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Molecular diversity analysis in Wheat genotypes using SSR markers

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Abstract
Molecular diversity of the seven wheat (*Triticum aestivum* L.) genotypes was evaluated using 50 SSR primers. Genetic distances were calculated using unweighted pair group of arithmetic mean (UPGMA) procedure. A high degree of genetic polymorphism was observed among the wheat varieties with average genetic polymorphism 70%. SSR diversity data was generated using above primers and a total of 114 alleles were detected with an average of 2.71 alleles per locus. The number of alleles per locus ranged from 2 to 6 and the percent of polymorphism ranged from 20% for the *Xwmc254* to 100% for the *Xbarc26.* The average genetic diversity based on SSR markers was 0.465 with a range of 0.037- 0.892. Our results suggested that the classification based on genotypic markers of these wheat genotypes would be useful for wheat breeders to plan crosses for positive traits. The results obtained suggested that the wheat microsatellite markers could be used to distinguish wheat genotypes and to estimate genetic diversity. The present study also indicates that microsatellite markers permit the fast and high throughout fingerprinting of accessions from a varieties collection in order to assess genetic diversity.

Keywords: Wheat (*Triticum aestivum* L.), Molecular markers, Simple Sequence Repeats, genetic diversity.

Introduction

Genetic diversity is the basis for launching an efficient breeding programme that aimed for the improvement of wheat productivity. Wheat breeding through hybridization also requires the selection of diverse genotypes, irrespective of whether the product is a pure line or a hybrid variety (Prasad *et al*., 2000; Zeb *et al*., 2009). The use of molecular markers for the evaluation of genetic diversity is very common. Simple sequence repeats (SSRs) (Tautz *et al*., 1989) have been widely exploited in wheat due to their high level of polymorphisms, co-dominant inheritance and equal distribution in the wheat genome (Khaled *et al*., 2015). SSRs are more abundant, ubiquitous in presence, hyper-variable in nature and have high polymorphic information content (PIC) (Gupta *et al*., 2009). SSR have been used to study genetic diversity of wheat cultivars by Eujay *et al*., 2001; Grewal *et al*., 2007; Hai *et al*., 2007; Ijaz and Khan, 2009; Khaled *et al*., 2015; Szucs *et al*., 2000; Mohammadi *et al*., 2009.

Microsatellites or simple sequence repeats (SSRs) provide an efficient tool in diversity studies for identifying the degree of genetic similarity. Due to their high rate of polymorphism, co-dominant character, selective neutrality, distribution across the genome and cost and labor efficiency, microsatellites markers are suitable for detecting allele frequency within the population and for assessing population structure (Khaled *et al*., 2015).

At present, SSR is one of most promising molecular markers which are able to identify or differentiate genotypes within a species. SSRs are ubiquitously interspersed in eukaryotic genomes and can find applications as highly variable and multi allelic PCR based genetic markers. The high level of polymorphism and easy handling has made SSRs extremely useful for different applications in crop improvement (Gupta *et al*., 2009). Keeping in view the advantage of SSR markers the present research work was carried out to study genetic variation among various wheat varieties using chromosome specific SSR markers and to find genetically most diverse genotypes of wheat which can further be used in hybridization programs to create genetically diverse germplasm of local wheat.

Materials and Methods

Seven wheat genotypes that are grown in the crop seasons 2009-10 studied for genetic polymorphism using chromosome specific SSR markers. Parentage and pedigree of the varieties is presented in Table 1. Genomic DNA was isolated using CTAB method of Saghai-Maroof *et al*. (1984) from a small amount of fresh leaf tissue (5.0 g) from each of parental genotypes. Agarose gel electrophoresis (0.8%) was used to check quality and quantity of genomic DNA. The DNA concentrations were estimated by visual assessment of band intensity in comparison with Lambda (λ) DNA of known concentration. Parental genotypes

