Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; SP1: 86-93

Pooja
Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Veena Chawla
Principal Scientist, Wheat & Barley Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Prince
Department of Horticulture, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Correspondence
Pooja
Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

(Special Issue- 1)
2nd International Conference
"Food Security, Nutrition and Sustainable Agriculture - Emerging Technologies"
(February 14-16, 2019)

Genetic diversity of bread wheat genotypes (*Triticum aestivum* L.) revealed by microsatellite SSR markers for leaf and stripe rust resistance

Pooja, Veena Chawla and Prince

Abstract
The present investigation was conducted to evaluate 40 diverse genotypes of bread wheat and to characterize these genotypes using Simple Sequence Repeat (SSR) markers. A total of 27 SSR primers were used in study. Three SSRs (*Xgwm 582*, *LrK10-1AS* 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 number of alleles detected.

Keywords: SSRs, polymorphism, Monomorphism

Introduction
Common wheat *Triticum aestivum* L. (2n = 6x = 42), belonging to the family *Poaceae*, which is considered the most diverse and important family of the plant kingdom, produces large edible grains and provides about one-half of humans food calories, a large part of their nutrient requirements and is among the most important grain crops in India. The availability of genetic variability in wheat material is a pre-requisite for any breeding program aimed towards the improvement of wheat productivity (Drikvand *et al*., 2013) [1]. 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 (Marsalis and Goldberg, 2006) [2]. 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. In north western plain zone black rust appears when the crop is near maturity but yellow and leaf rusts cause enormous reduction in grain weight and yield (Khan *et al.*, 2011) [8]. Unlike yellow rust, wheat leaf rust has a much extensive distribution (Gupta *et al.*, 2006) [9] 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 ^{o}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. 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 Bhardwaj *et al.* (2011) [7]. 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. However, use of disease resistant varieties is economical, efficient and most popular with resource poor farmers. The Indian wheat breeding programmes have also designated 49, 67 and 53 genes for resistance to stripe, leaf and stem rusts of wheat respectively. Many new genes are in the process of being designated as resistant sources

(Sharma 2014 b) [11]. Rust resistance genes most prevalent in Indian wheat varieties are *Lr1, Lr3, Lr10, Lr13, Lr19, Lr23, Lr24, Lr26, Lr28, Lr34* for leaf rust, *Sr2, Sr5, Sr7b, Sr8a, Sr8b, Sr9b, Sri, Sr24, Sr31* for stem 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. Molecular markers are considered as the best tool to more reliably select and deploy disease resistance genes among plants (Mir *et al*., 2012; Tomar *et al*., 2014) [3, 14]. SSR markers play an important role in cultivar identification, study of genetic diversity (Hao *et al*., 2011) [15], tagging of genes for stress resistance and other economical traits. In the present study, we used the SSR markers to investigate the genetic diversity among 40 genotypes. The objectives of this study were to use wheat microsatellite markers SSRs to assess levels and patterns of genetic variability among a representative sample of wheat genotypes and use wheat microsatellite markers for the characterization and assessment of the genetic diversity of forty wheat varieties for leaf and stripe rust resistance.

Material and Methods

Experimental site and Plant material

The field experiment was conducted in the Research Area, Wheat and Barley Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar located at 29°10'N latitude, 75°46'E longitude and altitude of 215.2 m above mean sea level. The present investigation was carried out on 40 diverse genotypes of bread wheat at Wheat and Barley Section, Department of Genetics and Plant Breeding CCSHAU, Hisar. A mixture of stripe rust pathotypes 46S119, 47S103 and 78S84 was used to create epiphytotic conditions for screening genotypes.

Experiment under natural field conditions

All diverse genotypes of bread wheat were grown in 2m paired rows, in 3 replications in randomized block design. Recommended package of practices were followed to raise the crop. The infector rows were grown after the interval of 10 entries as border rows of the experiment to ensure uniform infection. Data were recorded on the 5 plants/ replication/genotype.

Creation of epiphytotic conditions

Spray inoculums was done at tillering stage with urediospores of *Pst* (conc.10^6/ml). Genotypes were screened under epiphytotic conditions and data in terms of per cent leaf area infected was recorded using Modified Cobb's Scale (Peterson *et al*., 1948) [21]. Severity of disease was recorded in terms of per cent leaf area infection and pustule type was recorded as response.

Molecular marker Analysis

A total of 27 SSR molecular markers were used for studying molecular polymorphism and to detect *Lr/Yr* genes among genotypes. Genomic DNA was isolated from the young leaves of wheat plants by using CTAB (Cetyl trimethyl ammonium bromide) extraction method as given by Saghai-Maroof *et al.* (1984) [12] and Xu *et al*. (1994) [20]. PCR amplified DNA fragments for DNA markers were resolved by submerged horizontal electrophoresis in 2.5% (w/v) agarose gels PCR amplified products were viewed under UV light fluorescence using photo UV transilluminator. The size (in nucleotides base pairs) of the amplified bands was determined based on its migration relative to standard DNA marker (100 bp DNA ladder). The presence of band run on agarose gel was taken as one and absence of band was read as zero in different lines and promising RILs will be identified for *Yr* genes.

