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Detection of DNA Polymorphism, Genetic Diversity and Genotype Identification by using Microsatellites in Bread Wheat *(Triticum aestivum L.)*

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Abstract- By means of DNA fingerprinting techniques characterization of germplasm provides a utensil for specific germplasm identification and a quantitative estimate of genetic diversity. In this study, 36 wheat microsatellites (SSR) were used with 30 elite wheat varieties to scrutinize their service (1) in estimating genetic diversity (2) in the identifying genotypes and (3) in detecting DNA polymorphism, among wheat genotypes. The 30 elite varieties of Wheat used in this study originated in Pakistan and has been cultivating since long. A total of 155 alleles were detected at 36 loci using the above microsatellite primer pairs, all the primers amplified 1 locus each. Of the 36 primers amplifying 36 loci, 17 primers and their corresponding 18 loci were assigned to 13 different chromosomes (6 chromosomes of the A genome, 5 chromosomes of the B genome and 2 chromosomes of the D genome). The number of alleles per locus ranged from 2 to 16, with an average of 4.3 alleles per locus. The value of average polymorphic information content (PIC) for these markers was estimated to be 0.55. The dendrogram delineated the above genotypes into two major clusters (I and II), each with two sub clusters (Ia, Ib and IIa, IIb). Using a set of only 12 primer pairs, we were able to distinguish a maximum of 26 of the above 30 wheat genotypes. The weight of total grains per plant was trailed in RCBD design test and it is found that there is great difference among the varieties and the results demonstrate the effectiveness of microsatellite markers.

Keywords- Microsatellite markers · Wheat ·Genetic diversity · Genotype identification.

Abbreviations- PIC-Polymorphic information contents, SSR- Simple sequence repeats, GD- Genetic diversity, RCBD- Randomized complete block design.

1 INTRODUCTION

Man-madehexaploid wheat-rye hybrid triticale (x Triticosecale Witt.) is measured a capable crop with a broad genetic potential. Triticale adapted to a wide range of biotic and abiotic stressconditions, is asignificantchoice feed stock and produces similar grain yield but additional biomass compared to other (Altheit et al., 2011). It is becoming more and more important in agriculture and accepting its genetic diversity is vital for its continued development (Kuleung et al., 2006).Yet, it is hardly ever used for human utilization because of its poor bread-making quality (Lukaszewski, 2006).The awareness of diversity within the triticale gene pool is significant information for today´s line breeding and an essential requirement for future hybrid breeding (Tams et al., 2002).Simple Sequence Repeat (SSR) markers are a precious tool for a lot of purposes, such as fingerprinting, mapping, and breeding in many plant species (Röder et al., 1998, Gregáňová et al., 2005, Vyhnánek et al., 2009, Ražná et al., 2010, Labajová et al., 2011).

Though, they are no more than accessible in some economically imperative crops because of the sky-scraping cost and manual labor intensity involved in their advance (Kuleung et al., 2006).Microsatellite DNA markers are consistently found to be more informative than supplementary classes of markers in hexaploid wheat (Song et al., 2005).SSR markers are dear because of their superior level of transferability to interrelated species, and they can frequently be used as newscaster markers for comparative mapping and evolutionary studies (Varshney et al., 2005, Vyhnánek et al., 2009).Wheat is a major food source for most world population. However, its cultivation is strictly limited by such challenges as pests, diseases, droughts, extreme temperatures, and others. In order to manage sustainable wheat production, it is necessary to develop new cultivars of wheat resistant and tolerant to adverse environmental factors.

In this research work, we testimony the results of a study concerning the screening of 30 varieties using 36 microsatellite primers. The study was undertaken with the subsequent objectives: (1) to study the potential of microsatellite markers in general and specific SSRs in particular for detection of polymorphism and for genotype identification and (2) to evaluate the level of microsatellite-based genetic diversity among 30 spring wheat varieties that were potentially functional in wheat breeding programs.

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2 MATERIALS AND METHODS

2.1 Seed material

Seed of 30 varieties LU-26, PASBAN-90, BARS-2009, NARC-2009, FSD-2008, PIRSBAK-90, WAFAQ-2001, LASANI-2008, SEHAR-2006, FAREED-2006, SH-2003, BAHAWALPUR-2000, GA-2002, MERAJ- 2008, SHAFAQ-2006, SULEMAN-96, SHAHEEN-94, PIRSABAK-05, PASHKOO-03, PUNJAB-85, MANTHAR-03, BAKHAR-

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2002, AUQAB-2000, FAKHAR-E-SARHAD, SALEEM-2002, ZARDANA, SAUGHAT-90, PASTOR, ROHTAS-90 and KAGHAN-94 of spring wheat originating in Pakistan from last 30 years, was procured from the Directorate of Wheat Research institute Fateh Jang. DNA isolation and microsatellite primers DNA were extracted from leaves of each of the 30 varieties grown in the pots using a modified CTAB method (Saghai Maroof et al. 1984). Thirty six microsatellite primers were used, made available to us as a research scholar from Wheat Microsatellite Consortium (WMC) under a scholarship of Higher Education Commission batch VI. The clones bearing microsatellites belonged to a genomic library enriched for microsatellites (Edwards et al. 1996) and were sequenced by members of the WMC.

