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Use of Microsatellite Markers for Assessing Genetic Variability in Wheat Genotypes for Yellow Rust Resistance

Meenakshi Rathi*, Mukesh Kumar, Vijay Kumar, Pooja and Lakshmi Chaudhary

Department of Genetics and Plant Breeding, College of Agriculture
Chaudhary Charan Singh Haryana Agricultural University, Hisar 125001, India

**Corresponding author*

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A B S T R A C T

A set of sixty microsatellite markers were used to characterize 49 genotypes of wheat. Out of these, 23 markers showed polymorphism with a total of 170 alleles. Average number of SSR alleles per locus was 2.88 and ranges from 2 (Cfa2040, Xgwm261, Xgwm382, Xgwm413, Xgwm170, Wmx44, Cslv34, Xgwm501, Xgwm70, Barc136, Barc101, Xgwm352, Xgwm130, Barc187, Barc76, Wmc407) to 5 (Xgwm190). The PIC value varied from 0.11-0.65 and 7 out of 23 SSR loci, Xgwm261, Xgwm295, Xgwm190, Xgwm46, Gwm11, Xgwm273 and Xsps3000 had PIC value more than 0.5 thus considered very useful in estimating genetic diversity of the genotypes. The UPGMA cluster grouped into two, major cluster 1 consists of 6 sub clusters whereas major cluster 2 was not further fragmented and had only one genotype WH1191. Cluster pattern revealed that, sub-cluster 2 was the largest consisting maximum number of 24 genotypes. This cluster analysis grouped genotypes on the basis of similarity at DNA level, thus for selecting parents for hybridization program against yellow rust disease can be chosen from different clusters.

Introduction

Wheat (*Triticum aestivum*) is a hexaploid (2n=6x=42) with genome composition of AABBDD and belongs to family Poaceae. It is one of the leading sources of carbohydrates, which has about one half of the human's food calories thus fulfills the large part of nutritional requirements. With progressive global climatic change wheat production is influenced greatly (Singh and Chaudhary, 2006). Whereas on other hand world population is increasing continuously, thus developing high yielding genotypes is the major concerned of today's scenario. The major states involved in wheat production are Uttar Pradesh, Punjab and Haryana which accounts nearly 70 per cent of the total wheat produced in the country. India occupies 34.64 mha area under wheat cultivation with production and productivity of 98.76 mt and 30.60 q/ha respectively (Anonymous, 2016).

Genetic diversity in crop species is essential and basic for breeding program to develop varieties resistant to yellow rust disease. Reduction in the genetic variability makes the crops increasingly vulnerable to diseases and

adverse climatic changes (Aremu, 2012). Morphological traits are influenced by the environmental factors thus cannot be used to characterize genetic diversity (Spanic *et al.,* 2012).

These days DNA markers have been used extensively for estimation of genetic diversity in wheat. Among all the DNA markers, simple sequence repeats (SSR's) are frequently used markers for dissecting genetic diversity. These markers are highly reliable as they show high polymorphism, co-dominant inheritance and good reproducibility (Plaschke *et al.,* 1995; Roeder *et al.,* 1995; Ma *et al.,* 1996; Bryan *et al.,* 1997). Wheat has an extremely large genome size of 17 × 109 base pairs with more than 80% repetitive DNA (Bennett and Smith, 1976) thus SSR markers are best for use in this crop. In present research genotypes were evaluated for genetic diversity using SSR markers and their relatedness is measured. This information helps to select batter parents for further breeding programs and developing high yielding yellow rust resistant varieties.

Materials and Methods

Plant material

A total of 49 genotypes (Table 1) including released varieties and advanced lines were grown in paired rows and infector row on borders were evaluated. Artificial inoculation was carried out in field condition with pst (*Puccinia striiformis*) of races 46S102, 47S103 and 78S84 by spraying urediospores suspension.

The disease data were recorded following modified Cobb's scale (Peterson *et al.,* 1948). The screening, for reaction to stripe rust, was done and on the basis of that genotypes were classified as highly resistant (HR), moderately resistant (MR), moderately susceptible (MS) and highly susceptible (HS).