Results and Discussion

Screening of diverse wheat genotypes against leaf rust and stripe rust under natural field conditions

Out of 40 genotypes, 19 genotypes showed 0% infection against stripe rust and 15 genotypes showed 0% infection against leaf rust. Maximum disease infection (60S) and (20S) was observed in DBW17 and WH1142 respectively for leaf rust and stripe rust.

Characterization of wheat genotypes for leaf rust and stripe rust resistance

A total of 27 SSRs known to have association with *Lr*/*Yr* genes in available literature were used to characterize different wheat genotypes.

Qualitative and Quantitative Estimation of DNA

Genomic DNA was isolated from leaves of 2-3 weeks old wheat genotypes using CTAB extraction method (Saghai-Maroof *et al.* 1984) [12]. The DNA was quantified and diluted to a final concentration of 50 ng/µl. The quality of DNA was checked by agarose gel (0.8%) electrophoresis. A single discrete band near the wells was observed in all genotypes (Fig.1) showing that genomic DNA was intact, of high molecular weight and free from RNA contamination.

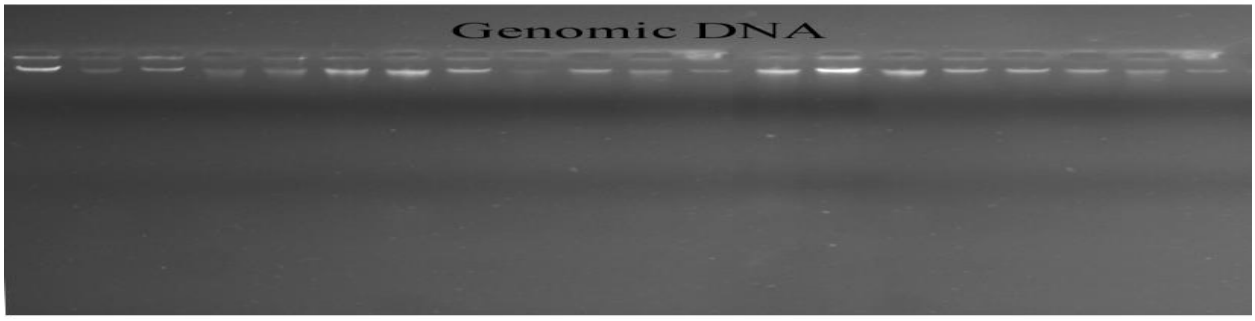

Fig 1: Agarose gel showing genomic DNA of bread wheat genotypes

Characterization of wheat genotypes using SSRs

Genomic DNA from all the genotypes used in the present studies was amplified using 27 SSRs (Table 2) 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%) (Table 2). 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.

Molecular characterization

SSR marker *WMC364* linked to *Yr 2* present a*t 7D chromosome* reported to be linked to

Yr 2 was used to amplify the genomic DNA and produced a monomorphic product of 250 bp in the present investigation while Feng *et al.* (2005) [17] reported the linkage of this marker at 5.6cM to the stripe rust resistance gene *Yr2* producing (207bp/201bp). *WMC 364* indicated that either all our wheat genotypes lacked this gene or this primer is not able to detect *Yr2* gene in our material. So available molecular marker showing association with only *Yr 2* were studied. *Xbarc181-1BL* at linkage distance of 6.7 cM from *Yr26* gene (Wang *et al* 2008) [18] was used to amplify the genomic DNA. Product of size varying from 300-375 bp was obtained. Since no specific product size had been reported. So the wheat genotypes were characterized on the basis of product size obtained in a particular genotype as given in binary data. Product of 300 bp was obtained in genotypes WH1126, WH1127, WH1128, WH1129, WH1133, WH1100, WH1080, WH1151 and PBW621, product size of 325 bp WH 1098, WH 542 and P11970, product of 350 bp was obtained by amplification of genotypes WH1136, WH1137, WH1139, WH1160, WH1124 and WH1153, A product of. 375 bp was obtained in WH1130, WH1132.