were screened using a total of fifty SSR markers for the molecular characterization, out of these 41 were polymorphic and used for diversity analysis. The PCR amplification conditions were optimized. The PCR reaction was conducted in a reaction volume of 20 µl containing 2ul of 1X PCR buffer, 100 µM dNTPs, 0.5 µl of each primer, 1.5 unit Taq DNA polymerase and 50 ng template DNA. The thermocycling program consisting of an initial denaturation at 95°C for 4 minutes followed by 40 cycles of 95°C for 1 minute, 1 minute and 20 second at annealing temperature (55-63 °C), 1 minute at 72°C and a final cycle of 72C for 10 minutes was used. Amplified products were resolved on 4% polyacrylamide gels using Amersham Biosciences system as described by Chen *et al.* (1997). Gels were pre-run until an adequate temperature (50-600C) was reached. DNA bands were visualized by using silver staining protocol (SILVER SEQUENCETM DNA Sequencing System, Promega Inc., Madison, WI, USA) after completion of electrophoresis. The frequency of polymorphism between different varieties of wheat for each type of marker was calculated based on presence (taken as 1) or absence (taken as 0) of bands (Ghosh *et al*. 1997). The 0/1 matrix was used to calculate similarity genetic distance using 'simqual' sub-program of software NTSYSPC (numerical taxonomy and multivariate analysis system programme) (Rohlf 1993). The resultant distance matrix was employed to construct dendrograms by the un-weighted pair-group method with arithmetic average (UPGMA) subprogram of NTSYS-PC.

Results and Discussion:

SSR markers are small (2-6 bp) DNA motifs, highly conserved and distributed among the genomes of all higher eukaryotes. SSR have been used extensively for designing primer sets which are not only highly polymorphic but also species specific (Pestova et al., 2000). Genetic diversity plays an important role in crop improvement and was demonstrated through SSR markers (Gupta *et al*., 2009) . In the present study, 50 SSR primers were used to estimate genetic polymorphism and to find out most diverse varieties for future breeding programs. The eight primers did not show amplification. Among the 42 primers producing amplification in parents the primers CFD 233 and WMC 421 produced only monomorphic bands. From 42 primers, a total of 114 bands were produced. Among these, 39 were polymorphic with an average of 70 per cent polymorphism.

The 0/1 data obtained using SSR primers were further used to construct similarity matrix among all seven genotypes using "simqual" sub-programme of software NTSYS-PC version 2.0. The allelic diversity data for SSR primers was used to produce a dendrogram for parental genotypes by using cluster, sub programme of the same software, which revealed the genetic relationship and proximity among all seven genotypes investigated. The calculations were based on Nei and Li (1979) equation.

SSR similarity matrices of seven wheat genotypes showed the relationship among them (Table 2). Maximum similarity value of 0.892 was observed between genotypes HS27 and HG2 whereas minimum similarity value of 0.037 was observed between HS27 and PBW502. The average similarity across all the genotypes was found out to be 0.464 indicating a high level of genetic diversity among seven wheat genotypes. The average linkage between wheat genotypes was used for constructing phylogenetic tree depicting the relationship among the seven parental wheat genotypes

The hierarchical cluster analysis revealed that the genotypes were mainly divided into two major clusters (Figure-1) at a similarity coefficient of 0.260. Cluster-1 is further subdivided into two sub-clusters A and B at similarity coefficient of 0.538. Sub-cluster A consists of only one genotype, HS67, whereas the sub-cluster B was divided into two groups C and D at similarity coefficient of 0.611. The group C comprised of one genotype, $HD2009_M$ and the group D further bifurcated into two subgroups E and F, which were comprised of Rm-Ts17 and HS27, HG2 respectively. Cluster-II divided in to two sub-clusters H and G that were comprised of two genotypes HJP81 and PBW502 with similarity coefficient of 0.862 in sub cluster H and G, respectively.

Two dimension PCA based on SSR diversity data (Figures 2) showed similar clusters of the seven wheat genotypes as was evident from cluster the analysis. The genotypes tended to cluster-I mainly two genotypes HS67 and PBW 502. The second cluster had HJP 81, $HD2009_M$, HS27, HG2 and Rm-Ts 17 genotypes. Similar type of studied were performed by Khaled *et al*., 2015; Mohammadi *et al*., 2009.

