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INVESTIGATION OF GENETIC DIVERSITY OF WHEAT GENOTYPES (*Triticum aestivum* L.) USING MICROSATELLITE MARKERS

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The study was devoted to evaluate genetic diversity of 48 winter wheat genotypes (varieties and advanced lines) with different origins using simple sequence repeat (SSR) markers. Out of 20 SSR primer pairs used for assessing the genetic diversity, 19 primer pairs were polymorphic. Microsatellite PIC values ranged from 0.26 to 0.93 with an average value of 0.740. The cluster analysis based on Nei genetic distance indices divided genotypes into four main groups. The Xwmc24, Xwmc169, Xgpw322, Xgwm297 and Xbarc267 primers amplified high number of alleles, showing relatively high level of polymorphism and evaluated as efficient primers for genetic diversity analysis of wheat populations.

Keywords: *bread wheat, microsatellite, polyacrylamide gel, genetic diversity*

Introduction

Wheat (*Triticum aestivum* L. em. Thell.) is one of the most important food crops in the world, and understanding its genome organization using molecular markers is of great value for genetic and plant breeding purposes [8]. Transcriptome studies have shown that over 30 000 genes are expressed in the developing wheat grain [15], while proteomic analysis of mature grain has revealed the presence of about 1125 individual components [11]. The haploid DNA content of hexaploid wheat (*Triticum aestivum* L. em Thell, 2n=42, AABBDD) is approximately 16 million bp, about 100 times larger than the Arabidopsis genome, 40 times that of rice and about 6 times that of maize [4]. The large genome of bread wheat has resulted from polyploidy and extensive duplications, such that over 80% of the genome consists of repetitive DNA sequences [12].

AFLP (Amplified Fragment Length Polymorphism) and RAPD (Random Amplified Polymorphic DNA) markers are most widely used for wheat among the others; however they define low level of polymorphism [9]. Microsatellites or simple sequence repeats (SSRs) are ubiquitous, hypervariable and abundant in many plant species, dispersed throughout the genome, and show higher levels of polymerphism [2]. These characteristics of microsatellites make them the most widely used markers in plant breeding, diversity analysis, mappings, and marker-assisted selection studies [14]. In eukaryotes, SSRs differ by wide variations in the repeat number resulting from dynamic and complex mutagenic events such as unequal crossing over, retrotransposition, and DNA polymerase slippage [5].

The objectives of the current study were to evaluate the use of SSR markers for assessing genetic diversity among 48 winter wheat genotypes.

Material and methods

A total of 48 winter bread wheat varieties (genotypes and advanced lines) originating from different countries/regions and obtained from the International Maize and Wheat Improvement Center (CIMMYT) were used for genetic diversity studies (table 1).

The experiments were carried out at the "Molecular Biology" laboratory of Warsaw University of Life Sciences. Genomic DNA isolation was performed using modified CTAB method (Murray and Thompson, 1980) [6]. The concentration of DNA was measured by Nanodrop 2000 spectrophotometer and diluted to a final concentration of 100 ng/μL.

Table 1

The name and the origins of the 48 winter bread wheat accessions

№	Genotypes	Origin	№	Genotypes	Origin
1	Sg-u 8069	CZ	25	Comp1/5/bez//tob/8156/4/on/	TCI
2	Gobustan	AZB	26	Agri/nac//attila	MX-CIT
3	Krasnovodopadskaya 211	KAZ	27	Destin	RO-FL
4	Prostor	BG	28	Demir	TR-ANK
5	Gerek	TR-ESK	29	Dyuopebusa	MOL
6	Ubileynaya 100	RUS-KR	30	Ok00421	USA-OK
7	Bayraktar	TR-ANK	31	Altay	TR-ESK
8	Hamsi-1	MX-TCI	32	Akinci-84	AZB
9	Bezostaya1	TR-ESK	33	Grs1201/tam202	US-ARS-Lincoln
10	Karahan	TR-KON	34	Mima	BG
11	Mvma/mv12//f2098	HU-MV	35	Nikoniya	TR-KON
12	Starshina	RUS-KR	36	Lc924/petja	UKR-OD
13	Co 970547-7	USA-CO	37	Podoima	BG-SAD
14	Zubkov	KYR	38	Steklovidnaya24	MOL
15	Mv06-02	HU-MV	39	Dalnitskaya	KAZ
16	Gloria	RO-FL	40	Vita	UKR
17	Lc 909 mima	BG-KC	41	Kharkovskaya107	RUS-KR
18	Tx96v2847	US-TX	42	Azeri	AZB
19	U1254-1-8-1-1/tam-202	USA-TX	43	453	KAZ
20	Sonmez	TR-ESK	44	Sg-s1915	CZ
21	Arlin/yuma	USA-KSU	45	Madsen/malcolm	OSU
22	Eritr 9945	KYR	46	7c/cno//cal/3/ymh/4/vp...	OSU
23	Gruia	RO-FL	47	Id 80-628/3/cer/ymh...	OSU
24	Mv dalma	HU-MV	48	U1254	TCI

Twenty pairs of microsatellite primers were downloaded from the GrainGenes database of United States Department of Agriculture (USDA) (http://wheat.pw. usda.gov/GG2) and cited from papers.

