KSSR and ISSR markers for assessing DNA polymorphism and genetic diversity among bread wheat varieties of India

Malik R, Kundu S, Sareen S, Kumar R, Shoran J and B Mishra
Directorate of Wheat Research, Post Box No. 158, Karnal-132001, India

ABSTRACT

SSR and ISSR marker systems were used to study the genetic diversity in bread wheat (*Triticum aestivum* L.) varieties released for high yield, quality and abiotic stress in India. A set of 27 varieties were screened with 20 wheat microsatellite markers (WMS), covering 15 wheat chromosomes , and 20 UBC series ISSR markers at the molecular level. A total 146 allelic variants were detected at 20 loci, ranging from 86 to 210 bp with an average of 7.3 alleles per marker. A high occurrence of rare alleles (frequency 33.56 %) was observed for 17 markers. The polymorphism information content (PIC) values of the markers ranged from 0.382 (WMS 186) to 0.826 (WMS 389) with an average of 0.628. During ISSR analysis, a total 176 bands were amplified of which 68.42% were polymorphic. The DNA fingerprint databases were used for UPGMA-based cluster analysis. The genetic relationships estimated by SSR and ISSR markers revealed substantial genetic variability reflecting wide adaptability and applicability of Indian bread wheat cultivars. Both marker types efficiently discriminated all 27 selected varieties at the DNA level. However, the SSR-based cluster tree grouping of these varieties was more in agreement with their known origin and pedigree as compared to the ISSR cluster analysis. Easy handling, reliability and high information levels are the features that justify the utility of SSR and ISSR markers in DNA fingerprinting of wheat for genetic variability analysis.

INTRODUCTION

In India, only a few (10-12) varieties having wide adaptability, high yield and tolerance against biotic stresses are contributing to the major proportion of total wheat produced in the country every year. Intensive agricultural practice has given rich dividends in terms of yield but this approach has narrowed down the genetic variability in the available cultivars (Gautam *et al*, 1997). Thus, it seems necessary to conserve genetic variation for the improvement of future varieties. That is why significant research on genetic resource characterization has been done on major cereals including wheat using morphological characters. Efforts have also been made to characterize wheat with molecular markers (Gupta and Roy, 2002). Microsatellite markers are one of the most efficient markers as they have high polymorphism levels, are reproducible, codominant with their chromosomal location known so they can be used for genotypic characterization and genetic relatedness of wheat. ISSR are arbitrarily primed random markers that also can be exploited for germplasm conservation and to determine the relationships among wheat genotypes. In the present investigation, these two marker systems were used for DNA polymorphism and genetic diversity studies in Indian wheat varieties.

MATERIALS AND METHODS

A set of twenty-seven wheat genotypes including prominent wheat varieties, landraces and a few exotic genotypes were used in the present study (Table 1). An equal number of fresh, young leaves (ten days old) of at least five plants from each genotype were bulked for DNA extraction. Total genomic DNA was isolated using the modified CTAB method (Saghai-Maroof *et al*, 1984). Twenty microsatellites (Roder *et al*, 1998) and 20 ISSR markers (Nagaoka and Ogihara, 1997; Pujor *et al*, 1999) of UBC set no 9 were used for the molecular studies. The PCR products of SSR markers were resolved on 6% polyacrylamide gel (PAGE) and visualized by silver staining (McCouch *et al*, 1997). Fragment sizes were approximately calculated by interpolation from the migration distance of marker fragments of 10-bp DNA ladder (Invitrogen, USA). The occurrence of 'null' alleles was verified by re-amplification using the same primer pair in the same conditions. ISSR primers that gave discrete bands in wheat genotypes during an initial screening were selected from set # 9 of the University of British Columbia Biotechnology Laboratory (UBC, Vancouver, Canada). The amplified fragments were separated on 1.5% agarose gels and the ethidium bromide-stained gel was documented under UV light (Alpha Innotech Corporation, California, USA). The molecular size of fragments was estimated by reference to a 1Kb ladder (Gibco, BRL, USA).

Alleles were calculated on the basis of presence (taken as 1) and absence (taken as 0) of common bands (Ghosh *et al*, 1997). The 0/1 binary matrix so generated was used to calculate genetic similarity (GS) using the “simqual” sub-program of software NTSYS-PC. A dendrogram was constructed using the GS matrix and the unweighted pair-group method with arithmetic average (UPGMA) subprogram of NTSYS-PC (Rohlf, 1990). Assuming the genotypes are homozygous for the respective loci, polymorphism information content (PIC) values were obtained for SSR analysis (Anderson *et al*, 1993). The percentage polymorphism was obtained for the ISSR markers. Principal Component Analysis (PCA) was carried out using NTSYS-PC 2.11 to summarize the variation of the original SSR database, and to represent the results in graphical form.

