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Original Research Article

SSR Molecular Marker are efficient tools for finding Genetic Diversity in Bread Wheat

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A B S T R A C T

A major effort of a plant breeder is the constant improvement of the best available genotypes for further enhancement in their yield potential either directly or through improvement of various factors which contribute indirectly to high yield. Genetic diversity of wheat cultivars is very important in reducing genetic vulnerability during plant breeding efforts. In order to estimate the genetic diversity, molecular markers provide excellent tools. The aim of this study was to molecularly characterize the fifty wheat accessions to assess phylogenetic relationship and mutual genetic distances. Twenty SSR primers amplified 45 bands out of which 25 were polymorphic and thus showed 55% polymorphism. The allelic polymorphism information content (PIC) value ranged from 0.173 to 0.433 with an average of 0.300. The **s**ignificant correlation (r = 0.597) for genetic distance among SSR markers was observed based on Mantel‘t’ test. Results show that high level of polymorphism among the wheat accessions. The genetic relationships estimated by the polymorphism of SSR markers revealed greater level of genetic variability in wheat accessions of wide adaptability and applicability. Simple sequence repeats (SSRs) occur frequently in most eukaryote genomes and can be very informative, multi- allelic and reproducible. SSRs are increasingly being used as genetic markers of chromosome segments and for the identification of individuals.

Keywords

Bread wheat, Genetic diversity, SSR markers and Wheat accessions

Introduction

Bread wheat (*Triticum aestivum* L. em. Thell), an allohexaploid (2n=6x=42), is the premier food crop of worldwide importance. It is also a crop where conventional plant breeding has paid rich dividend, as epitomized by the Green Revolution. Genetic diversity provides means of specific identification of species and strains. It describes genetic distance between genotypes and polygenic relationships are well traced through it and thus help in searching out genetic relationship among different genotypes. It will also be helpful in selection of better parent, therefore effective and efficient utilization of germplasm is needed. Knowledge of genetic diversity in a crop species is fundamental to its improvement. Evaluation of genetic diversity levels among adapted, elite germplasm can provide predictive estimates of genetic variation.

Applying genetic markers and recognition of polymorphic nucleotide sequences dispersed throughout the genome have provided new possibility for evaluating genetic diversity

and determining of inter- and intra-species genetic relationships (Gostimsky *et al*., 2005). Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred as gene 'tags'. Such markers themselves do not affect the phenotype of the traits of interest because they are located only near or 'linked' to genes controlling the trait.

Several PCR based molecular markers are available for investigation of genetic diversity. SSR (Tautz, 1989), RAPD (Williams *et al*., 1990), AFLP (Vos *et al*., 1995) and ISSR (Zietkiewicz *et al*., 1994) were the most important of them. The major limitations of these methods were low reproducibility of RAPD markers, high cost of AFLP and need to know the flanking sequences to design specific primers for SSR markers. SSR markers overcome most of these limitations. Easy handling, reliability and high information level are the salient features of SSR markers that justify the utility of these primers in DNA fingerprinting of wheat genetic analysis and germplasm management.

Materials and Methods

A total of fifty accessions of wheat germplasm was taken and evaluated at N. E. Borlaug Crop Research Centre, Pantnagar. Diversity at molecular level was studied at Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar using 20 SSR markers.

DNA extraction

Total genomic DNA was extracted from leaf tissue per each accession. Young leaves from fifteen day old plants were cut used as samples for DNA extraction. Cetyl trimethyl ammonium bromide (CTAB) method as described by Saghai-Maroof et al. (1984) and quantification was done in a Dynaquant™ 200 fluorimeter (Hoefer Instruments. USA)).

Data analysis

The SSR markers were scored for the presence (1) or absence (0) of amplified bands of microsatellites for each of 50 wheat accessions.

The amplification products were viewed under UV light and photographs were saved for the experimental evaluation. The amplification products were scored separately for each primer. The bands were scored for the presence or absence by binary coding i.e., assigning a value of 1 for presence and 0 for absence in a lane (Hartigan, 1975). Molecular size (bp) of amplified DNA fragment was determined by the DNA ladder marker which was used in the wells of agarose gel.

DNA fragment analysis was performed using the NTSYS-PC (Numerical Taxonomy System, version 2.11 W) software (Rohlf, 1992). The SIMQUAL programme was used to calculate Jaccard's coefficient, a common estimator of genetic identity and similarity matrices based on Jaccard coefficient were calculated.