DNA isolation

Genomic DNA was isolated from the young leaves of wheat plants using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method given by Murray and Thompson (1980) and modified by Saghai- Maroof *et al.,* (1984). DNA samples were treated with 1 µl of RNase A solution (10mg/ml) per 50 ng DNA sample to remove RNA contamination. Qualitative and Quantitative estimation of genomic DNA was estimated by UV spectrophotometer readings at 260nm as well as 280 nm wavelengths. Quantity of DNA is calculated by following formulae:

DNA (ng/µl) = A260 × Dilution factor × 50

The ratio of absorbance at 260 nm and 280 nm was calculated. Samples with a ratio of 1.7 to 1.8 were considered to be of good quality and band intensity was measured by running DNA on 0.8% agarose gel.

Molecular markers

A total of sixty molecular markers were used for studying molecular polymorphism in 49 genotypes of wheat consisting of released and advance lines.

Polymerase Chain Reaction (PCR) amplification

Applied biosystem thermocycler was used for PCR amplification. The Optimization of PCR reaction was done by varying the concentrations of master mix ingredients. The optimized reaction contained 50ng DNA template in 10.00 µl of master mix with 0.30 µl MgCl2 50 mM, 0.25 µldNTPs mix (10µM), 0.20 µl of Forward primer and Reverse primer each, 7.00 µl Sterile distilled water and 3 units of Taq polymerase. The PCR conditions were set at 94°C, 4 minutes for initial denaturation then again at 94°C, 1 minute for further denaturation. Primer annealing temperature

ranged from 48.5-70°C which existed for 1 minute and extension was attained at 72°C for 2 minutes then again 72°C, 10 minutes for final primer extension. The cycle was repeated from step (ii) to (iv) for 35 times and the amplified products were stored at -20^{0}C till further use.

Agarose gel electrophoresis

PCR amplified DNA fragments were resolved on 2.5% (w/v) agarose gels. Agarose was melted in 0.5X TBE buffer and ethidium bromide (1 µl/50ml) was added. Gel was submerged using 0.5X TBE buffer. Electrophoresis was carried out at constant voltage (3 v/cm of gel) for 2.5 hours. PCR amplified products were viewed under UV light using photo UV trans-illuminator and image was captured using gel documentation system.

Allele scoring

The size of amplified band of each primer was determined based on electrophoretic mobility relative to molecular weight of ladder (100 bp) used. The presence of band run on agarose gel was taken as one and absence of band was read as zero. The binary data was used to calculate similarity genetic distance using 'simqual' sub-program of software NTSYS–PC (Rohlf, 1992) and dendrogram was constructed on similarity bases.

Results and Discussion

Earlier genetic diversity analysis was limited to morphological variation seen in the genotypes under studies. However, there are some problems with the u e of morphological traits, first being limited in number and second; it is greatly influenced by the environment and by genotype × environment interactions. DNA based markers provide an alternative as they are unlimited in number and are not influenced by the environment. In present studies 60 microsatellite markers were used for diversity analysis of 49 wheat genotypes. Out of 60 primers57 primers were amplified and 23 gave polymorphic results. The remaining 34 gave monomorphic band. Twenty three polymorphic SSR markers were dispersed over the wheat genome (AABBDD), with allele range 1-5 and a total of 170 alleles were detected (Table 2).

The average number of SSR alleles per locus was 2.88, with a range from 2 (Cfa2040, Xgwm261, Xgwm382, Xgwm413, Xgwm170, Wmx44, Cslv34, Xgwm501, Xgwm70, Barc136, Barc101, Xgwm352, Xgwm130, Barc187, Barc76, Wmc407) to 5 (Xgwm190). Number of alleles detected per SSR locus as well as polymorphism information content (PIC) values for each of the SSR loci are summarized in Table 3 (Plates 1, 2 and 3). Prasad *et al.,* in 2000 used a set of 20 markers with 55 wheat genotypes for diversity analysis. Twenty-one loci detected a total of 155 alleles with average of 7.4 alleles per locus. In the studies by Pal *et al.*, 2015 alleles per marker varied from 2 to 5 with an average of 2.67 per locus.