2.1.1 DNA Extraction

Young leaf tissue was harvested from all the hexaploid wheat varieties for DNA extraction and placed in liquid Nitrogen for rapidly freezing the leaf material. The plant material was then crushed with a knitting needle while inside the tube. Five hundred µl DNA extraction buffer (1% SDS, 100mM NaCl, 100mM Tris base, 100mM Na2EDTA, pH 8.5 by HCl) were added to each appendorff tube containing the crushed leaf material and mixed well with the help of a knitting needle. Total 500 µl of phenol: chloroform: isoamylalchohol (25:24:1) mixture was added and tubes were vortex until a homogeneous mixture was made. Samples were then centrifuged at 5000 rpm for 5 minutes. The aqueous phase (supernatant) was transferred to a fresh appendorff tube. To precipitate DNA 50µl of 3M sodium acetate (pH 4.8) and 500 µl cold isopropanol was added to the tube and mixed gently. To make the DNA pellet, samples were centrifuged at 500 rpm for 5 minutes. The supernatant was decanted and the pellet was washed with 70% ethanol. Pellets were dried at room temperature for an hour and re-suspended in 50 µl TE (10mM Tris, 1mM EDTA pH: 8.0). To remove RNA, DNA was treated with 40µg RNAse-A (20 µl of commercially supplied RNAse-A) at 37°C for 1 hour.

2.1.2 DNA Quantification

Purity of the DNA in the samples, dissolved in TE buffer was analyzed by the checking the absorbance ratios at 280/260 nm on spectrophotometer while concentration was calculated assuming that 1 O.D (optical density) at 260nm corresponds to 50ng/ml DNA.

2.1.3 Single Sequence Repeats (SSR) Analysis

Polymerase Chain Reaction: PCR reactions were carried out in 25 µl reaction containing 50-100 ng total genomic DNA template, 0.25 µM of each primer, 200 µM of each dATP, dGTP, dCTP, dTTP, 50 mM KCl, 10 mM Tris, 1.5 mM MgCl2 and 2.5 units of Taq DNA polymerase (Dweikat et al., 1993). For SSR analysis SSR primers specific for A, B and D genome which are 276 in number were used. The sequences of these primers are given in Annexure II.

Amplification Conditions: The amplification conditions was as; an initial step of denaturation for 1 minute at 93ºC followed by 30 cycles each consisting of a denaturation step of 30 seconds at 93ºC, an annealing step of 1 minute at 60ºC and an extension step of 1 minute at 72ºC. Five minutes will be given after the last cycle to the extension step at 72ºC to ensure the completion of the primer extension reaction. Amplitrony x 6 was used for all amplification reactions. For electrophoresis of the amplification products, 1.5 % Agarose/TBE gel was used. Gels were visualized by Ethidium Bromide under the UV light chamber and observed using the computer program UVI Photo MW.

Chromosomal localization: PCR amplification using the above conditions was also carried out with target DNA samples from each of the 30 spring wheat varieties using the above 36 microsatellite primer pairs. This allowed chromosome localization of 26 out of the 30 loci sampled through the use of above microsatellite primer pairs. The different loci identified using individual primer pairs and assigned to specific chromosomes as above were given designations in accordance with the Rules of Nomenclature for DNA markers (McIntosh et al. 1998), as approved earlier at the 7th International Wheat Genetics Symposium, held at Cambridge (Hart and Gale 1988).

2.2 Statistical analysis

The fragment(s) sizes in 'Chinese Spring' were taken as standard, and the size differences of the fragments in other genotypes were considered to be the result of alterations in the repeat number of the simple sequences at the corresponding site(s). Allelic polymorphic information content (PIC) was calculated using the following formula.
PIC = 1- (Pi) 2

Where Pi is the proportion of the population carrying with allele, calculated for each microsatellite locus (Botstein et al. 1980).