Xgwm 582 (6D /6B) reported to be linked to *Yr15* gene at a distance of 0.4 cM did not amplify the genomic DNA of any of the genotypes indicating the absence of gene. *Xwgp 8- 1B* linked to *Yr9* amplified the genomic DNA with size of bands ranging from 150-200 bp while Shi *et al*., (2001) [10] reported a product of 365 bp indicating the absence of this gene in any of the 40 genotypes. *Xgwm130 -7DS* amplified *a* monomorphic product of 325 bp. *Xgwm295- 7DS* linked to *Yr18/Lr34* gave polymorphic product of size ranging from 200- 300. Spielmeyer *et al*. (2005) [13] reported a distance 2.7 cM between the primer and the *Yr18* gene with the product size of 258 bp. This shows the putative presence of gene in some of the genotypes like WH1130, WH1136, WH1137, WH11306, WH1164, DBW-17, PBW 550 and P11970. In the present investigation, PCR amplified DNA products were resolved by submerged horizontal electrophoresis in 2.5% (w/v) agarose gels in which difference of few bp products size can not be resolved. Using SSR *Xgwm 295-7DS* product size of 200 bp was obtained in genotypes, WH1129, WH1134, WH1142, WH1151, WH1153, WH1154 and WH1155 and product size of 225 bp was recovered in WH1126, WH1127, WH1128, WH1131, WH1132, WH1133, WH1135, WH542, WH1120 and WH1166, 300 bp product size was obtained in WH1105, WH1124, WH1157, WH1098, WH1158, WH1123, WH1081. Hiebert *et al*. (2008) [16] worked with *Xgwm251-4BL* to detect *Lr12*, a monomorphic product of 225 bp was recovered. As the *Lr12* was mapped using deletion mapping, the size of the product has not been mentioned, the presence of the gene could not be ascertained in the present investigation (Fig 4).

SSR *gwm344 -7B/7A* linked to *Lr34* did not amplify the genomic DNA. This marker did not seem to be strong enough to detect complex locus (Murphy *et al* 2009) [4]. *Xbarc 167-2B* and *Xbarc 349* -2B were reported to be linked with the *Yr5* gene as flanked by *Xbarc349* and *Xbarc167* on the proximal side at a distance of 2.6 cM and 0.4 cM respectively, although none of these markers were diagnostic in all backgrounds. In the present investigation former primer produced the polymorphic bands of size ranging from 200 -300 bp while the later produced a monomorphic band of 250 bp.

Genomic DNA was amplified with *Xpsp3000-1BS,* the marker reported to be in close genetic associations with *Yr10* by Bariana *et al* (2002). The *Yr10* linked *Xpsp3000* allele, 285 bp product was expected in case the gene was present in wheat genotypes under study. Polymorphic products of size ranging from 200-300 b p were obtained. Product of approximately 275bp was recovered in genotypes WH1126, WH1129, WH1130, WH1135, WH1153, WH1158, WH1155, WH1120, WH1166 and WH542, a product of 300bp was recovered in WH1154, WH1142, WH1139, WH1138, WH1137 and WH1128. These genotypes have probability of possessing *Yr*10 but this will have to be confirmed by visualizing the product using PAGE. Murphy *et al.* (2009) [4] reported that *Yr15, Xbarc8* and *Xgwm413* appeared to be completely linked with the gene in their population, along with resistance gene analog polymorphism marker *Xwgp34*.While *Xbarc8* produced a monomorphic band of 300 bp, polymorphism was observed with *Xgwm 413* with the product size of 200-325 bp. Peng *et al*. (2000) [19] located *Xgwm413,* 4.3 cM proximal to *Yr15*. However, the map generated by Murphy *et al*. (2009) [4] showed *Xgwm413* to be more tightly linked to the *Yr15* gene. Additionally, for *Yr15*, two SSR markers are located within 2 cM of the gene, although only the *Yr15* marker *Xgwm413* produced a positive result in all of the genotypes tested. In the present investigations band size ranging from 200 to 325 bp was observed in different genotypes as given (Fig.6) but presence of the *Yr15*, in the wheat genotypes could not be ascertained as the band size has not been reported. *Xgwm 6* and *Xgwm 538* were used to follow *Yr25* gene, both these markers produced monomorphic bands of 250 and 300 bp respectively and were not able to discriminate the bread wheat genotypes. Similarly, monomorphic bands of 200 and 275 bp were also produced by using *Xgwm 273-1B* and *Xgwm18-1BS* linked to the gene *Lr26* at 2cM. *Xwmc 44* SSR marker linked to *Yr29* yielded polymorphic bands of size 300-375 bp. Product size of 300 was observed in WH1127, WH1129, WH1133, WH1153, WH1151 and WH1155, 325 bp Product size of 300 was observed in WH1100, PBW621,P-11470, WH1154 and WH1120, 350 bp Product size of 350 was observed only in WH1156 and 375 bp Product size of 300 was observed in, WH1126, WH1130, WH1132, WH1136, WH1137, WH1139, WH1160, DBW -17, WH1105, WH1124, WH1098, WH1180, PBW 550and WH1153. *Xwmc198* linked to *Yr 32* and *Xgwm498 1A/1B* linked to *Yr CH2* also produced monomorphic bands of 275 and 250 bp and was thus unable to distinguish the genotypes. *Xbarc163* linked to *Lr13* gene at 5.1 cM gene produced polymorphic band of size ranging from 300- 375 bp. Bansal *et al*., 2008 reported a product of 310 bp with this marker. So, in present investigations the genotypes with a product of size 300 bp (Fig.2) may be expected to have *Lr13*, However this have to be confirmed by separating the product using PAGE. Maximum no. of polymorphic bands were observed with *BARC 7-2BS* linked to *Lr48*. *WMC 313 4A* linked to *Lr28* also produced polymorphic band was able to distinguish the genotypes for the presence of *Lr28* as given (Fig.5). Similarly, *Barc 149-1D* SSR was used to identify genotypes for *Lr60*. 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, which may further be misleading due to the over influence of the environment.

