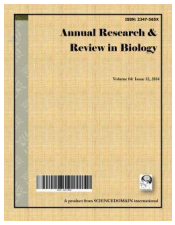

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Molecular Characterization of Diverse Genotypes of Indian Bread Wheat *(Triticum aestivum* L. Em. Thell) by Using SSRs Markers for Leaf and Stripe Rust Resistance

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Authors' contributions

This work was carried out in collaboration between all authors. Author Pooja designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VS and BS managed the analyses of the study. Author MR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Wheat (*Triticum* spp) is a crop of global significance and occupies a premier place among cereals. Due to its high nutritive value and huge acreage devoted for cultivation of wheat, it is a staple food supplying approximately 20% of total food calories. Although wheat has a wide range of climatic adaptability, it is usually affected by many biotic factors the most devastating of which are the rust diseases. All the three species of rusts viz. stem (black) rust *(Puccinia gramims)*; leaf (brown) rust *(P. triticina*) and stripe (yellow) rust *(P. striiformis*) infect wheat crop. Yellow and leaf rusts cause enormous reduction in grain weight and yield. Both usually occurs in cooler areas when temperature ranges between 10-21°C. Different rust races are evolving and a total of 49 races of leaf rust and 22

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of stripe rust identified. The wheat cultivars become susceptible to rusts due to their narrow genetic base and the rapid rate of evolution of the pathogen. In order to sustain wheat production, use of disease resistant varieties is economical and efficient with resource poor farmers. The Indian wheat breeding programmes have also designated 49 and 67 genes for resistance to stripe and leaf rusts of wheat. Rust resistance genes most prevalent in Indian wheat varieties are *Lr1, Lr3, Lr10, Lr13, Lr19, Lr23, Lr24, Lr26, Lr28, Lr34* for leaf rust, *YrA, Yr2, Yr9, Yr18, Yr 27* for stripe rust. So the ultimate objective of the wheat breeding is to have improved better yielding, resistant cultivars with combined resistance through pyramiding especially those *Lr/Yr* genes which act against important races of leaf and yellow rusts.

Keywords: Adaptability; pathogen; resistant varieties; pyramiding; races.

1. INTRODUCTION

Wheat (*Triticum* spp) is a crop of global significance due to which it occupies a premier place among cereals. It belongs to the family *Poaceae* which is the one of the most significant and diverse family of kingdom *Plantae.* Owing to its high nutritive value and huge acreage devoted for cultivation of wheat, it is a staple food of about 35% of the world population supplying approximately 20% of total food calories. About 90% of world wheat area is occupied by bread wheat (*Triticum aestivum* L.) because it has an extremely buffered genotype due to polyploidy [1] and three divergent alleles may be harboured at each locus [2]. At present in India, wheat is the second most important cereal crop after rice both in area and production. Approximately 90 per cent of the total wheat production is contributed by five states i.e. Uttar Pradesh, Punjab, Haryana, Madhya Pradesh and Rajasthan. Although wheat has a wide range of climatic adaptability, it is usually affected by many biotic factors the most devastating of which are the rust diseases. Management of rusts is, thus, the most demanding aspect of wheat production [3].