The marker index (MI) was calculated using the following formula (Powell et al. 1996). MI = Average polymorphic information content (PIC) Proportion of polymorphic bands Average number of loci per assay unit For the purpose of assessing genetic diversity leading to the preparation of a dendrogram, gels were scored in binary format, with the presence of a band scored as unity and its absence scored as zero. The binary data were used to compute pair-wise similarity coefficients (Jaccard 1908), and the similarity matrix thus obtained was subjected to cluster analysis using the UPGMA (unweighted pair-group method with arithmetic average) algorithm on NTSYS-PC, version 1.70 (Rohlf 1992).

3 RESULTS

The allelic information data for the 36 SSR markers which includes number of alleles and unique alleles for individual locus, predominant allele and its frequency, PIC and GD values is summarized in Table 2. The 36 markers and their

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corresponding 36 loci were distributed across all the 42 chromosomes comprising A, B and D genomes. The results of PCR amplification of a number of micro satellite loci in 30 wheat varieties using 36 wheat micro satellite primer pairs are summarized in Table (1). By micro satellite primer sequencing we detected a total of 155 alleles at 21 loci.

Table: 1 Details of micro satellite primer sequences, repeat motif and expected product size (*NA* not available)

S. no.	Primer designation	Primer sequence(5'-3')	Micro satellite	Annealing Temp-erature c	Expected product size (bp) in Chinese spring	Reference personal communication
1	CFD59	5' TCACCTGGAAAATGGTCACA 3'	1	72	296	Guyomarc'h H et al.
		5' AAGAAGGCTAGGGTTCAGGC 3'				(2002)
2	CFD81-5D	5' TATCCCCAATCCCCTCTTTC 3'	1	94	283	Guyomarc'h H et al.
		5' GTCAATTGTGGCTTGTCCCT 3'				(2002)
3	CFD82	5' GCTGATGCTGCTGTAAGTGC 3'	1	94	242	Guyomarc'h H et al.
		5' TGAAGAATACAATGGCAGCAA 3'				(2002)
4	CFD92	5' CTTGTTGATCTCCTTCCCCA 3'	1	72	253	Guyomarc'h H et al
		5' TTCTCTCATGACGGCAACAC 3'				(2002)
5	CFD83	5' AAGGATGGAGAGGACCCCTA 3'	1	94	233	Guyomarc'h H et al.
		5' GGAGGTGGAGCAACCTATCA 3'				
6	CFD2-1	5' GGTTGCAGTTTCCACCTTGT 3'	1	94	288	Somers DJ et al. (2004)
		5' CATCTATTGCCAAAATCGCA 3'				
7	CFD2-2	5' GGTTGCAGTTTCCACCTTGT 3'	1	72	228	Somers DJ et al. (2004)
		5' CATCTATTGCCAAAATCGCA 3'				
8	CFD2-3	5' GGTTGCAGTTTCCACCTTGT 3'	1	94	288	Somers DJ et al. (2004)
		5' CATCTATTGCCAAAATCGCA 3'				
9	BARC67-3A	5' GCGGCATTTACATTTCAGATAGA 3'	1	52	226	Somers DJ et al. (2004)
		5' GTGCCTGATTGTAGTAACGTATGTA3'				
10	CFD41-7D	5' TAAAGTCTCAGGCGACCCAC 3'	1	72	286	Guyomarc'h H et al.
		5' AGTGATAGACGGATGGCACC 3'				(2002)
11	CFD73-2B	5' GATAGATCAATGTGGGCCGT 3'	1	73	242	Guyomarc'h H et al.
		5' AACTGTTCTGCCATCTGAGC 3'				(2002)
12	CFD73-NA1	5' GATAGATCAATGTGGGCCGT 3'	1	72		
		5' AACTGTTCTGCCATCTGAGC 3'			242	Guyomarc'h H et al.
13	CFD73-NA2	5' GATAGATCAATGTGGGCCGT 3'	1	74		(2002)
		5' AACTGTTCTGCCATCTGAGC 3'				
14	CFD106	5' ACGGGTGGTTTTGCTCAGT 3'	1	72	187	Guyomarc'h H et al.
		5' ACTCCACCAGCGGAGAAATA 3'				(2002)
15	CFD9-3D	5' TTGCACGCACCTAAACTCTG 3'	1	72	209	Guyomarc'h H et al.
		5' CAAGTGTGAGCGTCGG 3'				(2002)
16	BARC45	5' CCCAGATGCAATGAAACCACAAT 3'	1	52	188	P. Cregan, Q. Song
		5' GCGTAGAACTGAAGCGTAAAATTA 3'				(2002)
17	CFD62-7A	5' CAAGAGCTGACCAATGTGGA 3'	1	72	220	Guyomarc'h H et al.
		5' ACGGCGGTGAGATGAG 3'				(2002)
18	CFD62-NA	5' CAAGAGCTGACCAATGTGGA 3'	1	72	220	Guyomarc'h H et al.
		5' ACGGCGGTGAGATGAG 3'				(2002)
19	CFD65-1D	5' AGACGATGAGAAGGAAGCCA 3'	1	72	199	Guyomarc'h H et al.
		5' CCTCCCTTGTTTTTGGGATT 3'				(2002)
20	CFD65-1B	5' AGACGATGAGAAGGAAGCCA 3'	1	72	178	Guyomarc'h H et al.
		5' CCTCCCTTGTTTTTGGGATT 3'				(2002)

