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Genic Microsatellite Markers for Genetic Diversity in Wheat Genotypes

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A B S T R A C T

Genetic diversity assessment is necessary to help tackle the threats of environmental fluctuations and for the effective exploitation of genetic resources in breeding program. Recent advancement in the field of molecular markers has made the genetic characterization of genotypes rapid, reliable and reproducible. In the present investigation, we have characterized 49 wheat genotypes at molecular level using 52 SSR primers (including *Yr* specific primers). 27 polymorphic SSR markers were dispersed over the *AABBDD* wheat genome, a total of 102 alleles were detected with allele range of 1 to 6. Polymorphism information content (PIC) values calculated to assess the informativeness of each marker ranged from 0.11 to 0.95 and there is significant that 5 out of 27 SSR loci, namely Xpsp 3000, Xwgp249, Wmc198, csLV34, Xgwm301 revealed PIC values above 0.70, can be considered highly useful for differentiation of wheat genotypes. The UPGMA cluster tree analysis led to the grouping of 49 wheat genotypes in two major clusters and nine sub clusters. Cluster pattern revealed that, sub-cluster six was the largest consisting maximum number of twelve genotypes. Our results suggested that the classification based on genotypic markers of these wheat genotypes would be useful for selection of varieties for wheat improvement program.

Introduction

Common wheat (*Triticum aestivum)* (2n = 6x = 42) is a versatile cereal crop belongs to family Poaceae, the most diverse and important family of the plant kingdom. It produces large edible grains and provides about one-half of human's food calories and a large part of their nutrient requirements. The substantial increase in world's population demands a consistent increase in the production of wheat. In India, Wheat is the second most important food crop after rice both in terms of area, production and consuming country in the world. Over the last 50 years, Indian agriculture has witnessed spectacular advances in both production and productivity after the introduction of dwarf

wheat during the mid-sixties. The major states involved in wheat production are Uttar Pradesh, Punjab and Haryana. They account for nearly 70 per cent of the total wheat produced in the country. Punjab and Haryana yield the highest amount of wheat because of the availability of better irrigation facilities and congenial weather condition. Haryana state on the whole has achieved a productivity level of 4.55 tons/ha on 2.5 million hectares (Anonymous, 2018).

Genetic diversity is basis for genetic improvement of crop plant and launching an efficient breeding programme that aimed for the improvement of wheat productivity. Therefore, it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic variation in future breeding work. The use of molecular marker for evaluation genetic diversity is receiving a much attention (Kumari *et al.,* 2017). Simple sequence repeats (SSRs) (Tautz, 1989) have been widely exploited in wheat due to their high level of polymorphisms, co-dominant inheritance and equal distribution in the wheat genome (Khaled *et al.,* 2015). SSRs are more abundant, ubiquitous in presence, hyper-variable in nature and have high polymorphic information content (PIC) (Gupta *et al.,* 2010). SSR have been used to study genetic diversity of wheat cultivars by (Eujay *et al.,* 2001; Grewal *et al.,* 2007; Hai *et al.,* 2007; Ijaz and Khan, 2009; Khaled *et al.,* 2015)

The current research was conducted to estimate the genetic diversity of 49 different wheat genotypes by using 52 microsatellite markers. All the wheat genotypes could be distinguish from each other at molecular level. The phylogenetic relationships, genetic diversity and molecular characteristics concluded in current study will facilitate in breeding programs for the selection of parents and to derive a high yielding yellow rest resistance variety.

Materials and Methods

Plant materials

Isolation of genomic DNA

Genomic DNA was isolated from the young leaves of wheat plants by using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method given by Murray and Thompson (1980) modified by (Saghai *et al.,* 1984). The concentration and purity of DNA was determined at 260 nm and 280 nm by using UV-Vis spectrophotometer. The band quality of genomic DNA was observed with the help of electrophoresis on 0.8% agarose gel. The DNA samples were diluted to a concentration of 2.0 ng/µl with TE buffer for SSR analysis.