All the three species of rusts viz. stem (black) rust *(Puccinia gramims* Pers. f. sp. tritici Eriks. & E. Henn); leaf (brown) rust *(P. triticina* Eriks.) and stripe (yellow) rust *(P. striiformis* Westend f. sp. tritici) infect wheat crop. The black or stem rust is important in warmer areas. In north western plain zone it appears when the crop is near maturity but yellow and leaf rusts cause enormous reduction in grain weight and yield [4]. Unlike yellow rust, wheat leaf rust has a much extensive distribution [5] and occurs in the entire country. The yellow rust usually occurs in cooler areas or early in the growth season, when temperature ranges between 10-21°C. High humidity and rainfall are favorable conditions for increasing its infection on both leaf blade and leaf sheath, even on spikes when it comes in epidemic form. Heavy infection by the rusts may result in stunted and weakened plants, shriveled grains, and fewer spikes, loss in no. of grains per spike and grain weight. The level of damage inflicted by rusts varies with the degree of infection and host plant resistance. Different rust races are evolving continuously and the use of resistant genes allows mutants or existing variants at low frequency to be selected and perpetuated. A total of 49 races of leaf rust, 22 of stripe rust and 31 of stem rust identified from different parts of the country since 1931 have been enlisted by [6]. The wheat cultivars become susceptible to rusts due to their narrow genetic base and the rapid rate of evolution of the pathogen, making it necessary to search for new source(s) of resistance. Molecular markers are considered as the best tool to more reliably select and deploy disease resistance genes among plants [7,8]. Microsatellites or simple sequence repeats (SSR) markers with tandem repeats of a basic motif of < 6 bp are highly useful markers in wheat [9] which combine the power of RFLPs (codominant markers, reliable, specific genome location) with the ease of RAPDs and have the advantage of detecting higher levels of polymorphism. SSR markers play an important role in cultivar identification, study of genetic diversity [10], tagging of genes for stress resistance and other economical traits. Hence, keeping in view the importance of disease and availability of SSR markers in wheat, the present study has been planned.

Hence, keeping in view the importance of disease and availability of SSR markers in wheat, the present study has been planned with following objective:

To characterize Indian wheat genotypes for leaf rust and stripe rust resistance using SSR markers.

2. METERIALS AND METHODS

The present investigation was conducted to evaluate 40 genotype of Indian wheat at the experimental area of Wheat Section of the Department of Genetics and Plant Breeding during *Rabi* 2013-14 for characterization of leaf rust and stripe rust resistance using molecular markers. A total of 27 SSR primer pairs showing association with *Lr/Yr* genes in available literature were used in this study. SSR amplification profiles were scored visually, based on presence (taken as +) or absence (taken as -) of bands. The 0 /1 matrix was used to calculate similarity index, genetic distance using 'simqual' sub-program of software NTSYS–PC [11]. Dendrogram was constructed by using distance matrix by the unweighted pair-group method with arithmetic average (UPGMA) sub-programme of NTSYS-PC. Principal component analysis (PCA) was done using the 'CPCA' sub-programme of NTSYS-PC software. Diagrams in both 2 and 3 dimensions were constructed. Presence or absence (+/-) of bands of particular bp may have putative association of *Lr* and *Yr* genes. The size of the intensely amplified band for each microsatellite marker in present investigation was determined based on its migration relative to molecular weight size marker (100 bp DNA/50 bp ladder from Sigma Chemicals Co., USA). For each SSR marker, total amplified bands, number of monomorphic bands, number of polymorphic bands, percentage of polymorphic bands (PPB) and polymorphism information content (PIC) were recorded. PIC was calculated according to the formula [12], as PIC = $1-\Sigma pi^2$, where, *pi* is the frequency of the i^{th} allele of the locus.

3. RESULTS AND DISCUSSION

Genomic DNA from all the genotypes used in the present studies was amplified using 27 SSRs known to be linked with *Lr* and *Yr* genes. Three SSRs (*Xgwm 582, Lr 10-1AS* and *gwm344)* did not show amplification of genomic DNA from different wheat genotypes. Only 24 SSRs (88.8%) showed amplification, of which 11 SSRs (40.7%) produced polymorphic bands while 13 SSRs produced monomorphic bands (44.4%). Size of amplified product in different genotypes ranged from 150-400 bp, a total of 44 alleles were detected with 1.83 as the average no. of alleles detected.

