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Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani wheat varieties

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Molecular diversity among one hundred and five bread wheat (*Triticum aestivum* L.) varieties was estimated with four SSR markers. All wheat varieties used in present study showed a high degree of polymorphism. Number of alleles produced were 9 revealing average of 2.25 alleles per locus. The number of alleles per locus were ranged from 2 to 3. The Polymorphic information content (PIC value) ranged from 0.55 (WMC-215) to 0.84 (Hap-6A-P1), revealing 0.67 average value per locus. Similarity matrix indicated, least genetic similarity value of 0.00 (Fakhre-sarhad and Zarlashta-99) and highest value 1.00 (Sariab-92 and Sandal-73) revealing average value of 0.50. Cluster analysis was applied by using UPGMA method. Cluster analysis showed that 105 wheat genotypes were grouped in to four clusters. Results revealed that high genetic diversity were showed by Pakistani bread wheat genotypes. To identify wheat genotypes and for estimation of genetic diversity these wheat microsatellite markers could be used. The present study also confirms that for evaluation of genetic diversity microsatellite or SSR markers could be used for the fast and high throughout fingerprinting of wheat genotypes.

Keywords: Bread wheat, Genetic diversity, PCR, PIC, SSR

INTRODUCTION

Bread wheat consists of large and complicated genome among cereals. Bread wheat is allohexaploid (2n=6x=42, AABBDD) in nature and consist of three homoeologous genomes (Sears, 1954) and a genome size of 16Mb.

Estimation of genetic diversity is considered the base for enhancement of crop plants and an essential step for adaptation of new varieties to new environmental conditions. Many studies have reported modern breeding has caused loss of genetic diversity (Fu et al. 2005). Many studies reported that the genetic diversity narrowness in crops is increasing which can lead to vulnerability of crop plants to diseases and pests (Fu and Somers., 2009). Genetic diversity narrowness could cause the incompetency of crop plants and can affect their response to various environmental constraints (Gorji and Zolnoori, 2011). Hence, for broadening the genetic diversity in coming future wheat breeding programs, it is crucial to investigate the level of genetic diversity in current bread wheat varieties.

Many criteria are being used for evaluation of genetic diversity, between them most commonly used methods are morphological traits, pedigree record and molecular markers (cox et al. 1985). For assessment of genetic diversity morphological traits are crucial, and could be used but problem associating with them they are influenced by environment. Microsatellites are receiving much attention for assessment of genetic diversity. Microsatellites had been used successfully for analysis of genetic variability among self-pollinating crops because they are inherited co-dominantly and are highly polymorphic in nature (Akkaya et al. 1992; Plaschke et al. 1995). Studies showed that for identification of genotypes within species microsatellites are successfully used (Devos et al. 1995).

In this research, we used twenty microsatellite markers in order to evaluate the genetic diversity between 105 varieties of bread wheat. The aims of present study are (i) For one hundred and five bread wheat varieties characterization and estimation of the genetic diversity, (ii) selection of parents for advancement of high yield bread wheat varieties in future plant crop breeding programs (iii) Evaluation of polymorphic information (PIC) content of microsatellite markers.

MATERIALS AND METHODS

One hundred and five bread wheat varieties were studied for investigation of genetic polymorphism by using four SSR markers. Name of varieties used in current study are presented in (Table 1). Extraction of DNA was done from two week old seedling of each plant by using protocol of Saghai-Maroof et al. (1984). In order to check quality of genomic DNA, 0.8% agarose gel electrophoresis was used. Final DNA concentration for individual sample was conformed to 50 ng/µl.

Wheat genotypes were screened with help of four SSR markers for the molecular characterization.

