discrimination power of accessions compared to phenotypic data used in this study, while both methods showed high genetic variation within the analysed wheat material, and demonstrated its potential for association studies of agronomically important traits in wheat.

Acknowledgements

This research is funded by the Ministry of Education, Science and Technological Development of Serbia (TR 31066).

Received 26 07 2018
Accepted 04 02 2019

References

1. Balfourier F., Roussel V., Strelchenko P., Exbrayat-Vinson F., Sourdille P., Boutet G., Koenig J., Ravel C., Mitrofanova O., Beckert M., Charmet G. 2007. A worldwide bread wheat core collection arrayed in a 384-well plate. Theoretical and Applied Genetics, 114: 1265–1275. https://doi.org/10.1007/s00122-007-0517-1
2. Borges A., Rosa M. S., Recchia G. H., de Queiroz-Silva J. R., de Andrade Bressan E., Veasey E. A. 2009. CTAB methods for DNA extraction of sweetpotato for microsatellite analysis. Scientia Agricola, 66 (4): 529–534. https://doi.org/10.1590/S0103-90162009000400015
3. Chao S., Zhang W., Akhunov E., Sherman J., Ma Y., Luo M.-C., Dubcovsky J. 2009. Analysis of gene-derived SNP marker polymorphism in US wheat (*Triticum aestivum* L.) cultivars. Molecular Breeding, 23: 23–33. https://doi.org/10.1007/s11032-008-9210-6
4. Earl D. A., von Holdt B. M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4: 359. https://doi.org/10.1007/s12686-011-9548-7
5. Hao C., Wang L., Ge H., Dong Y., Zhang X. 2011. Genetic diversity and linkage disequilibrium in Chinese bread wheat (*Triticum aestivum* L) revealed by SSR markers, PLoS ONE, 6 (2): e17279. https://doi.org/10.1371/journal.pone.0017279
6. Huson D. H., Scornavacca C. 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. Systematic Biology, 61: 1061–1067. https://doi.org/10.1093/sysbio/sys062
7. Kalia R. K., Rai M. K., Kalia S., Singh R., Dhawan A. K. 2011. Microsatellite markers: an overview of the recent progress in plants. Euphytica, 177: 309–334. https://doi.org/10.1007/s10681-010-0286-9
8. Kamran A., Iqbal M., Spaner D. 2014. Flowering time in wheat (*Triticum aestivum* L.): a key factor for global adaptability. Euphytica, 197: 1–26. https://doi.org/10.1007/s10681-014-1075-7
9. Kobayashi F., Tanaka T., Kanamori H., Wu J., Katayose Y., Handa H. 2016. Characterization of a mini core collection of Japanese wheat varieties using single-nucleotide polymorphisms generated by genotyping-by-sequencing. Breeding Science, 66: 213–225. https://doi.org/10.1270/jsbbs.66.213
10. Lai K., Duran C., Berkman P. J., Lorenc M. T., Stiller J., Manoli S., Hayden M. J., Forrest K. L., Fleury D., Baumann U., Zander M., Mason, A. S., Batley, J., Edwards D. 2012. Single nucleotide polymorphism discovery from wheat next-generation sequence data. Plant Biotechnology Journal, 10: 743–749. https://doi.org/10.1111/j.1467-7652.2012.00718.x
11. Langer S. M., Longin C. F. H., Würschum T. 2014. Flowering time control in European winter wheat. Frontiers in Plant Science, 5: 537. https://doi.org/10.3389/fpls.2014.00537
12. Le Couviour F., Faure S., Poupard B., Flodrops Y., Dubreuil P., Praud S. 2011. Analysis of genetic structure in a panel of elite wheat varieties and relevance for association mapping. Theoretical and Applied Genetics, 123: 715–727. https://doi.org/10.1007/s00122-011-1621-9
13. Najaphy A., Parchin R. A., Farshadfar E. 2012. Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. Australian Journal of Crop Science, 6: 326–332.
14. Neumann K., Kobiljski B., Denčić S., Varshney R. K., Börner A. 2011. Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L). Molecular Breeding, 27: 37–58. https://doi.org/10.1007/s11032-010-9411-7
15. Nielsen N. H., Backes G., Stougaard J., Andersen S. U., Jahoor A. 2014. Genetic diversity and population structure analysis of European hexaploid bread wheat (*Triticum aestivum* L) varieties. PLoS ONE, 9 (4): e94000. https://doi.org/10.1371/journal.pone.0094000
16. OECD. 2018. Crop production (indicator). https://doi.org/10.1787/49a4e677-en
17. Pagnotta M. A., Mondini L., Codianni P., Fares C. 2009. Agronomical, quality, and molecular characterization of twenty Italian emmer wheat (*Triticum dicoccon*) accessions. Genetic Resources and Crop Evolution, 56: 299–310. https://doi.org/10.1007/s10722-008-9364-4
18. Peakall R., Smouse P. E. 2012. *GenAlEx 6.5*: genetic analysis in *Excel*. Population genetic software for teaching and research – an update. Bioinformatics, 28: 2537–2539. https://doi.org/10.1093/bioinformatics/bts460
19. Rode J., Ahlemeyer J., Friedt W., Ordon F. 2012. Identification of marker-trait associations in the German winter barley breeding gene pool (*Hordeum vulgare* L.). Molecular Breeding, 30: 831–843. https://doi.org/10.1007/s11032-011-9667-6
20. Sorrells M. E., Yu J. 2009. Linkage disequilibrium and association mapping in the *Triticeae*. Feuillet C., Muehlbauer G. J. (eds). Genetics and genomics of the *Triticeae*. Springer, p. 655–683. https://doi.org/10.1007/978-0-387-77489-3_22
21. Soto-Cerda B. J., Cloutier S. 2012. Association mapping in plant genomes. Caliskan M. (ed.). Genetic diversity in plants. InTech, p. 29–54.
22. Spiertz J. H. J., Ewert F. 2009. Crop production and resource use to meet the growing demand for food, feed and fuel: opportunities and constraints. NJAS – Wageningen Journal of Life Sciences, 56: 281–300. https://doi.org/10.1016/S1573-5214(09)80001-8
23. Van Inghelandt D., Melchinger A. E., Lebreton C., Stich B. 2010. Population structure and genetic diversity in a commercial maize breeding program assessed with SSR and SNP markers. Theoretical and Applied Genetics, 120: 1289–1299. https://doi.org/10.1007/s00122-009-1256-2