3.1 SSR Polymorphism

The 27 SSR primers amplified a total of 88 alleles in a set of 40 wheat accessions, of which 44 alleles were polymorphic. These microsatellites were selected on the basis of their known genetic locations to give coverage for all three wheat genomes (A, B and D). The efficiency of molecular markers could be assessed with such parameters as PIC, MI and RP. PIC coefficient is relatively used to assess the potential of molecular markers information. The PIC values for the 11 primers varied from 0.56 to 0.74 with an average of 0.60 (Table 1). The high values of PIC for the SSR primers could be attributed to the diverse nature of the wheat accessions and/or highly informative SSR markers used in this study. The MI could be considered as an overall measure of marker utility. The MI values ranged between 1.68 and 3.68 with an average of 2.42. EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands and MI is the product of PIC and EMR, therefore, the higher polymorphism provides higher EMR. The primers which showed higher polymorphism had higher EMR values. In our assessment, EMR varied from 3 to 5 with a mean value of 4 recommended the parameters MI and RP to be used for selecting informative primers [13]. Previously, GD (genetic diversity), PIC, EMR and MI were used to identify the most suitable primer for SSR marker-based classification of germplasm, observed a highly significant positive correlation between them [14]. RP seems to be the perfect coefficient to analyse such ability. The RP values varied from 0.03 to 1.85. The highest RP was recorded for the primer *Xbarc163* (1.85) and the lowest for the primer *Xgwm413* (0.03). The highest PIC, MI, EMR and RP values (0.74, 3.71, 5.0 and 1.85) were observed for primer combination (*Barc149* and *Xbarc163*). This primer combination can be considered to be most informative and discriminative for the molecular characterization of wheat genotypes.

3.2 Screening of Bread Wheat Genotypes against Brown and Yellow Rust under Natural Field Conditions

For sustaining and realizing future goals, wheat crop has to be protected against biotic stresses. Among biotic stresses, the rust pathogens challenge wheat production globally with highly virulent and diverse races. Much has been accomplished in controlling the wheat rusts through deploying resistant cultivars carrying diverse resistance genes in India. Among 40 wheat genotypes of bread wheat used in present investigations DBW 17 and WH1142 showed maximum infection (60S and 20S) against yellow

and brown rust. Zero % infection was observed, in 19 genotypes with yellow rust and in 15 genotypes with brown rust. This indicated quite a good variability for resistance to yellow and brown rust in the present material. However, the data was recorded under natural conditions.

3.3 NTSYS-PC UPGMA Cluster Analysis

The NTSYS-PC UPGMA cluster tree analysis led to the grouping of 40 genotypes into eight different clusters in such a way that genotypes within each cluster had higher similarity than between clusters. Cluster pattern revealed that cluster I was the largest consisting of 13 genotypes (Table 2). This was followed by cluster II (12 genotypes), cluster VII (5 genotypes), cluster V (3 genotypes), cluster III, IV and VI (2 genotypes each) and cluster VIII (1 genotype). The association among the different genotypes is presented in the form of dendrogram in Fig. 1. First two clusters were formed at similarity index of 0.69. At similarity index 0.69, first main cluster consisted of 32 genotypes diverged into two sub clusters; Cluster I consists of 6 genotypes, cluster II consists of 26 genotypes. Cluster II further diverged into two further clusters consisted of 3 and 23 genotypes cluster. Further 23 genotypes cluster diverged into 2 and 21 genotypes and then 21 genotypes cluster divided in to 9 and 12 genotypes. Similarly second main cluster consisted of 8 genotypes in Fig. 2. As per the results of clustering pattern, obtained through molecular analysis, it can be concluded that the genotypes from different breeding groups were grouped together associated with related pedigrees (Table 3). Genotypes with the most distinct DNA profiles are likely to contain the greatest number of novel genes inherited from pedigrees and are likely to carry unique and potentially agronomically useful genes. The results have shown that it is possible to both classify the genetic diversity of elite genotypes and varieties for the highest genetic diversity using SSRs, as indicated by cluster analysis.

3.4 NTSYS-PC Based Principal Component Analysis (PCA analysis)

Genetic relationships as determined by NTSYS-PCA two-dimensional and 3 dimensional scaling of 40 genotypes shown in Fig. 1 and Fig. 2. The clustering of lines was essentially similar to that as obtained by NTSYS-PC (UPGMA). Two-dimensional PCA analysis showed that the genotypes were scattered in two major groups which were further divided into different subgroups. Three primers produced genotype specific amplifications. Primer (*Xwmc44*) produced unique band (350 bp) in the genotype WH1156, *Xbarc163* (400 bp) in genotype PBW550 and *Xbarc167* gave a unique band of 225 bp in WH1080. The uniqueness of the specific bands amplified in different genotypes would help in classification of genotypes for stripe and leaf rust resistance as well as in protection of breeder's right. So molecular markers linked to resistance genes can be used to characterize the genotypes provided a specific product is amplified by that marker. This will save the time and resources incurred to grow the crop and then screen it against a particular disease in field.