Results and Discussion

Twenty SSR primers amplified 45 bands out of which 25 were polymorphic and thus showed 55% polymorphism. The similarity coefficient ranged from 0.53 to 0.89. The allelic polymorphism information content (PIC) value ranged from 0.173 for the marker *Xwmc25* to 0.433 for the marker *Xwmc169* with an average value of 0.300. The significant correlation (r = 0.597) for genetic distance among SSR markers was

observed based on Mantel 't' test. The accessions, IC 532263 and IC 104659 showed lowest similarity value of 0.53, demonstrating a distant relationship between them. The accessions, IC 104577 and IC 533741 were found to be highly similar with a similarity value of 0.88. The accessions, IC 534808 and IC 104659 were grouped in two different and farthest clusters and found to be the most diverse.

Genetically diverse groups were formed based on the dendrogram. The difference in the genotypes may be attributed to different geographical locations of the place of release of varieties and different ploidy levels. Similar types of studies in wheat were performed by Pasqualone et al. (2000), El-Maati *et al.* (2004) and Carvalho *et al*. (2005).

Table.1 Details of 20 SSR primers used for PCR amplification of 50 wheat germplasm accessions, their genes and associated characters

Marker designation	Chromosome assignment	Associated characters
Xwmc24	1AS	Plant height, spikelets/spike
Xwmc25	2BS, 2DS	Days to flowering
Xwmc35	4B-	NA
Xwmc44	1BL	Harvest index
Xwmc47	4BS, 5AS, 5BS	NA
Xwmc76	7BL	NA
Xwmc83	7AS	Days to flowering, tiller no., harvest index, grain yield
Xwmc120	1A-	NA
Xwmc149	2AS, 2BL, 5BS	Spikelets/spike, days to flowering
Xwmc167	2DL	Days to flowering, tiller no., grain/spike, harvest index
Xwmc169	3AL	Days to flowering
Xwmc170	2A-	Days to flowering, biological yield, harvest index, days to maturity
Xwmc177	2AS	NA
Xwmc216	1BL, 1DL	Grain/spike, days to flowering, days to maturity
Xwmc221	7DL	NA
Xwmc243	2BS, 2DL, 6AS	grain yield
Xwmc245	2BL, 2DL	NA
Xwmc254	4B-	spikelets/spike
Xwmc256	6A, 6D	NA
Xwmc267	5A-	Days to maturity

Table.2 SSR Primers their sequences data on DNA profile and polymorphism generated in 50 germplasm accessions of wheat

S. No.	Primer Code	Percentage Polymorphism	PIC Values	Range of amplified loci (bp)
1.	WMC 24	66	0.413	200-400
2.	WMC 25	50	0.173	100-200
3.	WMC 35	50	0.313	100-200
4.	WMC 44	50	0.420	100-200
5.	WMC 47	66	0.260	100-500
6.	WMC 76	50	0.180	100-300
7.	WMC 83	50	0.300	100-200
8.	WMC 120	50	0.360	100-200
9.	WMC 149	50	0.260	100-200
10.	WMC 167	50	0.353	100-200
11	WMC 169	50	0.433	100-200
12	WMC 170	50	0.340	100-200
13	WMC 177	66	0.207	100-500
14	WMC 216	50	0.320	100-200
15	WMC 221	50	0.247	100-200
16	WMC 243	50	0.240	100-200
17	WMC 245	66	0.207	100-500
18	WMC 254	66	0.287	100-500
19	WMC 256	50	0.260	100-200
20	WMC 267	50	0.410	100-200