21	CFD141-3D	5' CGTAAAGATCCGAGAGGGTG 3'	1	72	155	Guyomarc'h H et al.
		5' TCCGAGGTGCTACCTACCAG 3'				(2002)
22	CFD143-3B	5' TTCTCCATGGGCAGCTACTT 3'	1	72	262	Guyomarc'h H et al.
		5' ACTACTTGCGGACGGCTG 3'				(2002)
23	CFD156	5' AGCAGTGTAATAAAAGGGCG 3'	1	72	300	Guyomarc'h H et al.
		5' GTATTCGCACCAGAATCCGT 3'				(2002)
24	GWM2	5' TCTCCCTTGTTTCCGGGATT 3'	1	68	256	Roeder MS et al.
		5' GGAAGATGAGAAGGAAGCCA 3'				(1995)
25	WMC215	cATgcATggTTgcAAgcAAAAg3'	1	72	207	Somers DJ and Isaac P
		cATcccggTgcAAcATcTgAAA3'				(2004)
26	CFD13	5' CCACTAACCAAGCTGCCATT 3'	1	72	254	Guyomarc'h H et al.
		5' TTTTTGGCATTGATCTGCTG 3'				(2002)
27	CFA2134	5'TTTACGGGGACAGTATTCGG3'	1	94	210	Sourdille P et al. (2001)
		5' AAGACACTCGATGCGGAGAG3'				
28	WMC149	5 ACAGACTTGGTTGGTGCCGAGC3	1	61	230	S.G. Rogers (USA)
		5 ATGGGCGGGGGTGTAGAGTTTG3				(2001)
29	WMC11	5' TTgTgATccTggTTgTgTTgTgA3'	1	61	177	Somers DJ and Isaac P
		5'cAcccAgccgTTATATATgTTgA3'				(2004)
30	WMC489	5'cgAAggATTTgTgATgTgAgTA3'	1	51	232	Somers DJ and Isaac P
		5'ggAcAAcATcATAgAgAAggAA3'				(2004)
31	WMC532	5'gATAcATcAAgATcgTgccAAA3'	1	61	176	Somers DJ and Isaac P
		5'gggAgAAATcATTAAcgAAggg3'				(2004)
32	WMC527	5'AcccAAgATTggTTgcAgAA3'	1	61	386	Somers DJ and Isaac P
		5'gcTAcAgAAAAccggAgccTAT3'				(2004)
33	CFD193	5' GCTGCCGCTACTGTCTGTC 3'	1	72	199	Guyomarc'h H et al.
		5' GGCACACTCACACACCACAC 3'				(2002)
34	WMC428	5'TTAATccTAgccgTcccTTTTT3'	1	51	257	Somers DJ and Isaac P
		5'cgAccTTcgTTggTTATTTgTg3'				(2004)
35	WMC264	5' cTccATcTATTgAgcgAAggTT3'	1	61	133	Somers DJ and Isaac P
		5'cAAgATgAAgcTcATgcAAgTg3'				(20049
36	WMC269	5'gcAccTTcTAAccTTccccAgc3'	1	61	147	Somers DJ and Isaac P
		5'cccTAATccAggAcTcccTcAg3'				(2004)

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The polymorphism information content (PIC) value is frequently taken in genetics as an evaluation of polymorphism for a marker locus used in linkage analysis. In this conversation we have derived the consistently minimum variance impartial estimator of PIC along with its accurate variance. We have also calculated the accurate variance of the maximum likelihood estimator of PIC which is asymptotically an unbiased estimator. To find out the variance we have derived a recursive formula to calculate the moments of every polynomial in a set of variables that are multinomial distributed. The PIC values that estimate the discriminating ability of any locus by considering the number of alleles per locus and their relative frequencies (Anderson et al. 1993) varied according to the markers and ranged from 0.04 to 0.76, with an average of 0.35.