Table 1. Chromosomal location (CL), polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI) and resolving power (RP) of polymorphic markers

Primer	CL	PB (%)	PIC	EMR	MI	RP
Xbarc181	1B	100	0.68	4	2.70	1.05
Xgwm295	7D	100	0.74	4	2.96	1.60
xgwm413	1B	100	0.56	3	1.68	0.03
Xwmc 44	1B	100	0.60	4	2.42	1.45
Xwgp 8	1B	100	0.62	3	1.85	0.80
Barc 149	1D	100	0.74	5	3.71	1.45
Xbarc163	4B	100	0.74	5	3.68	1.85
Xbarc167	2B	100	0.68	4	2.74	1.45
Xpsp3000	1B	100	0.69	4	2.75	1.15
Wmc313	4A	100	0.70	4	2.80	1.45
Barc 7	2B/3B	100	0.67	4	2.66	1.15
Mean		100	0.67	4	2.72	1.22

Table 2. Cluster analysis of forty wheat genotypes

Sr. no.	Clusters	Genotypes number of	Genotypes
1	I	WH1126, WH1130, WH1132, WH1134, HD2967, WH1097, PBW550,WH1142, WH1154, PBW621, WH1100, WH1155,WH1080	13
2	II	WH1131,WH1138, WH1135, WH1105, WH1157, WH1124,WH1158, WH1123, WH1136, WH1137, WH1139	12
3	III	WH1080, WH1081	2
4	IV	WH1153, WH1156	2
5	V	WH1127, WH1133, WH1151	3
6	VI	WH1128, WH1129	2
7	VII	WH1160, WH1164, DBW17, WH1120,WH1166	5
8	VIII	P11970	1