In the present study 42 Simple Sequence Repeat (SSR) primer sets were used to characterize seven wheat varieties to know about the diverse varieties for future breeding programs to enhance wheat production. Microsatellites displayed a high level of polymorphism in the present study. It is recommended that diverse wheat variety HS27 and PBW502 can be used in future breeding programs aimed at creating genetic variability in Pakistani wheat germplasm. The current data will enhance the breeding

efficiency and will add the strength of marker assisted selection (MAS). In the light of information about the genetic diversity in seven wheat varieties, it is therefore suggested that the breeding programs with the help of DNA fingerprinting technology will be helpful to utilize the local varieties to produce cultivars / varieties by crossing them with different elite varieties.

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References

Chen, H.B., J.M. Martin, M. Lavin and V. Talbert (1994). Genetic diversity in hard red spring wheat based on sequence tagged-site PCR markers. *Crop Sci*., **34**: 1629-1632.

Eujay, I., M. Sorrells, M. Baum, P. Woltersand and W. Powell (2001). Assessment of genotypic variation among cultivated durum wheat based on EST-SSRs and genomic SSRs. *Euphytica*, **119**: 39-43.

Fischer, R.A. and Edmeades, G.O. (2010). Breeding and cereal yield progress. *Crop Science*, **50:** S85–S98.

Ghosh S, Karanjawala Z E and Houser E R. (1997). Methods for precise sizing automated inning of alleles and reduction or error rates in large scale genotyping using fluorescently labelled dinucleotide markers. *Genome Research* **7**: 165–78.

Grewal, S., Kharb, P., Malik, R., Jain, S. and Jain, R. K. (2007). Assessment of genetic diversity among some Indian wheat cultivars using random amplified polymorphic DNA (RAPD) markers. *Indian. J. Biotech.* **6**: 18-23.

Gupta, P. K., Peter Langridge and Mir, R.R. (2009). Marker-assisted wheat breeding: present status and future possibilities. *Mol Breed*. DOI 10.1007/s11032-009-9359-7.

Hai, L., Wagner, C., & Friedt, W. (2007). Quantitative structure analysis of genetic diversity among spring bread wheats (*Triticum aestivum* L.) from different geographical regions. *Genetica*, **130**:213–225. http://dx.doi.org/10.1007/s10709-006-9008-6.

Ijaz, S., & Khan, I. A. (2009). Molecular characterization of wheat germplasm using microsatellite markers. *Genetics and Molecular Research*, **8** (3): 809-815. http://dx.doi.org/10.4238/vol8-3gmr608.

Khaled F. M. Salem, Marion S. Röder, Andreas Börner (2015). Assessing genetic diversity of Egyptian hexaploid wheat (*Triticum aestivum* L.) using microsatellite markers. *Genetic Resources and Crop Evolution* **62**(3): 377-385

Mohammadi, S. A., Khodarahmi, M., Jamalirad, S.,and Jalal Kamali, M. R. (2009). Genetic diversity in a collection of old and new bread wheat cultivars from Iran as revealed by simple sequence repeat-based analysis. *Annals of Applied Biology*, **154**(1): 67-76. http://dx.doi.org/10.1111/j.1744-7348.2008.00273.x.

Nei M, Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76: 5269-5273.

Röder, M.S., J. Plaschke, S.U. Konig, A., Börner, M.E. Sorrells, S.D. Tanksley and M.W. Ganal, 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet., 246: 327-333.

Rohlf F J. 1993. NTSYS-PC numerical taxonomy and multivariate analysis system. *Exeter Software*.

Saghai-Maroof M A, Soliman K M, Jorgensen R A and Allard R W. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of National of Academy Science* (USA) **81**: 8 014–8.

Szucs, P., A. Juhasz, I. Karsai, L. Lang, O. Veisz and Z. Bedo, 2000. Use of molecular markers for studying genetic diversity in durum wheat (*Triticum durum* Desf.). J. Genet. Breed., 54: 25-33.