The PIC were also estimated, and it can be seen from the data of Table (2) that the highest values of 0.90 and 0.89 were recorded for CFD-81 and CFD-59, respectively, and the lowest values of 0.24 were recorded for CFD2-1, CFD2-2 and 0.29 for CFD13, WMC489 respectively with the mean value of 0.55 for all the 36 used microsatellite markers. The genetic diversity (GD) coefficients for all the possible 30 spring wheat varieties ranged from 0.05 to 0.88 and averaged 0.23. The dendrogram prepared through cluster analysis is shown in Fig. 1 suggesting a high level of genetic diversity among the 30 wheat varieties The genotypes could be grouped into two major clusters, cluster I with 2 genotypes i.e. BARC-09 and NARC-09 and cluster II with rest of 28 wheat varieties. This indicates that these two

varieties might have some common origin because that do not having much genetic diversity in them. Cluster II is further subdivided into two sub clusters, sub-Cluster IIa containing 5 genotypes Auqab-2000, Kaghan-94, Manthar-03, Punjab-85 andLasani-08 and sub cluster IIb containing remaining 23 wheat genotypes. The remaining 23 genotypes belonging to cluster IIb were similarly grouped into two sub clusters, sub cluster IIb-1 containing two varieties SH-03 and S haheen-94 sub cluster IIb-2 containing rest of all varieties that were 21 in number.

Table: 2 Details of microsatellite primers used for the PCR amplification of alleles at 21 loci and the values of Polymorphic information content (PIC)

S. no.	Primer designation	Locus designation	No of alleles	Gene Diversity	PIC	Range of allele size (bp)
1	**CFD59**	Xcfd59-1B	13	0.90	0.89	230-310
2	**CFD81-5D**	xcfd81-5D	16	0.91	0.90	273-279
3	**CFD82**	Xcfd82	5	0.78	0.74	242-258
4	**CFD92**	Xcfd92-1D	3	0.53	0.47	253-256
5	**CFD83**	xcfd83	3	0.63	0.56	239-241
6	**CFD2-1**	Xcfd92-1D	2	0.28	0.24	350-350
7	**CFD2-2**	Xcfd2-2A	2	0.28	0.24	300-300
8	**CFD2-3**	Xcfd2-3A	4	0.74	0.70	168-172
9	**BARC67-3A**	Xbarc67	5	0.73	0.68	104-110
10	**CFD41-7D**	Xcfd41-7D	4	0.66	0.61	276-284
11	**CFD73-2B**	Xcfd73-2B	3	0.53	0.42	246-248
12	**CFD73-NA1**	Xcfd73-2D	3	0.64	0.56	280-282
13	**CFD73-NA2**	Xcfd73b	3	0.53	0.42	170-172
14	**CFD106**	Xcfd106-4D	3	0.61	0.52	198-200
15	**CFD9-3D**	Xcfd9-3D	8	0.79	0.77	180-256
16	**BARC45**	Xbarc45-3A	8	0.80	0.77	180-204
17	**CFD62-7A**	Xcfd62-7A	3	0.57	0.49	190-192
18	**CFD62-NA**	Xcfd62a	3	0.60	0.52	208-210
19	**CFD65-1D**	Xcfd65-1D	2	0.49	0.37	198-201
20	**CFD65-1B**	Xcfd65-1B	3	0.53	0.42	143-149
21	**CFD141-3D**	Xcfd141-3D	3	0.61	0.54	155-159
22	**CFD143-3B**	xcfd143-3B	6	0.78	0.74	205-500
23	**CFD156**	Xcfd156-5B	3	0.57	0.49	196-198
24	**GWM2**	xgwm2	4	0.69	0.64	132-140
25	**WMC215**	Xwmc215-5D	5	0.64	0.60	196-204
26	**CFD13**	Xcfd13-6B	2	0.36	0.29	120-120
27	**CFA2134**	xcfa2134	5	0.73	0.69	220-280
28	**WMC149**	xWMC149	4	0.70	0.64	105-110
29	**WMC11**	Xwmc11a-3A	4	0.64	0.57	165-250
30	**WMC489**	Xwmc489a	2	0.36	0.29	230-232
31	**WMC532**	Xwmc532-3A	5	0.74	0.70	176-185
32	**WMC527**	Xwmc527	5	0.74	0.69	360-386
33	**CFD193**	Xcfd193-4D	2	0.39	0.31	199-203
34	**WMC428**	Xwmc428	3	0.46	0.41	157-257
35	**WMC264**	Xwmc264-3A	3	0.50	0.43	120-133
36	**WMC269**	Xwmc269-1B	3	0.55	0.46	147-153
	Mean		4.3	0.61	0.55	