Table 1: The bread wheat varieties used in this study

S.No	Genotypes	S.No	Genotypes	S.No	Genotypes	S.No	Genotype
1	Rawal	29	Khyber87	57	Bahawalpur97	85	Seher2006
2	SA75	30	Noshera96	58	Potohar-90	86	Galaxy2013
3	Sandal73	31	C250	59	Punjab76	87	Faisalabad08
4	Raskoh05	32	LU26	60	Punjab81	88	PS2004
5	Bakhtawar94	33	MH97	61	NIAB-83	89	Gomal08
6	Dera98	34	Kohsar95	62	Shafaq2006	90	Siran2010
7	SH2002	35	Punjab88	63	yecora70	91	PS85
8	Pirsabak91	36	Shahkar95	64	Faisalabad85	92	AQ2000
9	Zarlashta99	37	DIRK	65	NARC2011	93	Kaghan94
10	sutlaj86	38	Punjab96	66	C119	94	SH2006
11	GA2002	39	Pirsabak2009	67	DWR	95	PS2008
12	Pari73	40	Imdad2005	68	Faisalabad83	96	Atta Habib
13	Rohtas90	41	Sandal	69	Sariab92	97	PAK81
14	SKD1	42	Nuri70	70	Tarnab-73	98	Janbaz
15	Wafaq2001	43	Anmol91	71	C271	99	ZAM04
16	Kaghan93	44	Inqilab2000	72	C228	100	AARI10
17	SA42	45	C591	73	Meraj08	101	Hashim70
18	Blue-silver	46	Soghat90	74	Chakwal86	102	KT2000
19	Fakhre-sarhad	47	Zarghoon79	75	Anmol90	103	Shalimar-88
20	NARC2009	48	Saleem2000	76	Tatara	104	Suleman96
21	Sariab-97	49	Kiran95	77	Raskoh	105	FPD-08
22	Khirman	50	Parwaz94	78	Lasani-08		
23	Haider2000	51	C273	79	LocalWhite		
24	Daman	52	Chenab2000	80	Margalla99		
25	Zardana	53	Punjab85	81	Pirsabak08		
26	Marvat01	54	Bahawalpur2000	82	Kohistan97		
27	Barani83	55	Kohinoor83	83	Auqab2000		
28	Barani70	56	Farid2006	84	Chakwal84		

The PCR was performed in a reaction volume of 20 µl, of which template DNA was 50 ng, Taq DNA polymerase 1.5 unit, dNTPs 100 µM, PCR buffer 2ul of 1X and each primer 0.5 µl. Microsatellite amplification carried out initial step for 4 minutes at 95°C, then 40 cycles for 1 minute at 95°C, 1 minute at (55°C - 61°C) annealing temperature, extension at 72°C for 2 minutes, and in last and a final extension for 7 minutes at 72°C after completion of 40th cycle. Amplified fragments consist of genomic DNA were electrophoresed on 2% agarose gels. Agarose gel was stained with ethidium bromide.

Microsatellite amplification data were recorded as Presence (1) and absence (0) of bands. Similarities among genotypes were investigated by using DICE coefficient of similarity and tree showing genetic distance was constructed by use of the NTSYS-pc software version 2.02 conforming to the (UPGMA) un-weighted pair group method with arithmetic average (Rohlf, 1992). Allele frequency was calculated by using Microsoft Excel software. Anderson et al. (1993) method was used for calculation of PIC (Polymorphism information content) value. PIC=1-ΣPij2, hence Pij is the frequency of jth allele of ith locus, summing entire alleles over all genotypes.

RESULTS AND DISCUSSION

In this study four primers were used (Table 2) for one hundred and five wheat genotypes. Total number of alleles identify were 9 revealing average of 2.25 alleles per locus. Total number of bands identified were 236. All primers (Figure 2) were polymorphic and bands were ranged from 207bp (WMC-215) to 939bp (HAP-6A). Varieties Sandal-73, Kaghan-93, DIRK, Anmol, C-273, Sariab-92, Tarab-73, and C-271 produce four alleles which were highest number of alleles, Contrary varieties Blue-silver, Imdad-2005, C-591, and PS-2008 did not produce any allele. The marker produced maximum number of polymorphic alleles (3) was BF-WR1, while the rest of markers produced two alleles.

Results revealed that all markers showed high PIC value and therefore they were highly polymorphic. These markers (Table 3) could be used in order to investigate genetic diversity and association studies in wheat. All primers indicate Polymorphic Information Content values were ranged from WMC215 (0.55) to Hap-6A (0.84) showing an average value of 0.67 (Table 2). The PIC value revealed all the markers used in this research were highly informative. A study conducted by Botstein *et al*. (1980) explained that markers are highly informative if PIC value>0.5 while markers showing PIC≤0.50 are just informative, hence PIC≤0.25 are least revealing markers. Bryan *et al*. (1997) revealed average PIC value of 0.51 in bread wheat with the help of 49 SSR primers. In past studies, wheat cultivated in Bulgaria showed PIC value ranged from 0.10 to 0.81. Studies from Hungary, Austria and Germany using 42 SSR markers for 60 wheat varieties showed average PIC value of 0.57 (Stachel *et al*., 2000). In past studies, Röder *et al*. (2002) used five hundred bread wheat varieties and find out of 0.67 as an average PIC value, and present study also confirmed it.

SSR similarity matrices of one hundred and five wheat varieties confirmed the relationship between them. Lowest similarity value of 0.00 was obtained among genotypes Fakhre- sarhad and zarlashta-99, whereas highest similarity value of 1.0 was obtained between Sariab-92 and Sandal-73. All the genotypes revealed average similarity of 0.50 which indicate a high level of genetic diversity between one hundred and five wheat varieties.