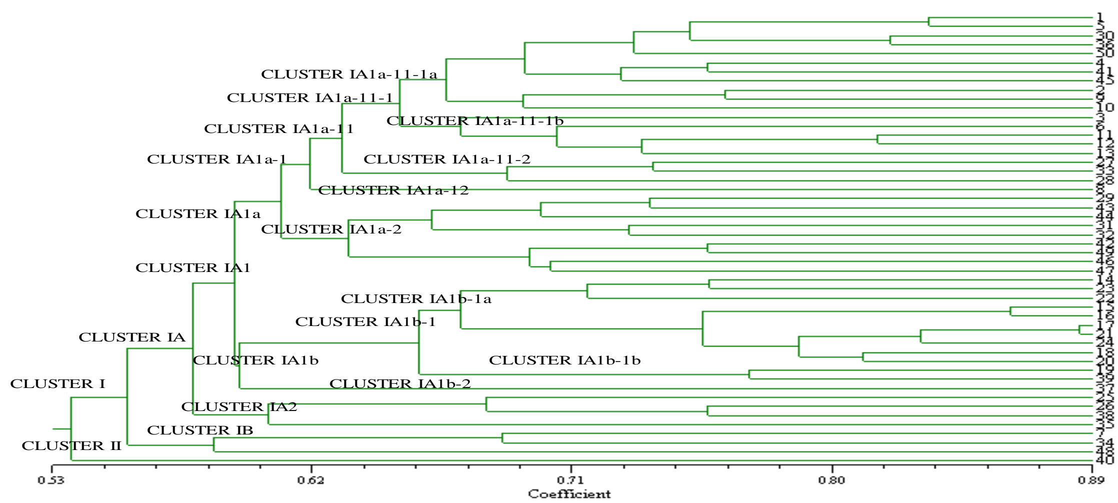
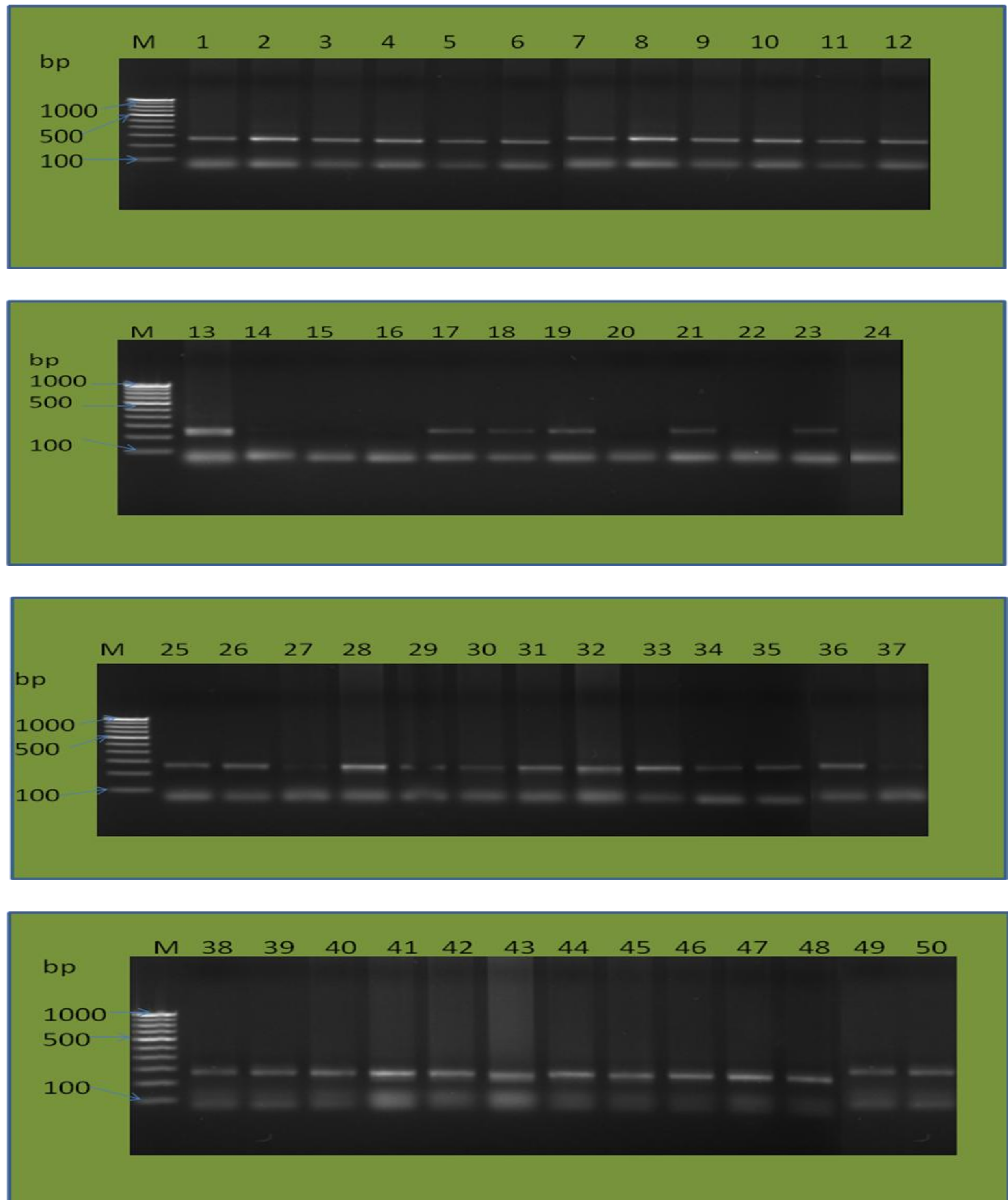


Figure b Dendrogram depicting the classification of 50 wheat accessions constructed through UPGMA method and based on SSR marker. The scale at the bottom is Jaccard's coefficient of genetic similarty.