Table 1: A brief description of primers used in the present investigation

Sr. No	SSRs Primer	Location on Chromosome	Forward Primer	Reverse Primer	Annealing Temp. (ºC)
1.	*WMC 364*	7D	5'ATCACAATGCTGGCCCTAAAAC3'	5'CAGTGCCAAAATGTCGAAAGTC3'	51
2.	*X barc 181*	1B	5'CGCAAATCAAGAACACGGGAGAAAGAA3'	5'CGCTGGAGGGGGTAAGTCATCAC3'	58
3.	*Xgwm 582*	1BL	5'AAGCACTACGAAAATATGAC3'	5'TCTTAAGGGGTGTTATCATA3'	43
4.	*Xwgp 8*	1B	5'GAGGAAGGACAGGTTGCC3'	5' CTCTGTATACGAGTTGTC3'	53
5.	*Xgwm 130*	7A	5'AGCTCTGCTTCACGAGGAAG3'	5'CTCCTCTTTATATCGCGTCCC3'	50
6.	*Xgwm 295*	7DS	5'GTGAAGCAGACCCACAACAC3'	5' GACGGCTGCGACGTAGAG3'	52
7.	*LrK10-1AS*		5'GTGTAATGCATGCAGGTTCC3'	5'AGGTGTGAGTGAGTTATGTT3'	48
8.	*Xgwm 251*	4B	5'CAACTGGTTGCTACACAAGCA3'	5'GGGATGTCTGTTCCATCTTAG3'	51
9.	*gwm 344*	7B	5'CAAGGAAATAGGCGGTAACT3'	5'ATTTGAGTCTGAAGTTTGCA3'	48
10.	*Xbarc 167*	2B	5'AAAGGCCCATCAACATGCAAGTACC3'	5'CGCAGTATTCTTAGTCCCTCAT3'	52
11.	*Xbarc 349*	2B	5'-CGAATAGCCGCTGCACAAG-3'	5'TATGCATGCCTTTCTTTACAAT3'	49
12.	*Xpsp 3000*	1 BS	5'GCAGACCTGTGTGTCATTGGTC3'	5'GATATAGTGGCAGCAGGATACG3'	50
13.	*Xbarc 8*	3D	5'GCGGGAATCATGCATAGGAAAACAGAA3'	5'GCGGGGGCGAAACATACACATAAAAACA3'	56
14.	*Xgwm 413*	1A/1B	5'TGCTTGTCTAGATTGCTTGGG3'	5'GATCGTCTCGTCCTTGGCA3'	52
15.	*Xgwm 6*	5A	5'CGTATCACCTCCTAGCTAAACTAG3'	5'AGCCTTATCATGACCCTACCTT3'	50
16	*Xgwm 538*		5'GCATTTCGGGTGAACCC3'	5'GTTGCATGTATACGTTAAGCGG3'	50
17.	*Xgwm 273*	1B	5'ATTGGACGGACAGATGCTTT3'	5'AGCAGTGAGGAAGGGGATC3'	51
18.	*Xgwm 18*	1BS	5'TGGCGCCATGATTGCATTATCTC3'	5'GGTTGCTGAAGAACCTTATTTAGG3'	51
19.	*Xwmc 44*	1D	5'TGTTGCTAGGGACCCGTAGTGG3'	5'GGTCTTCTGGGCTTTGATCCTG3'	59
20.	*Xwmc 198*		5'TTGAAGTGGTCATTGTTGCT3'	5'CACGCTGCCATCACTTTTAC3'	48
21.	*Xgwm 498*	1A/1B	5'GGTGGTATGGACTATGGACACT3'	5'TTTGCATGGAGGCACATACT3'	55
22.	*Xbarc 163*		5'GCGTGTTTTAAGGTATTTTCCATTTTCT3'	5'GCGCATCCTGTTCCTCCATTCATA3'	52
23.	*WMC 313*	4A	5'GCAGTCTAATCTGCTGGCG3'	5'GAGGCTTGCATGTGCTTGA3'	51
24.	*Barc 7*		5'CGCCATCTTACCCTATTTGATAACTA3'	5'GCGAAGTACCACAAATTTGAAGGA3'	55
25.	*Barc 149*	1D	5'ATTCACTTGCCCCTTTTAAACTCT3'	5'GAGCCGTAGGAAGGACATCTAGTG3'	52
26.	*Barc 321*		5'TCCACTTCCCACAACACATC3'	5'TTGCCACGTAGGTGATTTATG3'	50
27.	*Barc 104*	6A	5'GCGCTTCCAAGGCTTAGAGGCT3'	5'CGAGCATCAATAATTGAGAAATACATAGA3'	53