Selection of markers

A total of 52 molecular markers were used for studying molecular polymorphism in 49 genotypes based on different research paper used in analysis of genetic diversity of wheat. All these primers were custom synthesized from Sigma Chemicals Co. USA. The chromosome locations, base sequences of forward and reverse primers of SSR markers and their annealing temperature are given in (Table 2.)

Microsatellite marker analysis

PCR amplification reaction was carried out in applied biosystem thermocycler. The optimized PCR reaction contained DNA template 50 ng, 10X PCR buffer 2.0 µl, $MgCl_2$ 50mM 0.6 µl, dNTPs mix (10µM) 0.5 µl, Forward primer (10 µM) 0.4 µl, Reverse primer (10 µM)m 0.4 µl, *Taq* DNA Polymerase (5 U/µl) 0.3 µl in total volume of 20 µl. The PCR reaction (20 µl) was set up in thin walled 0.2 ml PCR tubes in applied biosystems thermocycler under following reaction conditions:

94 °C for 4 minutes (initial denaturation)
94 °C for 1 minute (denaturation)
48.5-73 °C for 1 minute (primer annealing)
72 °C for 2 minutes (primer extension)
72 °C for 10 minutes (final primer extension)

The amplification reaction was set to repeat the step (ii) to (iv) for 35 times and the amplified products were stored at -20 °C till further use. The PCR products were electrophoresed on 2.5% agarose gels containing at 100 V for 2 h and observed under a UV transilluminator.

Allele scoring and data analysis

The size of amplified band of each microsatellite marker was determined based on electrophoretic mobility relative to molecular weight of ladder (100 bp) used.

Amplified products from microsatellite analysis were scored qualitatively for presence and absence of each marker allele genotype combination. Binary matrix is used for data analysis 1 for present of band and 0 for absence of band.

The binary data was used to calculate similarity genetic distance using JMP 8.0 software, SAS Institute Inc., Carry, NC, 1989-2007. Dendrogram was constructed by using distance matrix by the unweighted pair group method using arithmetic averages (UPGMA) of JMP 8.0 Software.

Anderson *et al.,* (1993) formula is used for calculating the polymorphic information content (PIC) value of marker which is used in amplification

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$$PIC_i = 1 - \sum_{j=1}^{k} P^2_{ij}$$

Where, Pij is the frequency of the j th allele for I th marker and summation extends over the alleles.

Results and Discussion

In the present investigation, a total of 52 SSR primers (including *Yr* specific primers) were used for amplification in different wheat genotypes as shown in (Table 3). Out of these 52 primers only 49 primers gave amplification and remaining 3 were not amplified. Out of these amplified primers, 22 primers were found to be monomorphic and 27 gave polymorphic bands with a total of 102 alleles amplified with a range of 1-6 per primer. Maximum number of allele was observed in 6 in case of marker Xgwm408 whereas the minimum number of allele is 2 (Barc8, Wmc31, Xgwm341, Gwm11, csLV34, Psp2999, Wmc170, Xgwm95, Xgwm140, Wmc25, Barc76, Xgwm261). PIC values of various SSR loci across all the 49 genotypes ranged from 0.11 (Wmc31) to 0.95 (csLV34).

It is significant to note that 5 out of 27 SSR loci, namely Xpsp 3000, Xwgp249, Wmc 198, csLV34, Xgwm301 revealed PIC values above 0.70. The detail of PIC values of all 23 markers used in study is presented in (Table 4). Agarose gel displaying allelic polymorphism among wheat genotypes for some of the SSR markers have been shown in (Plates 1.) The size of amplified DNA fragments varied from approx. 100 bp to 500bp. The UPGMA cluster tree analysis led to the grouping of forty nine wheat genotypes in 2 major clusters and 9 sub clusters (Table 5) (Fig 1). Cluster pattern revealed that, sub-cluster 6 was the largest consisting maximum number of 12 genotypes. This way followed by sub-cluster 4 (8 genotypes), sub-cluster 3 and 8 (6 genotypes), sub-cluster 9 (5), sub-cluster 1 (4 genotypes), sub-cluster 2 and 7 (3 genotypes) and sub-cluster 5 (2 genotypes).