PCR reactions were performed in 15 µl reaction mixture containing 100 ng/µl of genomic DNA template, 1X green PCR Buffer, 0,5mM of $MgCl_2$, 0,2mM of dNTP, 0,2mM of forward primer, 0,2 mM of reverse primers and 0,02U/µl Taq DNA polymerase. The cycling program for DNA amplification (Veriti və Cycler və Gene Amp., PCR System 9700) consisted of 3 stages and 41 cycles. The samples were initially subjected to one times of 10 repeats of following cycle: 1 min at 94°C, 30 s at 65°C and 1 min at 74°C. Then reaction mixture subjected to another 30 cycle, which annealing temperature set to decrease -1°C for every cycle from 65°C to 55°C. The second cycling program were: 94.0°C for 15 s, 55.0°C for 30 s, 72.0°C for 1 min. Final extension was 72.0°C for 5 min 5µl LB (loading buffer) were added into PCR amplification products, denatured 4 min and separated using 6% polyacrylamide gels at 2900 V for 3 hours. Polyacrylamide gels were silver stained using combination of the different steps proposed by Basşam et al. [3], and Sanguinetti et al. [10]. Amplified bands were scored in binary format as present (1) or absent (2) and polymorphism information content (PIC) was calculated using the following formula: $PIC= 1- \Sigma Pi^2$, where P_i is the proportion of the population carrying i^{th} allele, calcu-

lated for each microsatellite locus. The binary data were used to compute Nei's genetic distance index. Cluster analysis was performed using the Unweighted Pair-Group Method with Arithmetical Average (UPGMA). The SPSS 12 and PAST software packages were used for statistical analysis of amplified alleles.

Results and discussion

Selected 20 primers on the base of their genetic location covered all three wheat genomes (A, B, and D) (table 2). Out of 20 SSR primer pairs used 19 primers produced sufficiently polymorphic alleles, whereas XBARC1239 primer pairs were found to be monomorphic in the studied wheat genotypes. A total of 104 alleles were detected with 19 primers. The number of alleles per locus ranged from 3 to 9, with a mean of 5.4 alleles per locus. The highest number of alleles was detected with a XGPW 322 primer pairs, while XWMC 304, XBARC 72, XWMC 47, XPSP 3094 and XGWM 33 primers were distinguished with a lower number of alleles amplified. The polymorphism information content values ranged from 0.26 to 0.93, with an average value of 0.74. Among the primers used in the present study, the lowest PIC values with 0.26 and 0.33 were recorded with XWMC 47 and XBARC 72, whereas XPSP 3094 and XGWM 33 primers showed the middle PIC values. The rest of 16 primer pairs observed with the highest polymorphism values. The PIC value of XWMC (0.93) was higher than the other studied primers.

Table 2

Number of alleles, repeat units, chromosomal location and polymorphism detected by SSR markers

№	Primer designation	Repeat units	Chromosomal location	Number of alleles	PIC
1	Xwmc24	Simple perfect	1A	8	0.93
2	Xwmc169	Simple perfect	3A	8	0.92
3	Xbarc20	Simple perfect	4B, 7B	5	0.81
4	Xbarc24	Compound imperfect	6B	7	0.82
5	Xwmc304	Simple perfect	1A	3	0.74
6	Xwmc47	Simple perfect	7A	3	0.26
7	Xbarc54	Simple perfect	3A, 6D	5	0.82
8	Xpsp3094	Compound imperfect	7A	3	0.61
9	Xbarc72	Compound imperfect	7B	3	0.33
10	Xgpw322	Simple perfect	3D	9	0.92
11	Xgpw2233	Simple perfect	7A	5	0.80
12	Xbarc79	Compound imperfect	6B	5	0.81
13	Xgwm33	Simple perfect	1A, 1B, 1D	3	0.55
14	Xwmc264	Simple perfect	3A	5	0.81
15	Xbarc94	Compound imperfect	5A, 7B	5	0.86
16	Xgwm297	Compound imperfect	7B	8	0.86
17	Xbarc267	Simple perfect	7B	8	0.84
18	Xbarc168	Simple perfect	2D	7	0.82
19	Xbarc10	Simple perfect	2B, 4B, 5A, 7B	4	0.77
20	Xbarc1239	Simple perfect	1B	Mon	Mon
	Total	-	-	105	-
	Minimum	-	-	3	0.26
	Maximum	-	-	9	0.93
	Mean	-	-	5.47	0.74