Polymorphic information content is a measure of informativeness or usefulness of a DNA marker for linkage studies. The PIC value reflects allele diversity and frequency among the wheat cultivars.PIC values of various SSR loci across all the 49 genotypes ranged from 0.11(Xgwm130) to 0.65 (GWM11). Seven out of twenty three SSR loci, Xgwm261, Xgwm295, Xgwm190, Xgwm46, Gwm11, Xgwm273 and Xsps3000 had PIC value more than 0.5 which can be considered highly useful for differentiation of wheat genotypes, studying their genetic diversity and phylogenic relationships. Medini *et al.,* (2005) found highest PIC value as 0.68 by SSR markers in the analysis of genetic variability. Whereas Prasad *et al.,* (2000) found it 0.71.

Table.1 List of 49 wheat genotypes used in the present study

Sr. No.	GENOTYPES	Sr. No.	GENOTYPES	Sr. No.	GENOTYPES
1s	C -306	18	WH 1166	35	WH 1193
2	WH-542	19	WH 1164	36	WH 1194
3	WH 711	20	WH 1157	37	WH 1197
4	WH 730	21	WH 1156	38	RAJ 3765
5	WH 1021	22	WH 1154	39	PBW 698
6	WH 1025	23	WH 1142	40	PBW 550
7	WH 1080	24	WH 1182	41	PBW 373
8	WH 1097	25	WH 1183	42	PBW 343
9	WH 1105	26	WH 1184	43	PBW 175
10	WH 1124	27	WH 1185	44	HD 3086
11	WH 1181	28	WH 1186	45	HD 2967
12	WH 1180	29	WH 1187	46	DPW 621-50
13	WH 1173	30	WH 1188	47	DBW 88
14	WH 1172	31	WH 1189	48	DBW 17
15	WH 712	32	WH 1190	49	WH 1195
16	WH 1169	33	WH 1191		
17	WH 1167	34	WH 1192		

Plate.1 Allelic pattern in 49 genotypes of wheat using marker Cfa2040

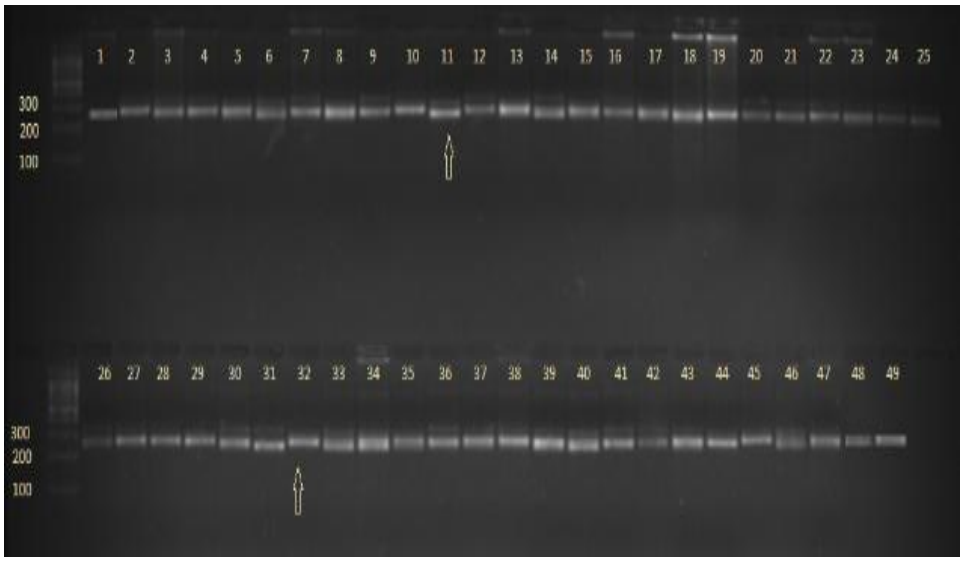


Table.2 Allelic diversity among forty nine genotypes as assessed by SSR markers

Number of markers used	**60**
Polymorphic primers	23
Monomorphic primers	34
Range of alleles	1-5
Total number of alleles	170
Average number of alleles	2.88