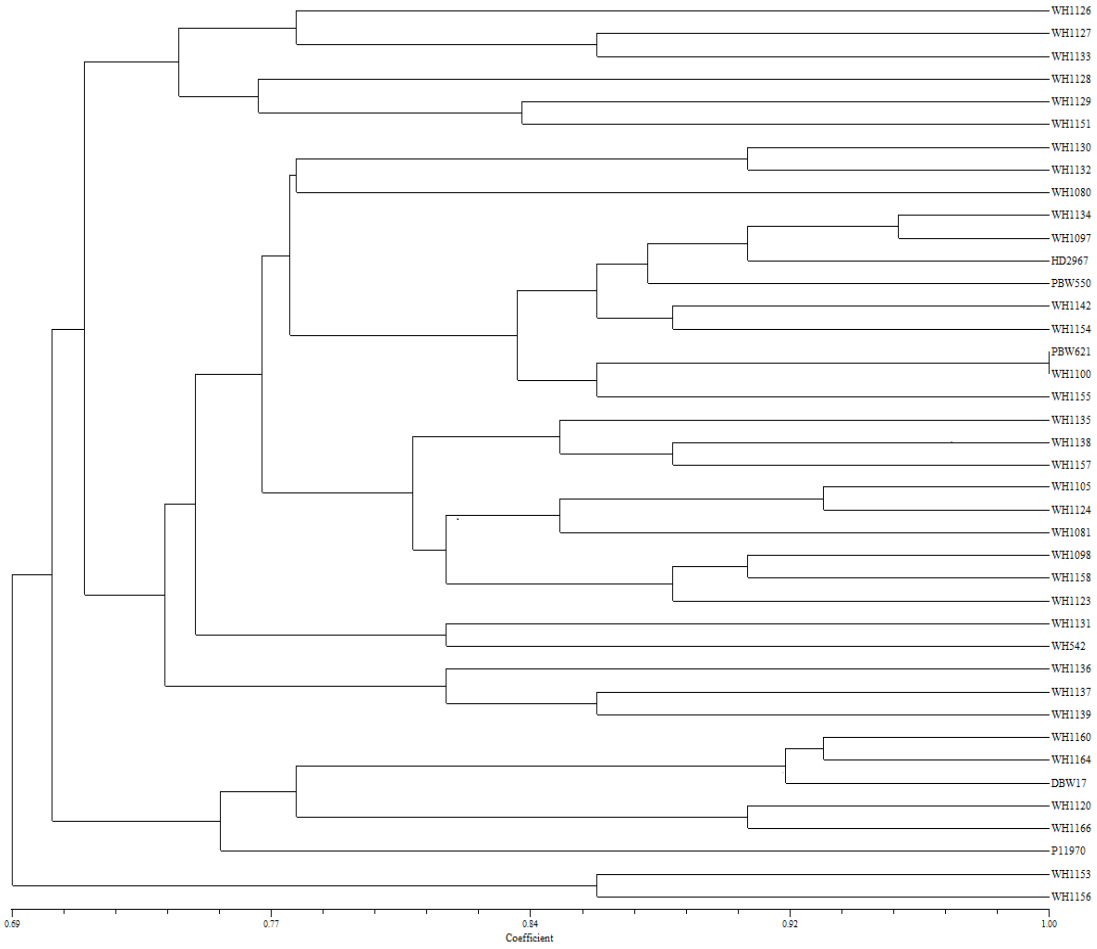

Fig. 1. Dendrogram of 40 genotypes based on SSR diversity data

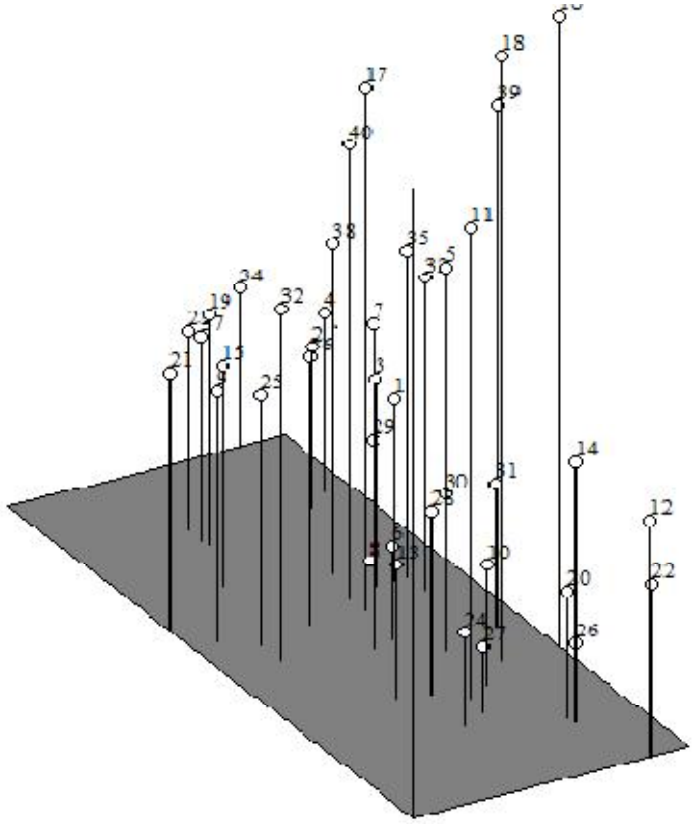

Fig. 2. Three dimensional PCA scaling of 40 genotypes based on SSR diversity data