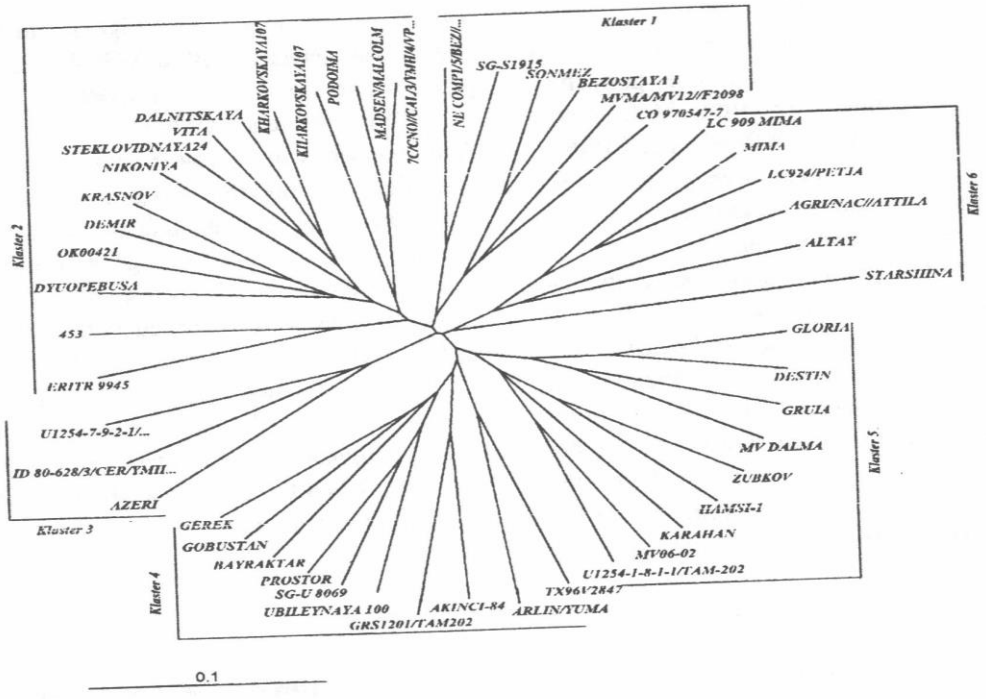

Figure. A dendrogram showing the genetic distance among 48 wheat accessions

SSR markers have been used to evaluate genetic diversity in cultivated hexaploid wheat genotypes and other plant species by different researchers. Genetic diversity of thirteen wheat cultivars of diverse origins have been evaluated using 43 SSR markers by Ahmad (2002) and a total of 156 polymorphic alleles at 43 loci with a wide range of allelic variants for each locus detected [1]. Another study by Prasad et al. (2000) examined the utility of a set of 20 wheat SSR markers to detect DNA polymorphism, identify genotypes, and estimate genetic diversity among 55 elite wheat genotypes [7]. The range of alleles amplified for per locus was 1-13, averaging 7.4, and the PIC range was 0.21–0.90, with an average value of 0.71. We observed a similar PIC value (respectively 0.26–0.93). The observed PIC value indicates that all these primers were highly informative and capable of distinguishing between studied genotypes.

The *Nei* genetic distance index was used to assess genetic distance between the 48 wheat genotypes. Cluster analysis by using UPGMA method grouped 48 wheat genotypes into 6 main clusters (figure).

The first cluster contained 6 genotypes (Turkey, Europe, and USA) from different origins and covered.12.5% of total genotypes. According to the Nei genetic distance indices, minimum and maximum values of genetic similarity coefficient were found to be 0.27 and 0.13, respectively. The lowest genetic distance value was calculated as 0.13 between Bezostaya and Sonmez genotypes, whereas the highest value was noted between CO 970547-7 and SG-S1915 (0.27). The second cluster was the largest and consisted of 14 (29%) genotypes. The genetically closest genotypes were 45 and 46 from USA, while the lowest similarity (0.32) was observed between ERITR 9945 (USA) and MADSEN/MALCOLM (Kyrgyzstan) genotypes, respectively. The third cluster identified the smallest group (6%) and contained 3 genotypes from Azerbaijan and USA. Two advanced lines from USA and Azeri genotypes were found to cluster in the same group, however the less genetic distance was observed (0.37) among mentioned genotypes. The fourth, fifth and sixth cluster consisted of 10, 9 and 6 genotypes, respectively. The corre-

lation between origin and localization of genotypes was not observed in the intra groups. The genetically closest and most distant genotypes for the same and various geographic regions were identified as a result of cluster analysis.

Knowledge of the genetic diversity of a species is important for the choice of parents in plant breeding [13]. The microsatellite markers are being widely used for genetic diversity and distance evaluation, genetic mapping studies and marker-assisted selection (MAS) in breeding. The results of studies on genetic diversity analysis using molecular markers contribute to development of appropriate strategies for selecting genotypes in breeding programs.

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