The development of molecular marker technologies during the last twenty years has revolutionized the genetic analysis of crop plants.

Today, molecular markers are the best tools used to determine the level of genetic diversity among plants and can provide detailed characterization of genetic resources (Manifesto *et al.,* 2001; Mir *et al.,* 2012). SSR have been used extensively for designing primer sets which are not only highly polymorphic but also species specific (Pestova *et al.,* 2000). Genetic diversity plays an important role in crop improvement and was demonstrated through SSR markers (Gupta *et al.,* 2009; Plaschke *et al.,* 1995) has used wheat microsatellite for the first time for studying the genetic diversity in closely related European bread wheat varieties.

The present study addressed the utility of SSR markers in revealing assessment of genetic variability and diversity at the molecular level among 49 wheat genotypes wherein 52 SSR primers were used, which were earlier identified in the genomic regions of A, B, and D genomes of wheat. The SSR marker loci generated by the 49 primer pairs were used to assess the genetic diversity among 49 wheat genotypes. The microsatellite or SSR primers generated 102 alleles with the number of alleles per locus varying from 0 to 6. Maximum number of allele was observed in 6 in case of marker Xgwm408 whereas the minimum number of allele is 2 (Barc8, Wmc31, Xgwm341, Gwm11, csLV34, Psp2999, Wmc170, Xgwm95, Xgwm140, Wmc25, Barc76, Xgwm261). A similar pattern of allelic variation was also observed earlier (Schuster *et al.,* 2009; Emon *et al.,* 2010; Zhang *et al.,* 2011). Contrarily the number of alleles detected in the present study was significantly higher than the average number of alleles in previous reports (Schuster *et al.,* 2009) which has reported 3.2. The presence of unique alleles in the set of cultivars may indicate that these materials are useful for plant breeders and geneticists as a rich source of genetic diversity for wheat.

The PIC value is a reflection of allele diversity and frequency among the wheat cultivars and also varied from one locus to another locus.

The level of polymorphism determined by PIC values was quite high and varied range 0.11 (Wmc31) to 0.95 (csLV34).

It is note that 5 out of 27 SSR loci, namely Xpsp 3000, Xwgp249, Wmc 198, csLV34, Xgwm301 revealed PIC values above 0.70, can be considered highly useful for differentiation of wheat genotypes. Similarly, (Ijaz and Khan 2009) reported high level of polymorphism ranging from 10.52% to 98.42%. (Manifesto *et al.,* 2001) reported PIC values ranged from 0.40 to 0.84 with an average value of 0.72.

The DNA fragments varied from approx. 100 bp to 500bp. Similarly, (Abbas *et al.,* 2008) obtained amplified DNA fragments that varied in size ranging from 250bp to 1000bp and (Manifesto *et al.,* 2001) obtained amplified DNA fragments that varied in size from 115bp to 285bp.

Cluster analysis using UPGMA method delineated the 49cultivars into 2 main clusters and 9 sub clusters. Cluster pattern revealed that, sub-cluster 6 was the largest consisting maximum number of 12 genotypes. 9 sub-clusters showing the effectiveness of microsatellite markers in genetic diversity assays.

Several studies using SSR have resulted in successful clustering of wheat cultivars (Amer *et al.,* 2001; Zhang *et al.,* 2005; Hao *et al.,* 2008; Ijaz and Khan *et al.,* 2009; Schuster *et al.,* 2009). This type of markers is very effective in delineating diversity based on parental source by grouping cultivars with similar pedigree information as well as grouping based on agronomic characteristics and geographical origin.