Table.3 Band size, allele number and PIC value of polymorphic markers

MARKER		**Number of**	**Bp**	**PIC**
Xcfa2040		2	255-270	0.30
Xgwm261		2	180-200	0.50
Xgwm295	Yr18	3	230-620	0.57
Xgwm382	Yr1	2	130-600	0.17
Xgwm413	Yr15	2	100-120	0.30
Xgwm170		2	230-250	0.32
Wmc44	Yr29	2	200-250	0.22
Barc181	Yr26	2	180-210	0.49
Xgwm190		5	150-600	0.56
Cslv 34	Yr18	2	170-220	0.25
Xgwm501	Yr5	2	150-170	0.18
Xgwm46		3	140-450	0.56
Gwm11	Yr15 Yr24	3	200-550	0.65
Xgwm70		2	170-190	0.15
Barc136		2	280-300	0.40
Barc 101	Yr36	2	115-130	0.27
Barc352		2	250-270	0.21
Xgwm130	Yr7	2	110-130	0.11
Barc187	Yr24	2	110-190	0.15
Xgwm273	YrH52	3	150-200	0.63
Barc76		2	200-220	0.18
Xwmc407		2	120-150	0.18
Xsps3000	Yr10	3	200-270	0.51

Table.4 Distribution of forty nine wheat genotypes in different clusters based on SSR markers

Major cluster	Sub cluster	Genotypes	Number
Cluster1	Subcluster 1	C306, WH1021, WH1025, WH542, WH711, WH730, WH1190,	10
	Subcluster 2	WH1175, WH1157, WH1195, WH1172, WH1166, WH1164,	24
	Subcluster 3	WH1080, WH1105, WH1097, WH1188, WH1181, WH1167,	8
	Subcluster 4	WH1186, WH1187	2
	Subcluster 5	PBW343	1
	Subcluster 6	PBW698, WH1124, PBW550	3
Cluster 2		WH1191	1

Plate.2 Allelic pattern in 49 genotypes of wheat using marker Barc136

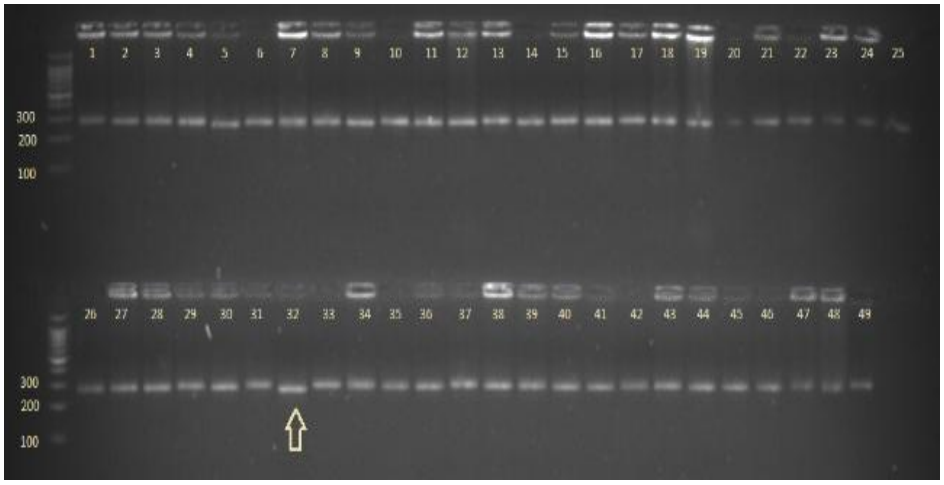
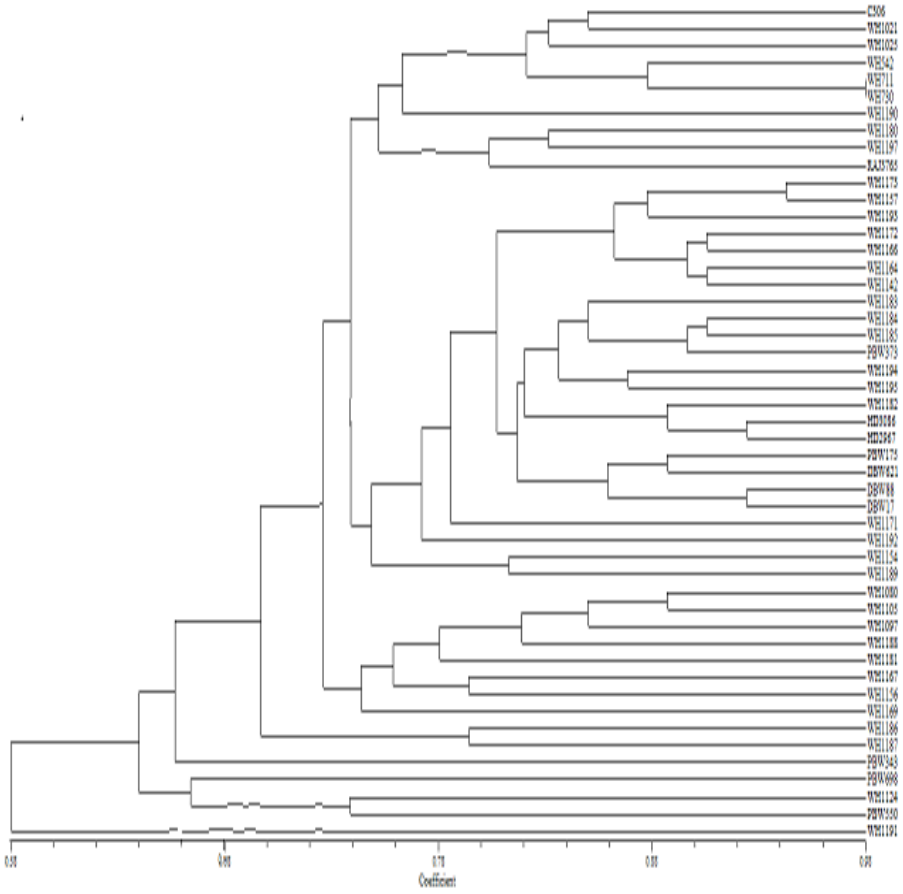