24. Wickham H. 2016. *ggplot2*: elegant graphics for data analysis. Springer, Basel. https://doi.org/10.1007/978-3-319-24277-4
25. Wingen L. U., Orford S., Goram R., Leverington-Waite M., Bilham L., Patsiou T. S., Ambrose M., Dicks J., Griffiths S. 2014. Establishing the A. E. Watkins landrace cultivar collection as a resource for systematic gene discovery in bread wheat. Theoretical and Applied Genetics, 127: 1831–1842. https://doi.org/10.1007/s00122-014-2344-5
26. Würschum T., Langer S. M., Longin C. F. H., Korzun V., Akhunov E., Ebmeyer E., Schachschneider R., Schacht J., Kazman E., Reif J. C. 2013. Population structure, genetic diversity and linkage disequilibrium in elite winter wheat assessed with SNP and SSR markers. Theoretical and Applied Genetics, 126: 1477–1486. https://doi.org/10.1007/s00122-013-2065-1
27. Zhang L., Liu D., Guo X., Yang W., Sun J., Wang D., Sourdille P., Zhang A. 2011. Investigation of genetic diversity and population structure of common wheat cultivars in northern China using DArT markers. BMC Genetics, 12: 42. https://doi.org/10.1186/1471-2156-12-42

ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 106, No. 3 (2019), p. 257–264
DOI 10.13080/z-a.2019.106.033

Paprastojo kviečio pagrindinės kolekcijos genetinė struktūra ir alelių gausumas sąsajoms su derliumi nustatyti

D. Trkulja[1], A. Kondić-Špika[1], L. Brbaklić[1], B. Kobiljski[2], S. Mikić[1], M. Mirosavljević[1], S. Glogovac[1], G. Šurlan-Momirović[3]

[1]Serbijos augalininkystės ir daržovių institutas
[2]„Biogranum“, Serbija
[3]Belgrado universiteto Žemės ūkio fakultetas, Serbija

Santrauka

Kuriant šiuolaikines kviečių veisles, pasižyminčias atsparumu įvairiems abiotinio ir biotinio streso veiksniams, panaudojant kryptingą / tikslingą tėvinių formų atranką, itin svarbios yra žinios apie paprastojo kviečio (*Triticum aestivum* L.) kolekcijų genetinę struktūrą ir alelių įvairovę. Be to, informacija apie populiacijos struktūrą yra pirmoji prielaida sudaryti sąsajų žemėlapį, užkertantį kelią klaidingų sąsajų tarp žymeklio ir požymio nustatymui. Tyrimo tikslas – įvertinti genetinę įvairovę ir populiacijos struktūros egzistavimą kviečių genotipų grupėje, pasirinktoje derliaus ir su derliumi susijusių požymių sąsajų žemėlapiams sudaryti. Medžiaga, sudaryta iš 282 kviečių genotipų, besiskiriančių 10-čia agronomiškai svarbių požymių, buvo įvertinta naudojant mikrosatelitinius žymeklius, kurie buvo pasiskirstę trijuose kviečių genomuose. Trisdešimt viename paprastųjų pasikartojančių sekų (SSR) žymeklyje buvo nustatyti 397 aleliai (vidutiniškai 12,4). Didžiausia įvairovė buvo nustatyta mikrosatelitų lokusuose B genome, mažiausias alelių skaičius – D genome. Struktūrinė analizė parodė, kad egzistuoja trys subpopuliacijos, kurių genotipai buvo sugrupuoti pagal kilmę ir genealoginius duomenis. Kiekvienoje subpopuliacijoje buvo nustatyti atskiri aleliai, rodantys analizuojamų mikrosatelitų lokusų informatyvumą nustatant populiacijos struktūrą. Esminiai skirtumai tarp grupių buvo nustatyti 8-iems iš 10-ies kviečių agronomiškai svarbių požymių, o pagrindinių komponentų analizė (PCA) neparodė aiškaus genotipų išsiskyrimo. Išanalizuoti kviečių genotipai buvo pakankamai aukšto genetinės įvairovės lygio, atsižvelgiant ir į molekulinius bei fenotipinius duomenis, todėl šie genotipai yra tinkami kviečių selekcinių požymių sąsajų analizei.

Reikšminiai žodžiai: agronomiškai svarbūs požymiai, įvairovė, mikrosatelitų lokusai, *Triticum aestivum*.