Table 3. Pedigrees of wheat genotypes used in the present study

Sr. no.	Genotype	Pedigree
1	WH1126	WBLL1*2/VIVITSI
2	WH1127	RL6043/4/NAC//PASTOR/3/BABAX
3	WH1128	PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI
4	WH1129	CS/TH.CS//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI
5	WH1130	PRL/2*PASTOR/4/CHOIX/STAR/3/HE1*CN079
6	WH1131	MUNIA/CHTO//AMSEL
7	WH1132	PBW65/2*PASTOR
8	WH1133	BABAX/LR42//BABAX*2/3/VIVITSI
9	WH1134	PRL/2*PASTOR
10	WH1135	HD29/2*WEAVER
11	WH1136	NI5663/RAJ3765
12	WH1137	NI623/ATILLA/3*BCN/3/PASTOR
13	WH1138	PBW65*2/PASTOR
14	WH1139	CHIR/3/SIREN//ALTAR84
15	WH1142	CHEN/Ae.sq (TAUS)//FCT/3/2*WEAVER
16	WH1160	WAXWING*2/VIVITSI
17	WH1164	RL6043/4*NAC//2*PASTOR
18	DBW17	CMH79A.95/3*CN079//RAJ3777
19	DPW621-50	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES

Sr. no.	Genotype	Pedigree
20	WH1105	MILAN/S87230//BABAX
21	HD2967	ALD/CUC//URES/HD2160/HD2278
22	WH1124	MUNIA/CHTO//AMSEL
23	WH1100	PBW65/2*PASTOR
24	WH1157	MUNIA/CHTO//AMSEL
25	WH1097	ATTILA/BABAX//PASTOR
26	WH1098	TILHI/PASTOR
27	WH1158	PBW 65/2* PASTOR
28	WH1123	NI5663/CHTO//AMSEL
29	WH1080	PRL/2*PASTOR
30	WH1081	PBW65/2*PASTOR
31	WH542	JUPATECO/BLUEJA/URES
32	PBW550	WH594/RAJ3856//W485
33	P11970	IRENA/2*PASTOR
34	WH1151	RL6043/4*NAC//PASTOR
35	WH1153	PI5065/LH1750 (03-04)
36	WH1154	WH337/HD2255//RAJ3077
37	WH1155	SERI*3//RL6010/4*TR/3/PASTOR/4/BAU92
38	WH1156	TILHI/PASTOR
39	WH1120	PRL/2*PASTOR
40	WH1166	HD29/*WEAVER/3/VEE/PJN//2*WEAVER/3/VEE/PJN//2*TUI/4/ MILAN

4. CONCLUSION

The present investigation entitled “Molecular characterization of diverse genotypes of bread wheat *(Triticum aestivum* L. Em. Thell) by using SSRs markers for leaf and stripe rust resistance’’ was conducted using 40 diverse wheat genotypes. These genotypes were grown in *rabi* season at the research area of the Department of Genetics & Plant Breeding, CCS HAU, Hisar in 2 m paired rows in three replications under randomized block design. Molecular characterization of these genotypes was conducted in molecular laboratory of wheat section using SSR marker linked to *Lr, Yr* genes.

- Forty wheat genotypes were screened under natural field conditions against leaf and stripe rust and data in terms of per cent leaf area infected was recorded using Modified Cobb’s Scale. Out of 40 genotypes, 19 showed 0% infection against stripe rust and 15 showed 0% infection against leaf rust. Maximum disease infection (60S) and (20S) was observed in genotypes DBW17 and WH1142 respectively for leaf rust and stripe rust.
- Based on the genomic DNA amplification profile of different genotypes using SSR markers linked to a particular gene, presence or absence of a gene in the genotypes could be ascertained if a product of particular size is amplified. *Yr2* and *Yr15* seemed to be lacking in all the genotypes. Putative presence of *Yr18* gene and *Lr13* gene in some of the genotypes was indicated.
- From the present studies it could be concluded that evaluation of germplasm is an important step in plant breeding so that the genotypes having inherent ability to perform better for different traits can be selected. Leaf rust and stripe rust are the important diseases which require the simultaneous attention of pathologists and the breeder. With the help of the DNA based molecular markers the resistance gene can be identified in the particular genotype with more authenticity and can be exploited as and when required. However, the knowledge of amplification profile of the marker is essential so that a particular gene can be followed through the product it amplifies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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