Figure.1 Dendrogram showing the clustering pattern of forty nine genotypes of wheat on the basis of SSR marker



Cluster analysis

The data obtained using SSR microsatellite was further used to construct similarity matrices among 49 released and advance lines using 'SIMQUAL" sub- programme of software NTSYS-pc. The allelic diversity was used to produce a dendrogram using 'cluster tree analysis' which revealed the genetic linkage and proximity among all the genotypes. The unweighted pair group method using arithmetic averages (UPGMA) cluster tree analysis led to the grouping of forty nine wheat genotypes in 2 major clusters and major cluster 1 is fragmented into 6 sub clusters (Table 4) (Fig. 1). Cluster pattern revealed that, sub-cluster 2 was the largest consisting maximum number of 24 genotypes. This way followed by sub-cluster 1 (10genotypes), sub-cluster 3 (8 genotypes), sub-cluster 6 (3 genotypes), sub-cluster 4 (2 genotypes) and sub-cluster 5 had only 1 genotype. The major cluster 2 also had only one genotype WH1191.UPGMA algorithm, distributed the genotypes into two major clusters (I and II), each with two sub-clusters (Prasad *et al.,* 2000). Zhang *et al.,* (2005); Sehgal *et al.,* (2012); and Pal *et al.,* (2015) also did cluster analysis in their studies.

In conclusion, genetic variability in elite germplasm is a pre-requisite for any breeding programme aimed towards the improvement of traits like resistance and productivity. Selection of diverse parents in hybridization also needs molecular marker assistance. Keeping in view, efforts have been made to predict the prospects of developing superior genotypes through genotyping. Present study analyzes 49 genotypes using 60 microsatellite markers out of which 23 were polymorphic.PIC value obtained more than 0.5 shows high usefulness for differentiation of wheat genotypes. Different cluster formation depicts the diversity of the genotypes and selection of parents for hybridization can be done from different clusters. From the present studies it could be concluded that evaluation of germplasm is an important step in plant breeding programme so that the potential genotypes can be identified and used for further improvements. Molecular markers are essential tools for fastening the process of variety development programme. It is further suggested that more polymorphic wheat microsatellites could be used for efficient screening of the germplasm by saturating more regions of the wheat genome.

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