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

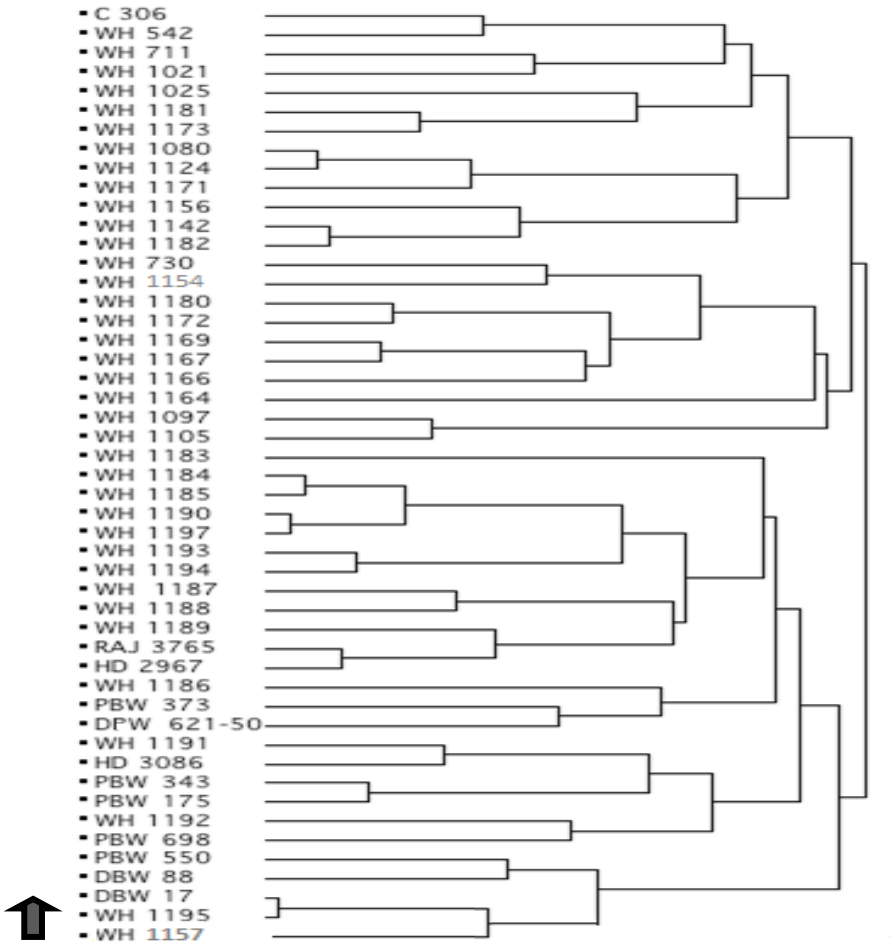

Plate.1 Polymorphism in different forty nine genotypes of wheat by using primer Xgwm349

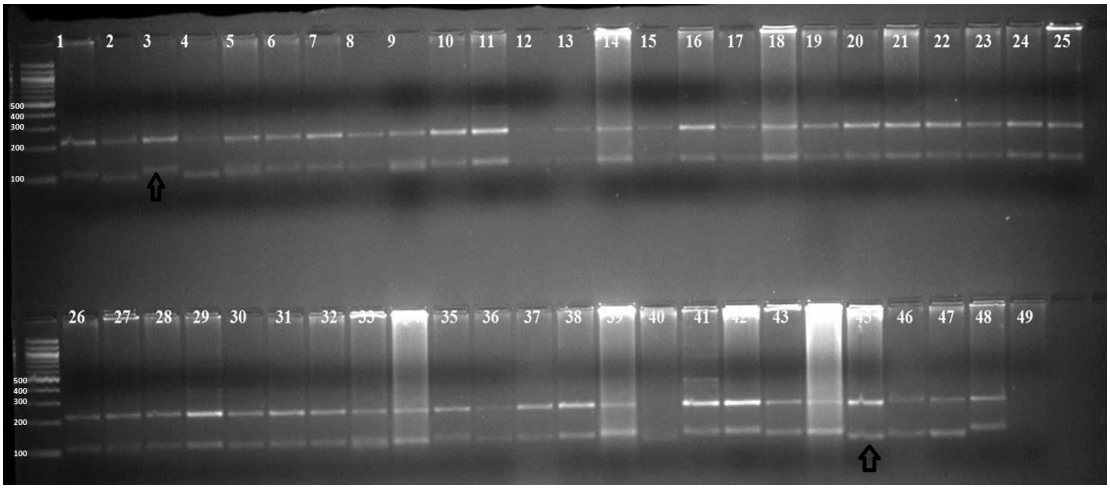

Plate.2 Polymorphism in different forty nine genotypes of wheat by using primer csLV34