In order to indicate the relationship among one hundred and five wheat varieties, a phylogenetic tree was constructed by using an average linkage among wheat varieties. A dendrogram was constructed in order to indicate the genetic relationship among one hundred and five wheat genotypes (Figure 1).

Table 2: Names of wheat microsatellite markers, their loci, annealing temperature, expected fragment sizes, chromosomal locations, no. of alleles, and PIC values

S.NO	Marker	Annealing temperature	Expected fragment	Chromo- some	No of alleles	PIC value
1	WMC-215	55	207	5D	2	0.55
2	BFWR1	56	237	4B	3	0.58
3	BFMR1	56	237	4B	2	0.71
4	Hap-6A	58	949	6A	2	0.84

Table 3: Description of SSR markers used in the study

Primers	Forward Primer	Reverse Primer
WMC 215	CATGCATGGTTGCAAGCAAAAG	CATGCATGGTTGCAAGCAAAAG
BFWR1	GGTAGGGAGGCGAGAGGCGAG	CATCCCCATGGCCATCTCGAGCTG
BFMR1	CCAGATACACAACTGCTGGC	TGATCTTGAGGTTCTCGTCG
HAP-6A	CGTTACCTCTGGTTTGGGTGTCGTG	CACCTCTCGAAAATCTTCCCAATTA