RESULTS AND DISCUSSION

A total of 146 microsatellite alleles, ranging from 3 to 14 alleles per SSR, were amplified for 27 genotypes with an average of 7.3 alleles per locus as reported for wheat in different regions of world (Roder *et al*, 2002; Landjeva *et al*, 2006). A total of 49 unique alleles were observed for 17 primer pairs that might indicate the genetically diverse genotype selection in the present study. Four microsatellite primers (WMS 108, WMS 161, WMS 186 and WMS 389) generated 47 multiple alleles. These additional loci in wheat are homoeologous or non-homoeologous amplified products that were smaller in size and showed a lower level of polymorphism as reported for European wheats (Roder *et al*, 2002). PIC values ranged from 0.382 (WMS 186) to 0.826 (WMS 389) with an average of 0.628 for selected primers with positive correlations for amplification profiles and PIC values i.e. Coefficient of correlation for size range Vs PIC was calculated to be 0.726, was 0.890 for number of alleles Vs size range and 0.883 for PIC values with number of alleles. This reflected the efficiency and relevance of the DNA fingerprint database generated for selected genotypes of wheat with a given set of microsatellite markers. The number of alleles observed for respective repeat motifs was greatest for (GT)n loci suggesting that primers with (GT)n repeat motifs revealed higher polymorphisms in Indian wheat (Ammiraju *et al*, 2001). During ISSR analysis, primers of di-nucleotide repeat markers revealed maximum amplification and were found to be more amenable in wheat. A total of 176 bands were amplified with 68.42% polymorphic. During an initial screening, only 3’-anchored di-nucleotide motif primers gave clear amplified products whereas 5’-anchored di-nucleotide primers either gave poor or no amplification in wheat genotypes as reported (Pujor *et al,* 1999). Maximum polymorphism was observed in tetra-nucleotide motif markers (primer # 873 and 876). Therefore it is suggested that tetranucleotide repeat markers should be further screened for wheat fingerprinting.

The microsatellite and ISSR marker data were collected and used to analyze genetic diversity through cluster analysis. A UPGMA tree was prepared using the NTSYS-PC sub-program “Simqual” which used “Sham” coefficient to establish genetic relationships at the molecular level. The selected genotypes were differentiated and placed as individual entities for both SSR and ISSR marker system-generated cluster trees. In the microsatellite_based dendrograms, all genotypes were grouped in one major group as shown in Fig 1. The wheat varieties developed in India were clustered in two groups. Varieties derived from salt tolerant Indian landrace Kharchia like KRL 1-4, KRL 19 and Kharchia 65 were placed in one group with local varieties C 306 and C591. The second group contained local varieties NP 4, K 68 and HD 1982 and represented genotypes with good quality characters. Genotypes like PBW 343, CPAN 3004, and HD 2009 carry exotic pedigrees in their development and represented well adapted high yielding bread wheat varieties of India. Sufficient genetic variability was reflected during SSR analysis in the available wheat accessions. In the ISSR-based cluster analysis, some similar groupings were obtained with a few exceptions as represented in Fig 2. Varieties carrying indigenous pedigrees like NP4 were placed in one group with varieties NI 5439 and HD 1982 carring exotic pedigree. Local varieties developed in India like KRL 1-4, KRL 19, Kharchia 65, C 306 and C 591 were placed in two groups that were in contrast with the SSR-based analysis. Most of the genotypes having both exotic and indigenous parentage as well as genotypes with exotic parentage were placed separately in different groups as was obtained in the microsatellite-based dendrograms. Therefore, it is suggested that both marker systems were found to be efficient in discriminating each genotype at the molecular level and can be used for genetic diversity analysis for germplasm conservation.

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REFERENCES:

Ammiraju, J.S.S., Dholakia, B.B., Santra, D.K., Singh, H., Lagu, M.D., Tamhankar, S.A., Dhaliwal, H.S., Rao V.S., Gupta, V.S. and

Ranjekar, P.K. (2001). Identification of inter simple sequence repeat (ISSR) markers associated with seed size in wheat. *Theor. Appl. Genet.* 102: 726-732.

Anderson J.A., Churchill G.A., Autique J.E., Tanksley S.D. and Sorrells M.E. 1993. Optimizing parental selection for genetic linkage maps. Genome, 36: 181-186.

Gautam P.L., Mittal S.K. and Kochhar S.1997. Wheat genetic resource management and its use in National PGR system. *In:* Wheat, research needs beyond 2000A.D. (ed. Nagarajan S., Singh G. and Tyagi B.S.) Narosa Publishers, India, pp. 39-50.

Gupta P.K. and Roy J.K. 2002. Molecular markers in crop improvement: Present status and future needs in India. Plant Cell Tissue and Organ Culture, 70: 229-234.

Ghosh S., Karanjawala Z.E. and Hauser E.R. 1997. Methods for precise sizing, automated binning of alleles and reduction of error rates in large-scale genotyping using fluorescently labeled dinucleotide markers. Genome Res., 7: 165-178.

Landjeva S., Korzun V. and Ganeva G. 2006. Evaluation of genetic diversity among Bulgarian winter wheat (Triticum aestivum L.) varieties during the period 1925–2003 using microsatellites. Genet. Res. and Crop Evol, 53 : 1605-1614.