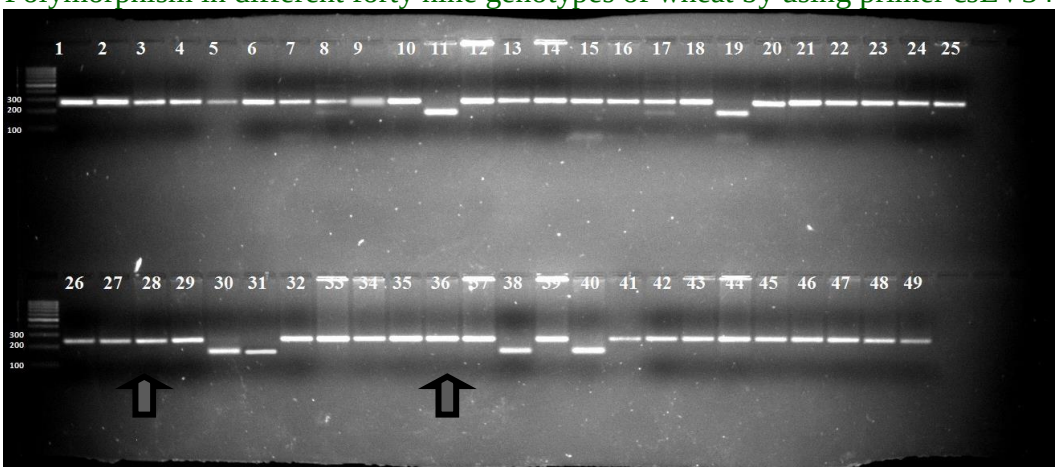

Plate.3 Polymorphism in different forty nine genotypes of wheat by using primer GWM11

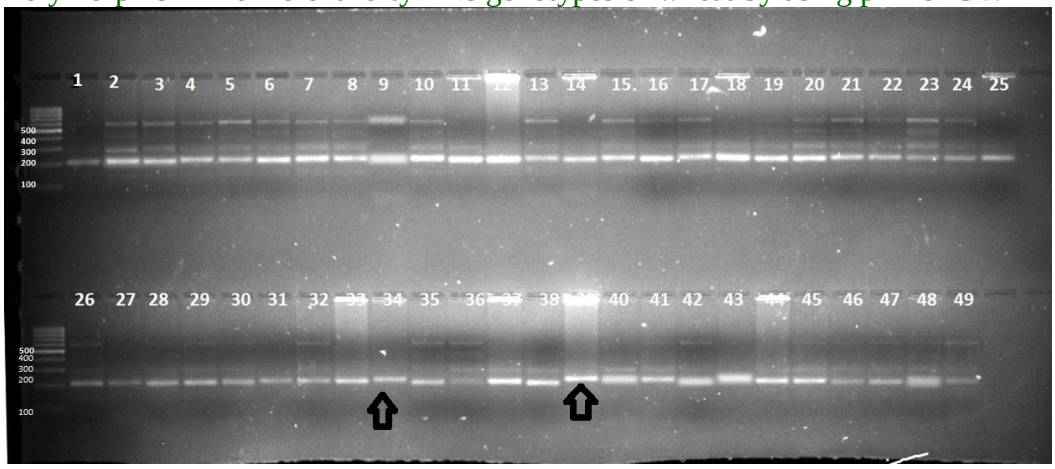

Table.1 List of all the 49 wheat genotypes under experiment

SR.NO.	GENOTYPE	SR.NO.	GENOTYPE	SR.NO.	GENOTYPE
1	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 1171	32	WH 1190	49	WH 1195
16	WH 1169	33	WH 1191		
17	WH 1167	34	WH 1192		

Table .2 List of 52 SSR markers (including Yr specific markers) used for studying polymorphism in 49 genotypes