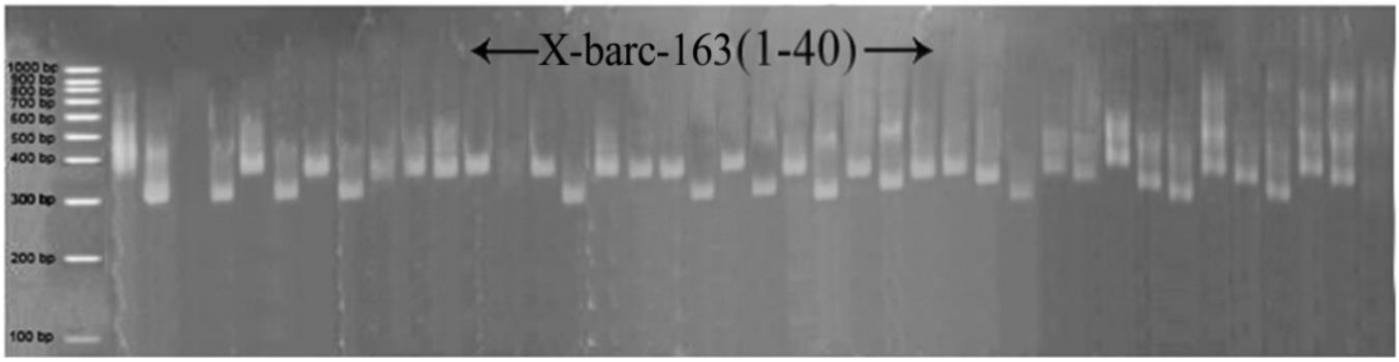

Fig 2: Polymorphism in different bread wheat genotypes by using primer *Xbarc163* (*Lr13)*

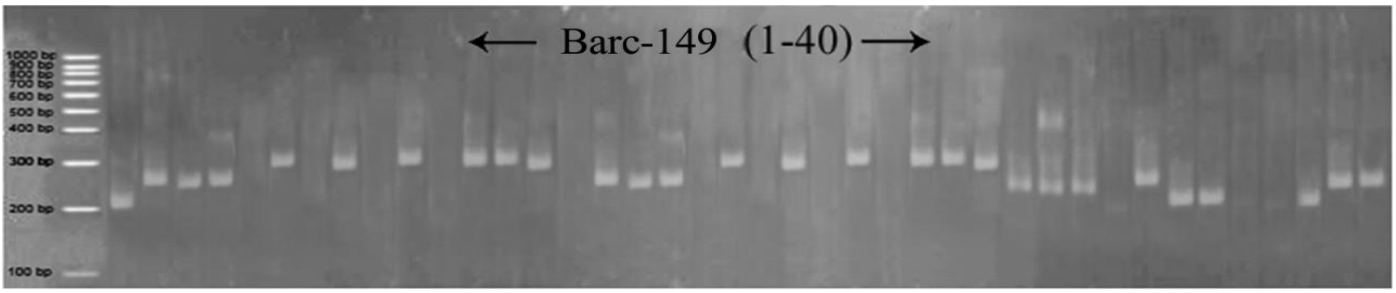

Fig 3: Polymorphism in different bread wheat genotypes by using primer *Barc149* (*Lr 60*)

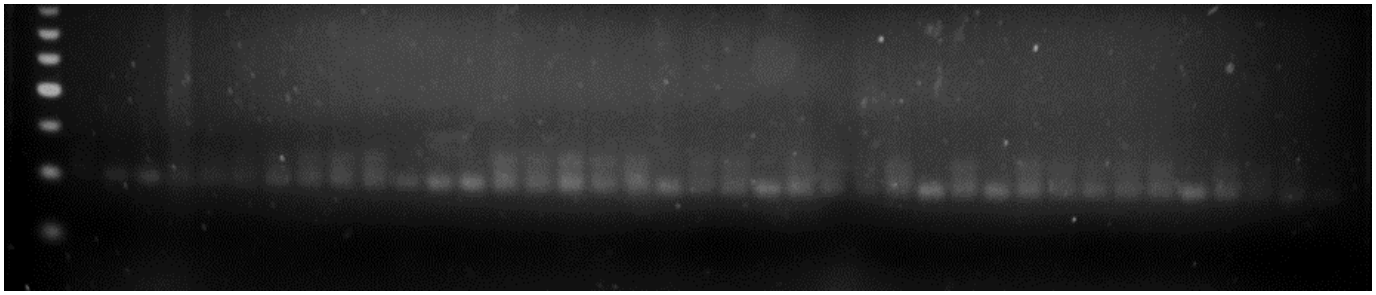

Fig 4: Monomorphism in different bread wheat genotypes by using primer *Xgwm251* (*Lr12*)