Fig.a The Simple Sequence Repeat (SSR) profiles of 50 accessions of wheat. Amplification was done with Primer WMC 76 on agarose gel. Lane M denotes DNA ladder and the lanes 1 to 50 denote 50 accessions of wheat



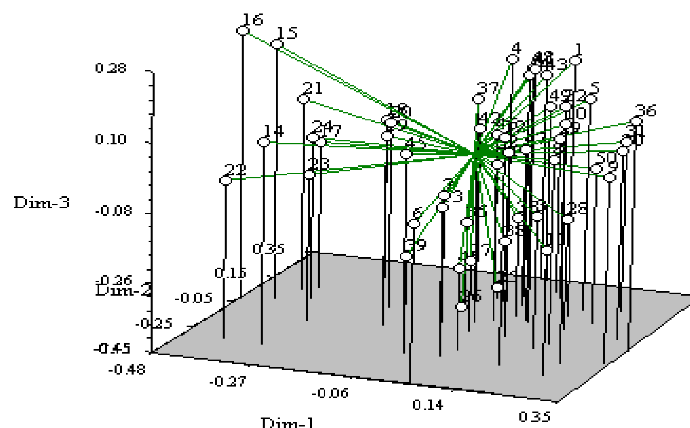


Figure c Three dimensational classification of 50 wheat accessions constructed through Principle Coordinate Analysis based on SSR markers

It can be concluded that the SSR markers are highly polymorphic and repeatable and are being used in revealing the genetic diversity at intra-specific level. The clusters, groups and subgroups formed in case of fifty wheat accessions, which are indigenous collections from different locations across the country suggest that SSRs are potential molecular markers are studying the genetic diversity. Molecular marker technology provides information that can help to define the distinctiveness of germplasm and their ranking according to the number of close relatives and their phylogenetic position. It is a complementary approach for genetic characterization.

SSR markers are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions. SSR are highly polymorphic PCR based markers and may be expected to be very powerful in cultivar discrimination. SSR have been used for cultivar identification in wheat by many researchers Prasad *et al*., (2000), Mangini *et al*., (2010) and Mandoulakani *et al.,* (2010).

In the present study 20 SSR primers yielded a total of 45 amplified fragments (100 to 500 bp in size) ranging from 2 to 3 polymorphic fragments per primer. Two alleles per locus were found for the primers WMC 25, WMC 35, WMC 44, WMC 76, WMC 83, WMC 120, WMC 149, WMC 167, WMC 170, WMC 216, WMC 221, WMC 243, WMC 256, WMC 267 where as three alleles per locus were found for WMC 24, WMC 47, WMC 177, WMC 245, WMC 254 with an average of 2.8 alleles per locus. None of the primers yielded any unique band in 50 germplasm accessions of wheat. The data scored from the SSR analysis of wheat accessions using SSR primers were used to generate pair-wise matrix based on Jaccard's similarity coefficient. The similarity coefficient ranged from 0.53 to 0.89 (i.e., 53 to 89 per cent similarity). The allelic polymorphism information content (PIC) value ranged from 0.173 for the marker *Xwmc25* to 0.433 for the marker *Xwmc169* with an average of 0.300. Jaccard's similarity coefficient ranged from 53 to 89% among the paired accessions. Significant correlation of microsatellite genetic distance tested by Mantel 't' test (r= 0.597).

Genetically diverse groups were formed based on the dendrogram. The difference in the genotypes may be attributed to different geographical locations from where they were collected of the place of release of accessions and different ploidy levels.

Since wheat is a self-pollinating species, variation within the same germplasm is expected to be very low. However, microsatellite markers have been found to be ideal markers for characterizing genetic diversity at the intra-species level by Olufowote *et al*. (1997). The variability in the number of alleles per locus may results from different locus specific mutation rates and reflects strong differences in allelic diversity between SSRs loci.