S. No	SSR Marker	Linkage group	Forward Primer sequence	Reverse Primer sequence	Ta (°C)
1	**Xbarc7-2B**	2B	GCGAAGTACCACAAATTTGAAGGA	CGCCATCTTACCCTATTTGATAACTA	51.5
2	**Xbarc8**	1B (Yr15)	GCGGGAATCATGCATAGGAAAACAGAA	GCGGGGGCGAAACATACACATAAAAAA	58
4	**Xbarc101**	3B (Yr36)	GCTCCTCTCACGATCACGCAAAG	GCGAGTCGATCACACTATGAGCCAATG	54
5	**Xbarc181**	1B (Yr26)	CGCTGGAGGGGGTAAGTCATCAC	CGCAAATCAAGAACACGGGAGAAAGAA	60
6	**Xbarc167**		AAAGGCCCATCAACATGCAAGTACC	CGCAGTATTCTTAGTCCCTCAT	65.2
7	**Xbarc187**	1BYr24	GTGGTATTTCAGGTGGAGTTGTTTTA	CGGAGGAGCAGTAAGGAAGG	62
8	**IAG95-STS**	Yr9/Lr26/Sr34	CGAATAGCCGCTGCACAAG	TATGCATGCCTTTCTTTACAAT	51
9	**Xbarc59**		GCGTTGGCTAATCATCGTTCCTTC	AGCACCCTACCCAGCGTCAGTCAAT	69
10	**Xbarc76**		ATTCGTTGCTGCCACTTGCTG	GCGCGACACGGAGTAAGGACACC	69.5
11	**Xbarc137**	1B	GGCCCATTTCCCACTTTCCA	CCAGCCCCTCTACACATTTT	61
12	**Xbarc352**		CCCTTTCTCGCTCGCCTATCCC	CTGTTTCGCCCAATCTCGGTGTG	64
13	**Xgwm261**	2D	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	62
14	**Xgwm273**	1B(YrH52)	ATTGGACGGACAGATGCTTT	AGCAGTGAGGAAGGGGATC	52.5
15	**Xgwm297**	2D	GCGTAGGAGAGATGCCCCAAAGGTT	GCGTGCGGACTCGTGAATCATTAC	54.5
16	**Xgwm408**	5B	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	63.9
17	**Xgwm437**	7D	GATCAAGACTTTTGTATCTCTC	GATGTCCAACAGTTAGCTTA	54.2
18	**Xgwm186**	5A	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	58.5
19	**Xgwm413**	1B (Yr15)	TGCTTGTCTAGATTGCTTGGG	GATCGTCTCGTCCTTGGCA	52.5
20	**Xgwm18**	1B (Yr26)	TGGCGCCATGATTGCATTATCTTC	GGTTGCTGAAGAACCTTATTTAGG	50
21	**Xgwm359**	2A	CTAATTGCAACAGGTCATGGG	TACTTGTGTTCTGGGACAATGG	58
22	**Barc72**		CGTCCTCCCCCTCTCAATCTACTCTC	CGTCCCTCCATCGTCTCATCA	68
23	**Barc353**		GAAGTTCCCAAAATGCCTCTGTC	GCGGATCGAAGACCTAAGAAAAG	71
24	**Xwmc120**		GGAGATGAGAAGGGGGTCAGGA	CCAGGAGACCAGGTTGCAGAAG	67.5
25	**wmc364**	Yr2	ATCACAATGCTGGCCCTAAAAC	CAGTGCCAAAATGTCGAAAGTC	52
26	**Xwmc44**	Yr29	GGTCTTCTGGGCTTTGATCCTG	TGTTGCTAGGGACCCGTAGTGG	60
27	**Xwgp8**	1B (Yr9)	CTCTGTATACGAGTTGTC	GAGGAAGCACAGGTTGCC	62
28	**Xgwm16**	2B/5D/7B	GCTTGGACTAGCTAGAGTATCATAC	CAATCTTCAATTCTGTCGCACGG	62
29	**Xgwm249**	2A (Yr16)	CAAATGGATCGAGAAAGGGA	CTGCCATTTTTCTGGATCTACC	48
30	**csLV34**	Yr18/Lr26/Sr39	CTTGGTTAAGACTGGTGATGG3	TGCTTGCTATTGCTGAATAGT3	62