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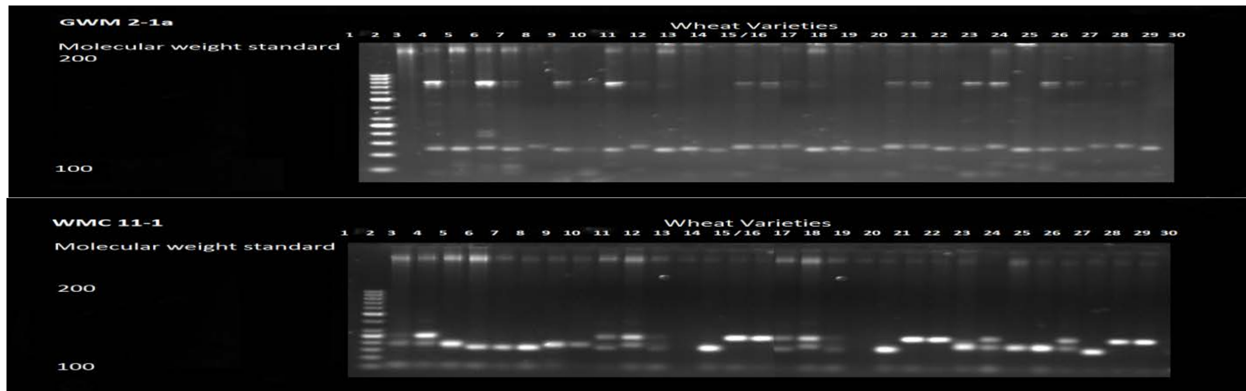
Fig. 1 Representative sample of amplification profiles in 30 wheat genotypes obtained using different microsatellite primer pairs:



4 DISCUSSION

A low polymorphism was experiential among the *Triticum aestivum* spring wheat varieties, with an average of 4.3 alleles per locus (range 2–16) and an average similarity coefficient of 0.61. The observed average number of alleles was higher than that reported in the majority of the studies on emmer wheat collections (Pagnotta *et al*. 2005) observed 2–8 alleles(with an average of 4) per locus in a collection of 39Italian emmer accessions, using 6 EST-SSR markers. And lower in a diversity analysis approved on 34 Ethiopian emmer landraces using 29 microsatellite markers, an average of 6.95 alleles per locus was prescribed by (Teklu *et al*. 2006). Regardless of this general single-locus nature of microsatellites, in the current study 55 microsatellite loci were identified using 36 primer pairs: each of 19 primer pairs detected only 1 locus and only 1 primer pair, WMC256, detected 2 loci. Since microsatellite primers are locus specific, only 1 specific locus was anticipated to be augmented by each primer, and it was unexpected that this 1 primer amplified 2 loci. The microsatellite loci are also multiallelic (1–13 alleles per locus with a mean of 7.4 alleles/locus in the present study) and the allele's co dominant, thus suggesting their comparative supremacy in detecting DNA polymorphism over some other markers (e.g. SNPs, single nucleotide polymorphisms) which are biallelic and dominant.

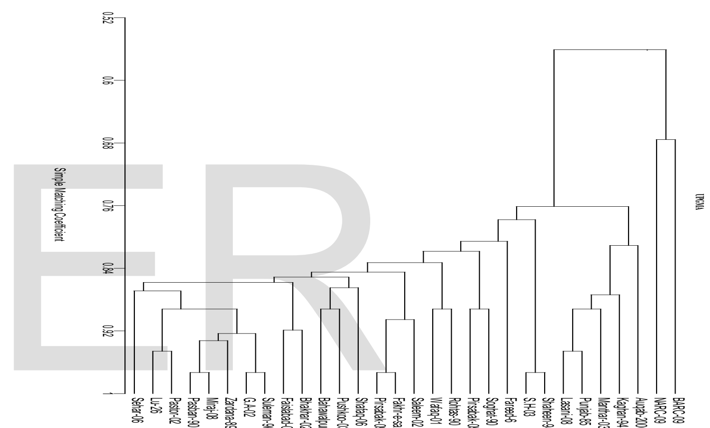


Fig. 1 Dendrogram of 30 wheat genotypes based on data on allelic profiles generated using 36 microsatellite primer pairs. I and II represent clusters, and Ia, Ib, IIa, IIb represent subclusters (for details, see Results)

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The number of alleles per locus ranged from 2 to 16, with an average of 4.3 alleles per locus. The maximum number of alleles (16) was detected at the locus CDF81-5D.A judgment of the outcome obtained in this study with those available earlier indicates that the average number of alleles per locus recorded at some point in the present study was comparatively higher that is 4.3 than those earlier reported for several self-pollinated and annual crops including wheat, with estimates of 3.8, 4.6 (Hokanson et al. 1998). This soaring number of alleles per locus definitely contributed to the usefulness of these markers, although there seems to be no straight correlation between the number of alleles at a locus and the PIC value. Genetic diversity among 30 wheat varieties in the course of cluster analysis is estimate of a genetic similarity (GS) coefficient among pairs of varieties ranged from 0.28 to 0.91. In the present study the average value of GS was as low as 0.61, suggesting that the 30 wheat varieties used were diverse. This GS value of 0.61 can be compared with those reported in three previous studies, where SSR-based GS coefficient values of 0.31 (Plaschke et al. 1995) and 0.57 (Bohn et al. 1999) and a STS-based GS coefficient value of 0.81 (Chen et al. 1994) were reported. In