Zeb , B., Khan, I. A., Ali , S., Bacha, S., Mumtaz, S. and Swati, Z. A. 2009. Study on genetic diversity in Pakistani wheat varieties using simple sequence repeat (SSR) markers. African Journal of Biotechnology, 8 (17): 4016-4019

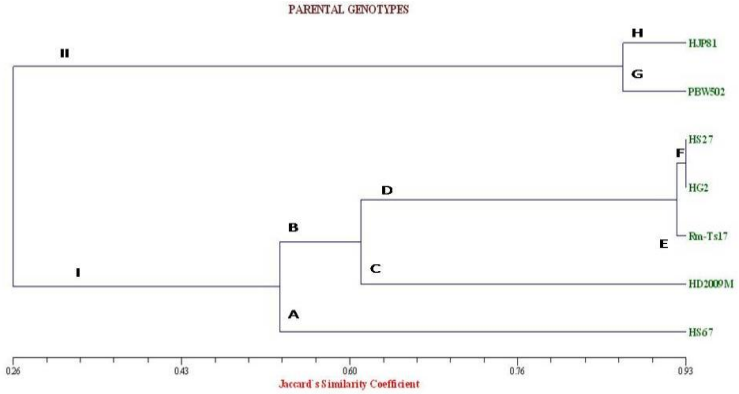

Figure-1: **Dendrogram showing relationship among seven wheat genotypes generated by UPGMA analysis based on single primers using polymorphic SSR primer pairs**

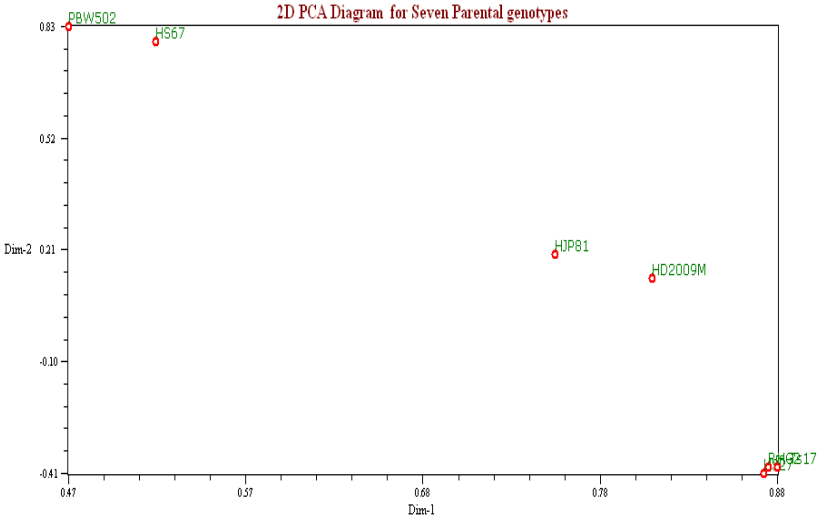

Figure-2: **Two dimensional PCA (Principal component analysis) scaling of seven wheat genotypes using SSR markers**

Table-1: Pedigree of parental genotypes involved in five wheat crosses

Genotype	Pedigree
PBW 502	WH485/PBW 343//RAJ/1482
HD 2009_M	A mutant of HD2009 (ARJUN): LR64A/NAI60
HG2	Advanced line derived from WH 157 x GP 104 (‘*gigas*’ spike)
HS27	RILL derived from S 308 : (1154-38-Andes x YT 54-N 103) LB and Harrier ‘*s’*
HS67	[(Cno”s’ – No. 66/C273 x NP 875 – E 853.5.8) 7C] Hock “s”
HJP81	Heritage collection Jai Parkash, a progressive farmer
Rm-Ts17	Advanced line derived from WH 157 x GP 104 (‘*gigas*’ spike)

Table-2: Average estimates of genetic distances among seven wheat genotypes using 50 SSR primer pairs similarity matrix

Genotype	HJP81	HS27	HS67	Rm-Ts17	PBW502	HG2	$HD2009_M$
HJP81	1.00						
HS27	0.096	1.00					
HS67	0.244	0.416	1.00				
Rm-Ts17	0.114	0.880	0.418	1.00			
PBW502	0.706	0.037	0.253	0.065	1.00		
HG2	0.105	0.892	0.422	0.882	0.066	1.00	
$HD2009_M$	0.219	0.430	0.295	0.450	0.147	0.456	1.00