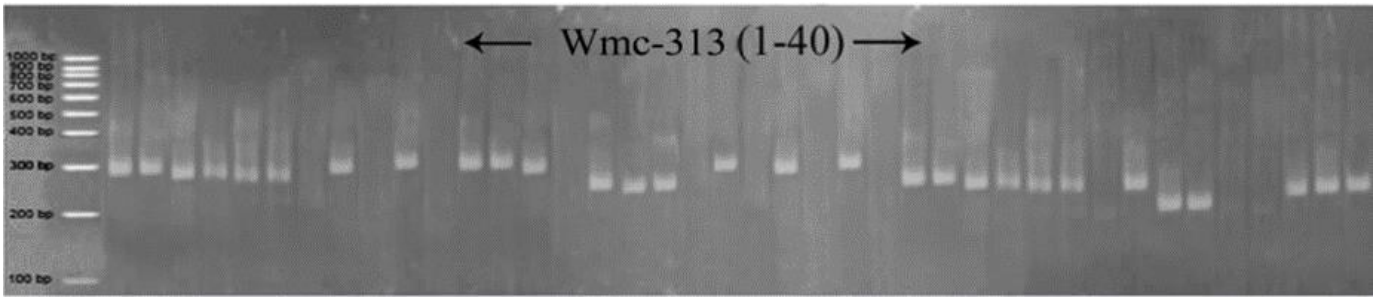

Fig 5: Polymorphism in different bread wheat genotypes by using primer *Wmc313 (Lr28)*

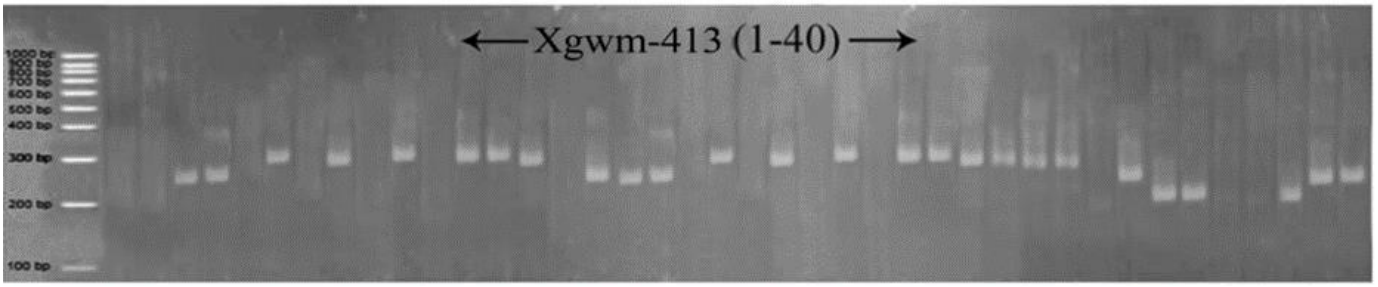

Fig 6: Polymorphism in different bread wheat genotypes by using primer *Xgwm413* (*Yr15*)

Table 2: DNA fingerprinting profile of different wheat genotypes using SSRs

Sr. No.	Ssr Primer	Linkage With Lr/Yr Gene	Size Of Bands (Bp)	Results
1.	*WMC364 7D*	*Yr2*	250	MONOMORPHIC
2.	*Xbarc181 1B*	*Yr2*	300-375	POLYMORPHIC
3.	*Xgwm 582*	*Yr9, Yr15*	0	NOT AMPLIFIED
4.	*Xwgp 8 1B*	*Yr9*	150-200	POLYMORPHIC
5.	*Xgwm130 7DS*	*Yr18, Lr34*	325	MONOMORPHIC
6.	*Xgwm295 7DS*	*Yr18/Lr34*	200- 300	POLYMORPHIC
7.	*LrK10-6**	*Lr 10*	0	NOT AMPLIFIED
8.	*Xgwm251 4B*	*Lr12*	225	MONOMORPHIC
9.	*gwm344 7B/7A*	*Lr 34*	0	NOT AMPLIFIED
10.	*Xbarc167 2B*	*Yr5*	200-300	POLYMORPHIC
11.	*Xbarc349 2B*	*Yr5*	250	MONOMORPHIC
12.	*Xpsp3000 1BS*	*Yr10*	200-300	POLYMORPHIC
13.	*Xbarc 8*	*Yr15*	300	MONOMORPHIC
14.	*Xgwm 413*	*Yr15*	200-325	POLYMORPHIC
15.	*Xgwm 6 5A*	*Yr25*	250	MONOMORPHIC
16.	*Xgwm 538*	*Yr25*	300	MONOMORPHIC
17.	*Xgwm 2731B*	*Yr26, YrH52*	200	MONOMORPHIC
18.	*Xgwm18 1BS*	*Yr26*	275	MONOMORPHIC
19.	*Xwmc 44*	*Yr29*	300-400	POLYMORPHIC
20.	*Xwmc 198*	*Yr32*	275	MONOMORPHIC
21.	*Xgwm498 1A/1B*	*Yr CH 42*	250	MONOMORPHIC
22.	*Xbarc 163*	*Lr13*	300-375	POLYMORPHIC
23.	*WMC313 4A*	*Lr 28*	225-300	POLYMORPHIC
4.	*Barc 7*	*Lr 48*	300-400	POLYMORPHIC
25.	*Barc 149 1D*	*Lr 60*	200-300	POLYMORPHIC
26.	*Barc 321*	*Lr 63*	250	MONOMORPHIC
27.	*Barc 104 6A*	*Lr 64*	250	MONOMORPHIC