31	**GWM11**	Yr15/Yr24	GGATAGTCAGACAATTCTTGT	GTGAATTGTGTCTTGTATGCTTCC	58
32	**Xgwm 6**	4B	CGTATCACCTCCTAGCTAAACTAG	AGCCTTATCATGACCCTACCTT	51.5
33	**Xgwm 37**	7D	ACTTCATTGTTGATCTTGCATG	CGACGAATTCCCAGCTAAAC	56
34	**Xgwm 120**	Yr5	GATCCACCTTCCTCTCTCTC	GATTATACTGGTGCCGAAAC	54
35	**Xgwm 140**	Yr29	ATGGAGATATTTGGCCTACAAC	CTTGACTTCAAGGCGTGACA	60
36	**Xgwm 192**	5D	GGTTTTCTTTCAGATTGCGC	CGTTGTCTAATCTTGCCTTGC	61
37	**Xgwm 210**	2B/5D/7B	TGCATCAAGAATAGTGTGGAAG	TGAGAGGAAGGCTCACACCT	60.5
38	**Xgwm 301**	2D	GAGGAGTAAGACACATGCCC	GTGGCTGGAGATTCAGGTTC	58
39	**Xgwm 319**	2B	GGTTGCTGTACAAGTGTTCACG	CGGGTGCTGTGTGTAATGAC	57
40	**Xgwm 349**	2D	GGCTTCCAGAAAACAACAGG	ATCGGTGCGTACCATCCTAC	61
41	**Xgwm146**	7B	CCAAAAAAACTGCCTGCATG	CTCTGGCATTGCTCCTTGG	48.5
42	**Xgwm268**	1B	AGGGGATATGTTGTCACTCCA	TTATGTGATTGCGTACGTACCC	60.2
43	**Xgwm537**	7B	ACATAATGCTTCCTGTGCACC	GCCACTTTTGTGTCGTTCCT	62
44	**Xgwm569**	7B	GGAAACTTATTGATTGAAAT	TCAATTTTGACAGAAGAATT	54
45	**Xgwm577**	7B	ATGGCATAATTTGGTGAAATTG	TGTTTCAAGCCCAACTTCTATT	57.5
46	**Xgwm247**	2B	GCAATCTTTTTTCTGACCACG	ATGTGCATGTCGGACGC	64
47	**Xgwm341**	3D	TTCAGTGGTAGCGGTCGAG	CCGACATCTCATGGATCCAC	51.5
48	**Xwmc25**	2D	TCTGGCCAGGATCAATATTACT	TAAGATACATAGATCCAACACC	55.8
49	**Xwmc31**		CTGTTGCTTGCTCTGCACCCTT	GTTCAAGTGGTCATTGTTGCT	54
50	**Xwmc170**	2A	ACATCCACGTTTATGTTGTTGC	TTGGTTGCTCAACGTTTACTTC	53.5
51	**Xwmc89**	6A	ATGTCCACGTGCTAGGGAGGTA	TTGCCTCCCAAGACGAAATAAC	52
52	**Xwmc198**	Yr32	CACGCTGCCATCACTTTTAC	TTGAAGTGGTCATTGTTGCT	51

Ta (^{0}c) - annealing temperature

Table.3 List of SSR marker primers showing amplification in different wheat genotypes