McCouch S.R., Chen X., Panaud O., Temnykh S., Xu Y., Cho Y.G., Huang N., Ishii T. and Blair M. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. Pl. Mol. Biol., 35: 88-99.

Nagaoka, T. and Ogihara, Y. (1997). Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor. Appl. Genet.* 94: 597-602.

Pujor, S., Tamhankar, S.A., Rao, V.S., Gupta, V.S., Naik, S. and Ranjekar, P.K. (1999). Arbitrarily primed-PCR based diversity assessment reflects hierarchical groupings of Indian tetraploid wheat genotypes. *Theor. Appl. Genet.*, 99: 868-876.

Roder M.S., Korzum V., Wendehake K., Plaschke J., Tixier M.H., Leroy P. and Ganal M.W. 1998. A microsatellite map of wheat. Genetics, 149: 2007-2023.

Roder M.S., Wendehake K., Korzum V., Bredemeijer G., Laborie D., Bertand L., Issac P., Rendell S., Jackson J., Cooke R.J.., Vosman B. and Ganal M.W. 2002. Construction and analysis of a microsatellite-based database of European wheat varieties. Theor. Appl. Genet., 106: 67-73.

Rohlf F.J. 1990. NTSYS-PC Numerical Taxonomy and Multivariate Analysis System Version. 1.60. Appl. Biostat., New York.

Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A. and Allerd R.W. 1984. Ribosomal spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci., USA.81: 8014-8019.

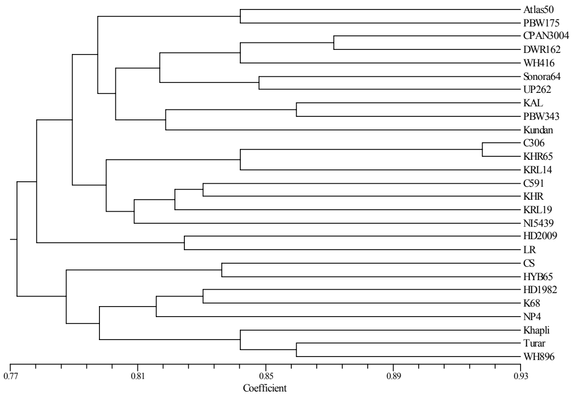

Fig.1: UPGMA based cluster tree of 27 wheat genotypes with 20 microsatellite markers

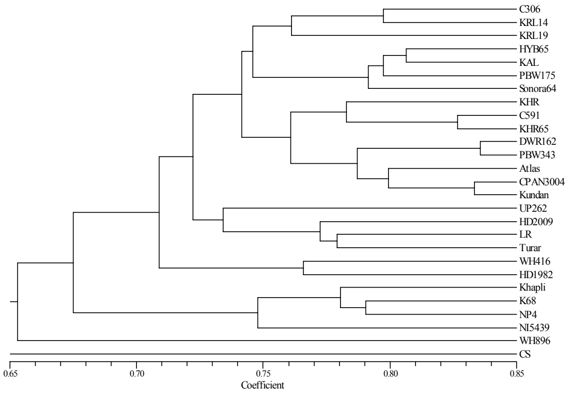

Fig.2: UPGMA based cluster tree of 27 wheat genotypes with 20 ISSR markers

Table1: Genotypes used for diversity analysis

Genotypes	Pedigree
C-306	RGN/CSK 3//2* C591/3/C217/ N14// C281
Kharchia 65*	KHR/EG 953
KRL 1-4	KHLC/WL711
KRL 19	PBW 255/KRL1-4
NI 5439	REP 80/3* NP 710
Kharchia	Local of Sanchore area, Rajasthan
C-591	Type 9/8B
HD 2009	LR64A/NAI 60
K 68	NP 773/K 13
Kalyansona*	PJ/S//GB 55
PBW 343	ND/VG 1944/KA//BB/3/ YACO$^{/}$S$^{/}$4/VEE # 5’S’
WH 416	WH147/UP 368
Atlas 50 (2373)	
CPAN 3004	GLL/AUST II 61-157 //CNO/NO/3/VEE
DL 153-2	Tanori 71/NP 890
DWR 162	KVZ/BUHO/KAL/BB
HD 1982	YT 54/N10B/HD 845
HYB 65	GB-AUS/115
NP4	Selected local Mundia
PBW 175	HD 2160/4/JN/GAGE / JN/KAL/3/PV 18/ C 273
Sonora 64	YT 54/N10B//2* Y54
UP 262	S 308/BJ 66
Lermarojo*	Y50/N10B// L52/3/2* LR
Chinese Spring*	---
WH 896	STN/S//WH 852
Khapli (E 3508)	Land Race
Triticum urartu*	Wild type

Abbreviated : Kalyansona – KAL; Lermarojo – LR; T. urartu – Turar; Chinese spring – CS
Kharchia 65 – KHR65