the present study, the highest GS value of 0.91 elite markers (CFD81-5D) discriminated19 genotypes, 3 (CFD65-1D WMC11,WMC489 and CFD193) microsatellite markers discriminated the 30 all the genotypes and 4 microsatellite markers (BARC67-3A, CFD73-2B, CFD73-NA2, CFD9-3D, BARC45, CFD65-1B and WMC215) discriminated 29 genotypes with the GS value 0f (.49, 64, .36, .70 and .73, .53, .64, .79, .80, .49, .64) respectively. Three ellite markers (WMC532, WMC264 and WMC269) discriminated the 28 genotypes with the GS value of (.74, .50 and .55) respectively. Four ellite markers (CFD41-7D, CFD62-7A, CFD143-3B and CFD156) discriminated the 27 genotypes with the GS value of (.66, .57, .78 and .57) respectively.

Only one ellite marker (CFD13) discriminated the only 7 genotype with the GS value of (.36) and three ellite markers (CFD81-5D CFD81-5D, CFA3193 and CFD81-6D) discriminated 19 genotypes with a GS value of (.91 and .90 and .70) and one (CFD83) ellite marker with GS value of (.63) discriminated 20 genotypes and two ellite markers (CFD82, CFD2-3) with GS value of (.74 and .24) discriminated 22 genotypes and four ellite markers (CFD59, CFD2-2, CFD73-NA1 and WMC428) with the GS value of (.90, .28, .53, .46) discriminated 24 genotypes and 25 respectively (CFD92 CFD2-1, CFD141-3D) discriminated 25 genotypes with the GS value of (.90, .28, .56 and .61) respectively . Which are below the average of 25.48 and all the other ellite markers discriminated the genotypes more than the average. However, supplementing the 3 markers with more markers did improve the discriminating ability of the set of ellite microsatellite markers. So that even 3 markers only discriminated the same 30 genotypes that could be discriminated by a set of 12 markers. A wide range of genetic diversity among all genotypes was experienced. In the light of these results it is possible for both to classify the genetic diversity of best genotypes and select genotypes or cultivars for the highest genetic diversity using SSRs, as pointed by cluster analysis.

An assessment was finished in a current study to agree on whether genetic distances calculated by means of molecular markers, as above, can be used for predicting the intensity of genetic variance (□2 g) among the progenies that would be derived from the crosses made between diverse genotypes (Bohn et al. 1999). Consequently, further studies may need to be carried out to find out the efficacy of the GS worked out using casual molecular markers rather than QTL-linked GS for predicting the □2g in the progeny of a projected cross. The above conversation sufficiently demonstrates the efficacy of microsatellites, which can be gainfully utilized in wheat not only for detecting polymorphism and tagging genes (Prasad et al. 1999; Roy et al. 1999) but also for genotype identification and for judgment of genetic diversity.

5 CONCLUSION

The present study of SSR, s genetic diversity and similarity among the wheat varieties under this research confirms that they had significant effect on the phylogenetic relationship of plant populations and an efficient means of introducing novel diversity into bread wheat gene pool.

Germplasm collections are our birthright for future generations for improving the quality and quantity of bread wheat.

6 REFERENCES

[1] Altheit, K.V., Reif, J.C., Maurer, H.P., Hahn, V., Weissmann, E.A., Miedaner, T. Wurschum, T. 2011. Detection of segregation distortion loci in triticale x Triticosecale Wittmack based on a high-density DArT marker consensus genetic linkage map. In BMC Genomics, vol.12, 2011, p. 1-13.

[2] Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993) Optimizing parental selection for geneticlinkage maps. Genome 36:181–186.

[3] Bohn, M., U.H. Friedrich and A.E. Melchinger. 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs and SSRs and their use for predicting progeny variance. Crop Sci., 39: 228-237.

[4] Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32: 314–331.

[5] Dweikat, I., S. Mac Kenzie, M. Levy, H. Ohm. 1993. Pedigree assessment using RAPD-DGGE in cereal crop species. Theor Appl Genet., 83: 497–505.

[6] Gregáňová, Ž., Kraic, J. - Gálová, Z. 2005. Effectiveness of microsatellites in differentiation of elite wheat cultivars. In Biologia, vol. 60, 2005, p. 665-670.