S.No.	SSR Marker	Amplification Result	S.No.	SSR Marker	Amplification Result
1	*Xbarc7-2B*	M	*27*	*Xwgp8*	M
2	*Xbarc8*	P	*28*	*Xgwm16*	M
3.	*Xbarc101*	M	*29*	*Xgwm249*	P
4.	*Xbarc181*	M	*30*	*csLV34*	P
5.	*Xbarc167*	M	*31*	*GWM11*	P
6.	*Xbarc187*	M	32	*Xgwm 6*	M
7.	*IAG95-STS*	P	33	*Xbarc137*	P
8.	*Xbarc59*	NA	34	*Xgwm 120*	M
9.	*Xbarc76*	P	35	*Xgwm 140*	P
10.	*Xbarc137*	P	36	*Xgwm 192*	M
11.	*Xbarc352*	M	37	*Xpsp3000*	P
12	*Xgwm261*	M	38	*Xgwm 301*	P
13	*Xgwm273*	P	39	*Xgwm 319*	P
14	*Xgwm297*	M	40	*Xgwm 349*	P
15	*Xgwm408*	P	41	*Xgwm146*	P
16	*Xgwm437*	M	42	*Xgwm268*	P
17	*Xgwm186*	M	43	*Xgwm537*	M
18	*Xgwm413*	M	44	*Xgwm569*	NA
19	*Xgwm18*	M	45	*Xgwm577*	P
20	*Xgwm210*	M	46	*Xgwm247*	M
21	*Barc72*	M	47	*Xgwm341*	P
22	*Barc353*	M	48	*Xwmc25*	P
23	*Xwmc120*	M	49	*Xwmc31*	P
24	*wmc364*	M	50	*Xwmc170*	P
25	*Xwmc44*	P	51	*Psp2999*	P
26	*Xgwm95*	P	52	*Xwmc198*	P

Table.4 Range and PIC value of polymorphic SSR primers

	Primer name	No. Of Alleles	Range (bp)	PIC values
1	Xpsp 3000	5	180-300	0.75
2	Barc8	2	280-500	0.49
3	Xwgp249	4	100-500	0.75
4	Xgwm273	3	200-400	0.66
5	Wmc31	2	100-170	0.11
6	Wmc 198	4	160-500	0.72
7	IAG95-STS	3	100-210	0.58
8	Xgwm341	2	140-180	0.50
9	Gwm11	2	200-210	0.19
10	csLV34	2	180-280	0.95
11	Xgwm349	3	100-210	0.53
12	Psp2999	2	180-190	0.23
13	Wmc170	2	220-230	0.21
14	Xgwm95	2	110-120	0.48
15	Xgwm140	2	210-210	0.30
16	Xgwm268	3	200-300	0.29
17	Wmc25	2	180-210	0.36
18	Xgwm577	4	100-200	0.53
19	Xgwm319	3	120-210	0.66
20	Barc76	2	210-220	0.40
21	Barc137	3	200-500	0.61
22	Xgwm44	3	200-500	0.40
23	Xgwm146	3	160-400	0.47
24	Xgwm674	5	150-500	0.60
25	Xgwm261	2	180-200	0.50
26	Xgwm301	6	160-160	0.77
27	Xgwm408	3	180-200	0.32

Table.5 Distribution of forty nine wheat genotypes in different clusters based on SSR markers

Major cluster	Sub- clusters	Genotypes	No of genotypes
Cluster A	**Cluster1**	C306, WH542,WH711,WH1021	4
	Cluster2	WH1025,WH1181,WH1173	3
	Cluster3	WH1080,WH1124,WH1171,WH1156,WH1142,WH1182,	6
	Cluster4	WH730, WH1154, WH1180, WH1172, WH1169, WH1167, WH1166, WH1164	8
	Cluster5	WH1097,WH1105	2
Cluster B	**Cluster6**	WH1183,WH1184,WH1185,WH1190,WH1197,WH1193, WH1194,WH1187,WH1188,WH1189,RAJ3765,HD2967	12
	Cluster7	WH1186, DBW621-50, PBW373	3
	Cluster8	WH1191, PBW698, HD3086, PBW343, PBW175, WH1192	6
	Cluster9	PBW550, WH1195, DBW88, DBW17, WH1157	5

Genetic diversity evaluation serves as a crucial platform in plant improvement. In the present study 52 Simple Sequence Repeat (SSR) primer sets were used to characterize 49 wheat varieties to know about the diverse varieties for future breeding programs to enhance wheat production. Microsatellites displayed a high level of polymorphism in the present study. The information about the genetic diversity of these wheat cultivars will be much useful for proper identification and selection of appropriate parents for use in the breeding programs, including gene mapping for wheat improvement, enhance the breeding efficiency and will add the strength of marker assisted selection (MAS).

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