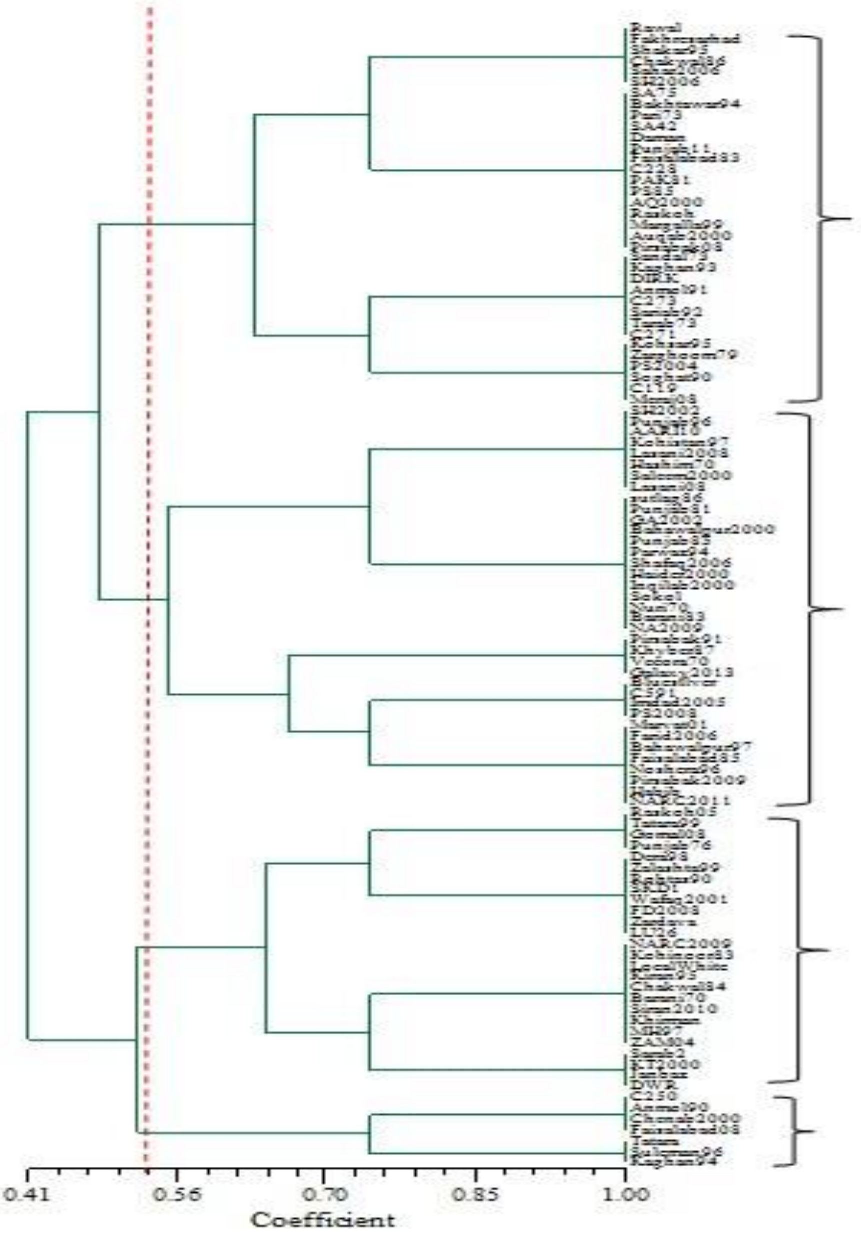
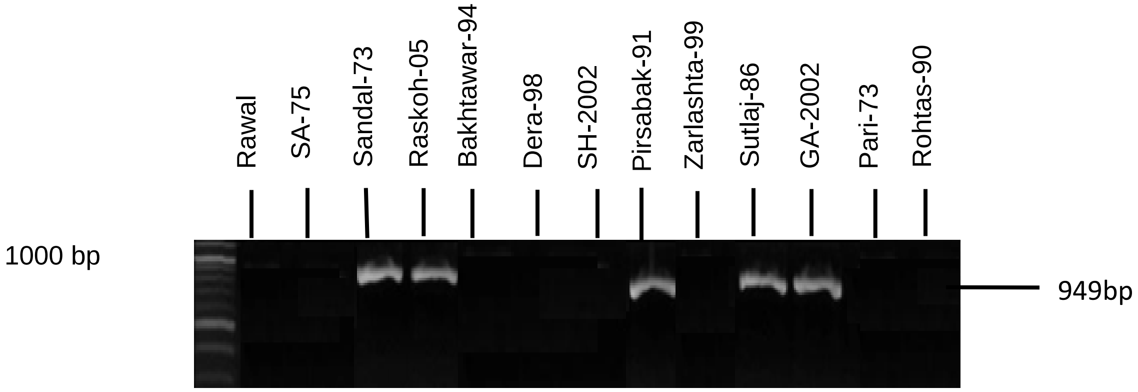
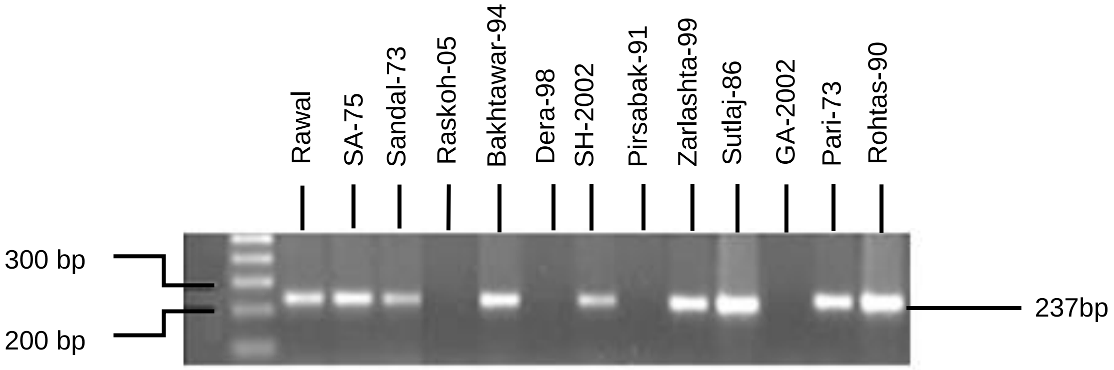


Figure 1: The dendrogram based on 105 Pakistani bread wheat (Triticum aestivum L.) varieties from UPGMA analysis

A) - HAP06A



B**) – BFWR1**



C) – BFMR1

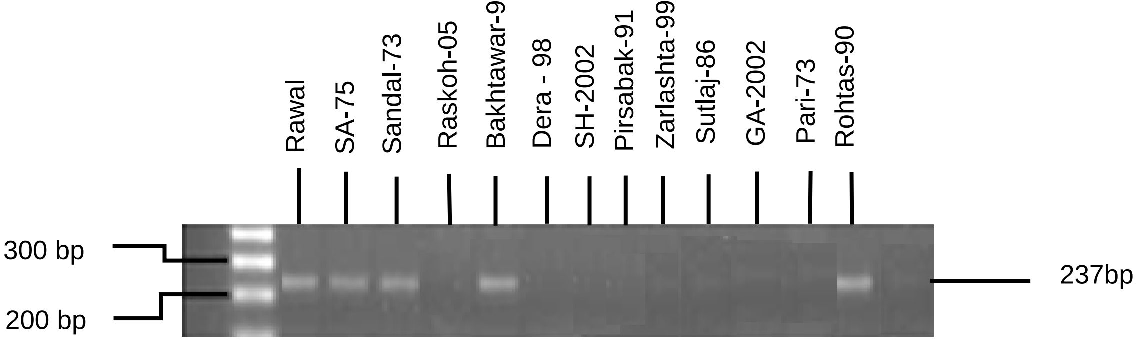


Figure 2 (A-C): PCR results with following primers: A) Hap-06: 949 bp, B) BF-WR1: 237 bp and C) BF-MR1: 237bp

Dendrogram illustrate the ability of microsatellite to identify large amount of genetic diversity in wheat varieties. Wheat varieties were divided into four major groups on the basis of cluster analysis. Cluster I included 35 varieties. Cluster II consist of 37 varieties. Clusters III comprised of 26 varieties. Cluster IV consist of 7 varieties.