[7] Hart G, Gale MD (1988) Guidelines for nomenclature of biochemical/molecular loci in wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp Vol. 2. Bath Press, Bath,

[8] Hokanson SC, Szewc-McFadden AK, Lamboy WF, McFerson JR(1998) Microsatellite (SSR) markers reveal genetic identities,genetic diversity and relationships in *Malus* □□*domestica*borkh. Core subset collection. Theor Appl Genet 97: 671–683

[9] Jaccard P (1908) Nouvelles recherches sur la distribution florale.Bull Soc Vaudoise Sci Nat 44: 223–270.

[10] Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaudoise Sci Nat 44: 223–270.

[11] Kuleung, C., Baenziger, P.S., Kachman, S.D., Dweikat, I. 2006. Evaluating the genetic diversity in triticale with wheat and rye SSR markers. In Crop Science, vol. 46, 2006, p. 1692-1700.

[12] Kuleung, C., Baenziger, P.S., Kachman, S.D., Dweikat, I. 2006.Evaluating the genetic diversity in triticale with wheat and rye SSR markers. In Crop Science, vol. 46, 2006, p. 1692-1700.

[13] Labajová, M., Senková, S.,Žiarovská, J., Ražná, K., Bežo, Mi.,Štefúnová, V., Zeleňáková, L. 2011. The potential of ISSR markers in amaranth gamma-radiance mutant's genotypying. In Journal of Microbiology, Biotechnology and Food Sciences, vol. 1, 2011, p. 507-521.

[14] Lukaszewski, A. J. 2006. Cytogenetically engineered rye chromosomes 1R to improve bread-making quality of hexaploid triticale. In Crop Science, vol. 46, 2006, p. 2183-2194.

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[15] McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed)Proc 9th Int Wheat Genet Symp Vol. 5. University ExtensionPress, University of Saskatchewan, Canada, pp 1–12.

[16] Pagnotta MA, Mondini L, Atallah MF (2005) Morphologicaland molecular characterization of Italian emmer wheataccessions. Euphytica 146:29–37

[17] Prasad, M., R.K. Varshney, J.K. Roy, H.S. Balyan and P.K. Gupta. 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat Theor Appl Genet., 100:584–592.

[18] Raziq, F., M. Ahmad and H. Kiran. 2005. Isolation of Trichoderma on selective media and it's in vitro efficacy against Fusarium oxysporus. Sarhad J. Agric. 21(1): 117-120.

[19] Ražná, K.,Bátovská, A., Bežo, M., Masnicová, S., Žiarovská, J. 2010.Analýza DNA polymorfizmu vybraných línií kukurice siatej (Zea mays L.) pomocou PCRISSR markérov. In Acta fytotechnica et zootechnica, vol. 13, 2010, p. 15-18.

[20] Röder, M.S. - Korzun, V. - Wendehake, K. - Plaschke, J. - Tixier, M.-H. - Leroy, P. - Ganal, M.W. 1998. A microsatellite map of wheat. In Genetics, vol. 149, 1998, p. 2007–2023.

[21] Rohlf FJ (1992) NTSYS-PC: Numerical taxonomy and multivariate analysis system. Exeter Software, New York.

[22] Saghai-Maroof, M.A., Biyashev, R.M., Yang, G.P. Zhang, Q. and Allard, R.W. (1994). Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc. Natl. Acad. Sci., USA, 91:5466-5470

[23] Song, Q.L., Shi, J.R., Singh, S., Fickus, E.W., Costa, J.M., Lewis, J., Gill, B.S., Ward, R., Cregan, P.B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. In Theoretical and Applied Genetics, vol. 110, 2005, p. 550-560.

[24] Tams, S.H., Melchinger, A.E., Oettler, G., Bauer, E. 2002. Assesment of genetic diversity in European winter triticale using molecular markers and pedigree. Proceedings of the 5thInternational Triticale Symposium, vol. 1, 2002, p. 95-102.JMBFS / Trebichalský et al. 2013 : 2 (Special issue on BQRMF) 1898-1906.

[25] Teklu Y, Hammer K, Huang XQ, Ro¨der MS (2006) Analysis ofmicrosatellite diversity in Ethiopian tetraploid wheatlandraces. Genet Resour Crop Evol 53:1115–1126. UK, pp 1215–1218.

[26] Varshney, R.J., Graner, A.,Sorrells, M. E. 2005. Genic microsatellite markers in plants: features and applications. In Elsevier, vol. 23, 2005, no. 1, p. 48-55

[27] Vyhnánek, T., Nevrtalová, E., Slezáková, K. 2009. Detection of the genetic Variability of triticale using wheat and rye SSR markers. In Cereal Research Communications, vol. 37, 2009, no. 1, p. 23-29.

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