*****STS marker

Table 3: Binary data profile of different genotypes of wheat using *Lr* & *Yr* linked markers

Sr. No.	Genotypes	*Xbarc 181 (Yr 2)*				*Xgwm 295* (*Yr18*)			*Xgwm 413* (*Yr15*)				*Xwmc 44* (*Yr29*)				*Xwgp 8* (*Yr 9*)		
		300	325	350	375	200	225	250	300	200	250	275	300	325	350	375	150	175	200
1	WH1126	+					+									+			+
2	WH1127	+					+						+						
3	WH1128	+					+				+								+
4	WH1129	+				+					+		+						
5	WH1130				+			+								+			
6	WH1131						+					+					+	+	
7	WH1132				+		+									+			
8	WH1133	+					+					+	+						
9	WH1134					+													
10	WH1135						+					+							+
11	WH1136			+				+								+	+	+	
12	WH1137			+				+				+				+			
13	WH1138											+							+
14	WH1139			+								+				+	+		+
15	WH1142					+													
16	WH1160			+				+			+					+			+
17	WH1164							+			+								
18	DBW17			+				+			+					+			
19	PBW621	+												+					
20	WH1105								+			+				+			
21	HD2967																		
22	WH1124			+					+			+				+			+
23	WH1100	+												+					
24	WH1157								+			+							
25	WH1097																		+
26	WH1098		+						+			+				+			
27	WH1158								+			+							
28	WH1123								+			+							
29	WH1080	+										+				+			
30	WH1081								+			+							
31	WH542		+				+					+						+	
32	PBW550							+								+			
33	P11970		+					+			+			+					
34	WH1151	+				+				+			+						
35	WH1153			+		+				+						+			
36	WH1154					+								+				+	
37	WH1155					+							+						
38	WH1156									+					+				
39	WH1120						+				+			+				+	
40	WH1166						+				+								

Table 3: Contd….

Sr. No.	Genotypes	*Barc 149* (*Lr60*)					*Xbarc 163* (*Lr13*)					*Xbarc* 167 (*Yr5*)			
		200	225	250	275	300	300	325	350	375	400	200	225	250	275
1	WH1126	+								+					
2	WH1127				+		+							+	
3	WH1128			+											+
4	WH1129			+			+								+
5	WH1130									+		+			
6	WH1131					+	+					+			
7	WH1132									+		+			
8	WH1133					+	+					+			
9	WH1134								+						
10	WH1135					+			+					+	
11	WH1136								+					+	
12	WH1137					+			+						+
13	WH1138					+									
14	WH1139				+				+						
15	WH1142						+					+			
16	WH1160			+						+				+	
17	WH1164			+						+				+	
18	DBW17			+						+				+	
19	PBW621							+							
20	WH1105					+				+					+
21	HD2967							+							

22	WH1124					+				+				+
23	WH1100							+						
24	WH1157					+				+				+
25	WH1097								+					
26	WH1098					+			+					+
27	WH1158					+			+					+
28	WH1123				+				+					+
29	WH1080		+			+		+					+	
30	WH1081		+							+		+		
31	WH542		+						+			+		
32	PBW550										+			
33	P11970		+					+						+
34	WH1151			+			+					+		
35	WH1153	+								+		+		
36	WH1154								+					
37	WH1155							+						
38	WH1156	+								+		+		
39	WH1120		+						+				+	
40	WH1166		+										+	

Table 3: Contd….

Sr. No.	Genotypes	*Xpsp 3000* (*Yr10*)				*WMC 313* (*Lr28*)				*Barc 7* (*Lr 48*)			
		225	250	275	300	225	250	275	300	300	325	350	375
1	WH1126			+					+	+			
2	WH1127		+						+	+			
3	WH1128				+			+					
4	WH1129			+				+		+			
5	WH1130			+				+				+	
6	WH1131							+					
7	WH1132											+	
8	WH1133								+	+			
9	WH1134												
10	WH1135			+					+				
11	WH1136											+	
12	WH1137				+				+			+	
13	WH1138				+				+				
14	WH1139				+				+			+	
15	WH1142				+								
16	WH1160	+					+					+	
17	WH1164	+					+						
18	DBW17						+					+	
19	PBW621		+							+			
20	WH1105		+					+				+	
21	HD2967												
22	WH1124							+				+	
23	WH1100		+							+			
24	WH1157								+				
25	WH1097		+										
26	WH1098							+			+		
27	WH1158			+				+					
28	WH1123						+						
29	WH1080							+					
30	WH1081			+				+					
31	WH542			+				+			+		
32	PBW550												
33	P11970						+				+		
34	WH1151					+				+			
35	WH1153					+							+
36	WH1154				+								
37	WH1155			+						+			
38	WH1156						+						+
39	WH1120			+			+					+	
40	WH1166			+			+						

References

1. Drikvand R, Bihamta MR, Najafian G, Ebrahimi A. Investigation of genetic diversity among bread wheat cultivars (*Triticum aestivum* L.) using SSR markers. Journal of Agricultural Science. 2013; 5(1):122-129.
2. Marsalis MA, Goldberg NP. Leaf, stem and stripe rust diseases of wheat. *New Mexico State University Guide.* 2006, A-415, http://www.cahe.nmsu.edij.