CONCLUSION

Primers showed a high polymorphic pattern in this study. Hence, these primers could be used for assessment of genetic diversity, association mapping studies and for screening of bread wheat varieties. In conclusion, for efficient screening of wheat varieties there is need for more polymorphic SSR markers. The data revealed by this study will further enhance the wheat breeding efficiency by adding strength of marker-assisted selection (MAS). The genetic variability in one hundred and five Pakistani wheat varieties, in the light of information above, would be more helpful by utilizing the local wheat cultivars or varieties to produce hybrid varieties by crossing them with different elite varieties.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

KZ: Sample collection, Conceptualization, Methodology, Writing and editing the manuscript, Laboratory work. Inamullah: Supervision and proofreading. AB: Data analysis and data interpretation.AJ and KA Data collection. NS Sample collection.NZ laboratory work. All authors read and approved the final manuscript.

REFERENCES

Akkaya SM, Bhagwat AA and Cregan PB, 1992. Length polymorphism of simple-sequence repeat DNA in soybean. Genetics 132: 1131-1139.

Anderson JA, Churchill GA, Autrique JETanksley SD and Sorrells M.E, 1993. Optimization parental selection for genetic linkage maps. Genome 36: 181-186. *http://dx.doi.org/10.1139/g93-024*.

Botstein D, White RL, Skolnick M and Davis RW, 1980. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. American Journal of Human Genetics 32: 314-331.

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB and Gale MD, 1997. Isolation and characterization of microsatellites from hexaploid bread wheat. Theoretical and Applied Genetics 94: 557-563. *http://dx.doi.org/10.1007/s001220050451.*

Cox TS, Lookhart GL, Walker D, Harrell ELG, Albers LD and Rodgers MD, 1985. Genetic relationships among hard red winter wheat cultivars as evaluated by pedigree analysis and gliadin polyacrylamide-gel electrophoretic patterns. Crop Science 25: 1058 1063.

Devos KM, Bryan GJ, Collins AJ and Gale MD, 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor Appl Genet 90: 247-252.

Fu YB, Peterson GW, Richards KW, Somers D, DePauw RM and Clarke JM, 2005. Allelic reduction and genetic shift in the Canadian hard red spring wheat germplasm released from 1845 to 2004. Theor Appl Genet 110: 1505-1516.

Ghosh S, Karanjawala ZE and Houser ER, 1997. Methods for precise sizing automated inning of alleles and reduction or error rates in large scale genotyping using fluorescently labeled dinucleotide markers. Genome Research 7: 165–78.

Gorji AH and Zolnoori M, 2011. Genetic diversity in Hexaploid Wheat Genotypes using Microsatellite Markers. Asian J Biotechnol 3(4): 368-377.

Plaschke J, Gangland WHMS and Röder MS, 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theoretical and Applied Genetics 91: 1001-1007.

Röder MS, Wendehake K, Korzun V, Bredemeijer G, Laborie D, Bertrand L, Isaac P, Rendell S, Jackson J, Cooke RJ, Vosman B and Ganal MW, 2002. Construction and analysis of a microsatellite based database of European wheat varieties. Theor Appl Genet 106: 67-73.

Rohlf FJ, 1992. NTSYS-pc numerical taxonomy and multivariate analysis system New York: Exeter Software. Retrieved from *http:/www.exetersoftware.com.*

Saghai-Maroof MA, Soliman KM, Jorgensen RA and Allard RW, 1984. Ribosomal DNase pacer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci 81; 8014–8019.

Sears ER, 1954. The Aneuploids of Common Wheat Bull 572, University of Missouri Agricultural Experiment Station Columbia, MO.

Stachel M, Lelley T, Grausgrube H and Vollmann J, 2000. Application of microsatellites in wheat (*Triticum aestivum* L.) for studying genetic differentiation caused by selection for adaptation and use. Theoretical and Applied Genetics 100: 242-248. *http://dx.doi.org/10.1007/s001220050032.*