SSR markers exhibited a remarkably strong association with genetic origin. The reduction in allelic diversity (absence of bands in particular genotypes) was not only due to plant breeding, but also largely due to the elimination of deleterious alleles by selection rather than erosion, which delineate the genetic diversity generated not only by different agro-ecological conditions but also by selection procedure among the germplasm collection. Similar types of studies in wheat have been done by Gupta *et al*. (2000), Dograr *et al.* (2000) and Hayden *et al*. (2006) in *Phaseolus vulgaris* done by Blair (2006) and Benchimol (2007). Diversity analysis is important for deciphering genetic relationship including parentage and for the efficient management of germplasm and thereby use in breeding of improved varieties. Establishing the identity of crop variety using diversity study has assumed greater importance for protecting plant breeder's and farmer's rights. In the present study, microsatellite marker were analyzed in the large number of genotypes mostly collected from different parts of India to get better information about the genetic diversity of wheat germplasm.

References

Benchimol L.L.; CamposT, Carbonell, SAM.; Colombo, C.A.; Chioratto, A.F; Formighieri, EFL; Gouve, R.M. and Souza APD 2007. Structure of genetic diversity among common bean varieties of Mesoamerican and Andean origins using new developed microsatellite markers. *Genet Resour. Crop Evol*. 54:1747-1762.

Carvalho, A.; Matos, M.; Lima, B.; Jose, M.; Guedes, P.H. and Benito, C. 2005. DNA Fingerprint of F1 interspecific hubrids from the triticeae tribe using ISSR. Euphytica 143.1-2: 93-99.

Dograr, N.; Akin Yalin, S. and Akkaya, M. S. 2000. Discriminating durum wheat cultivars using highly polymorphic simple sequence repeat DNA markers. *Plant Breeding* 119(4): 360-362.

El-Maati, F. B.; Jlibene, M. and Moumni, M. 2004. Study of the polymorphism of common wheat using ISSR markers. *Journal of Food, Agriculture and Environment* 2(314): 121-125.

Gupta P.K and Varshney R.K 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113:163–185.

Gostimsky, S. A.; Kokaeva, Z. G. and Konovalov, F. A. 2005. Studing plant genome variation using molecular markers. Russ. J. Genet. 41: 378– 388.

Hartigan, J.A. 1975. Clustering algorithms. John Wiley and sons, Inc. Newyork. Pp 154.

Hayden, M.J.; Stephenson, P.; Logojan, A. M.; Khatkar, D.; Rogers, C.; Elsden, J.; Koebner, R.M.D.; Snape, J.W. and Sharp, P.J. 2006. Development and genetic mapping of sequence-tagged microsatellitea in bread wheat. *Theoretical and Applied Genetics* 113: 1271-1281.

Karp A, Krosorich S, Bhat KV, Ayod IVG, Hodgin T 1997. Molecular tools in plant genetics resources conservation: A guide to technologies. Technical Bulletin no. 2, pp 13–29.

Mangini, G.; Taranto, F.; Giove, S.L.; Gadaleta, A. and Blanco, A. 2010. Identification of durum wheat cultivars by a minimum number of microsatellite markers. *Cereal Res. Commun.* 38: 155-162.

Mandoulakani, B.A.; Busheri, A.A.; Tabatabaei, B.E.; Torabi, S.;Hajiabad, A.M. 2010. Genetic diversity among wheat cultivars using molecular markers. J. *Crop Impov*. 24:299-309.

Olufowote, J.O., Xu, Y; Chen, X.; Park, W.O. and Beachell, H. M. 1997. Comparative evaluation of within cultivar variation of rice usind microsatellite and RFLP Markers. Genome, 40: 370-378.

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

Pasqualone, A., Lotti, C., Bruno, A., De Vita, P., Di Fronzo, N. and Blanco, A. 2000. Use of ISSR markers for cultivar identification in durum wheat. CIHEAM – IAMZ. 620 p.

Rohlf, F. J. 1992. NTSYS-pc Numerical taxonomy and multivariate analysis system. Exeter Software, New York, USA.

Tautz, D., 1989.Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res*., 17: 6463–71.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van Der Lee, T., Hornes, M, Frijters, A., Pot, J., Peleman, J., Kuiper M. and Zabeau, M. 1995. AFLP a new technique for DNA fingerprinting. *Nucleic Acids Res.,* 23: 4407–14.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., 18: 6531–5.

Zietkiewicz, E.; Rafalski, A. and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat SSR anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.