3. Mir R, Kumar D, Balyan. A study of genetic diversity among Indian bread wheat (*Triticum aestivum* L.) cultivars released during last 100 years. Genetic Resources and Crop Evolution. 2012; 59(5):717-726.
4. Murphy LR, Santrab D, Kidwellb K, Chena X, Yanc G. Linkage maps of wheat-stripe rust resistance gene Yr5 and Yrl5 for use in marker assisted selection. Crop Science. 2009; 4:1786-1790.
5. Bansal UK, Hayden MJ, Venkata BP, Khanna R, Saini RG, Bariana HS. Genetic mapping of adult plant leaf rust resistance genes Lr48 and Lr49 in common wheat. Theoretical and Applied Genetics. 2008; 117:307-312.
6. Bariana HS, Brown GN, Ahmed NU, Khatkar S, Conner RL, Wellings CR *et al*. Characterisation of Triticum vavilovii-derived stripe rust resistance using genetic, cytogenetic and molecular analyses and its marker-assisted selection. Theor Appl Genet. 2002; 104(2, 3):315-320.
7. Bhardwaj SC. Resistance genes and adult plant rust resistance of released wheat varieties of India. Research Bulletin No. 5: 31 pp. Regional Station, Directorate of Wheat Research Flowedale, Shimla 171002 (India), 2011.
8. Khan MA, Zargar SM, Saini RG. A novel hypersensitive stripe rust (*Puccinia striiformis* Westend f. sp. Tritici Resistance Gene in Bread Wheat Cultivar Cook Effective in India. Journal of Phytology. 2011; 3(7):44-46.
9. Gupta SK, Cherpe A, Prabhu KV, Haque QMR. Identification and validation of molecular marker linked to leaf rust resistance gene Lrl9 in wheat. Theor. Appl. Genet. 2006; 13:1027-1036.
10. Shi ZX, Chen XM, Line RF, Leung H, Wellings CR. Development of resistance gene analog polymorphism markers for the *Yr9* gene resistance to wheat stripe rust. Genome. 2001; 44:509-516.
11. Sharma I. Application of molecular markers for incorporation of resistance to rusts and Karnal bunt in wheat. Indian Phytopath. 2014; 66(4):329-340.
12. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allad RW. Ribosomal DNA spacer-length polymorphism in barley, Mendelian inheritance, Chromosomal location and Population dynamics. Proc. Natl. Acad. Sci. U.S.A. 1984; 81:8014-8019.
13. Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES. Powdery mildew resistance and *Lr34*/*Yr18* genes for durable resistance to leaf and stripe rust co-segregate at a locus on the short arm of chromosome 7D of wheat. Theoretical & Applied Genetics. 2005; 111:731-735.
14. Tomar SMS, Singh SK, Sivasamy M, Vinod. Wheat rusts in India: Resistance breeding and gene deployment – A Review. Indian J Genet. 2014; 74(2):129-156.
15. Hao C, Wang L, Ge H, Dong Y, Zhang X. Genetic Diversity and Linkage Disequilibrium in Chinese Bread Wheat (*Triticum aestivum* L.) Revealed by SSR Markers. Plant Sci. Journal. 2011; 6(2):772-779.
16. Hiebert CW, Thomas JB, McCallum BD, Somers DJ. Genetic mapping of the wheat leaf rust resistance gene Lr60 (LrW2). Crop Science. 2008; 48:1020-1026.
17. Feng L, Shichang X, Lijun Z, Qing M, Qiang Z, Nan L. SSR marker of wheat stripe rust resistance gene Yr2. Journal of Triticeae Crops. 2005; 25(1):17-19.
18. Wang CM, Zhang YP, Han DJ, Kang ZS, Li GP, Cao AH *et al*. SSR and STS markers for wheat stripe rust resistance gene Yr26. Euphytica. 2008; 159:359-366.
19. Peng JH, Fahima T, Roder MS, Li YC, Grama A, Nevo E. Microsatellite high-density mapping of the stripe rust resistance gene *YrH52* region on chromosome 1B and evaluation of its marker-assisted selection in the F2 generation in wild emmer wheat. New Phytologist. 2000; 146:141-154.
20. Xu Y, Shimoro X, Hofstra H. Plant DNA isolation protocol. Nucleic Acid Res. 1994; 22:2399-2403.
21. Peterson RF, Campbell AB, Hannah AE. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J Res. 1948; 26(5):496-500.