Int.J.Curr.Microbiol.App.Sci (2018) *7*(12): 2222-2232

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 12 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.712.253

Microsatellite based Genetic Divergence Study in Bread Wheat (*Triticum aestivum* L. em. Thell.)

Monu Kumar[1,2], R.K. Sharma[1]*, G.P. Singh[1,3] and Anima Mahato[4]

[1]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi – 110 012, India
[2]ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore – 700 120, India
[3]ICAR-Indian Institute of Wheat and Barley Research, Karnal – 132 001, India
[4]ICAR-Indian Institute of Seed Science, Mau – 275103, India

**Corresponding author*

Keywords

Bread wheat, Genetic diversity, SSR markers, Molecular characterization, DNA fingerprints

Article Info

Accepted:
17 November 2018
Available Online:
10 December 2018

A B S T R A C T

A set of 45 polymorphic microsatellite markers, representing markers for almost each chromosome, was used for the assessment of genetic diversity in 35 genotypes of bread wheat. A total of 127 alleles were detected which ranged from 2 to 6 with an average allele number of 2.82 per locus. The PIC value of the 45 SSRs ranged from 0.08 to 0.50 with an average PIC of 0.30. Resolving power and Marker index of all 45 markers varied from 0.17 to 2.82 and 0.16 to 1.52, respectively. Genome wise comparison of diversity recorded maximum genomic diversity in genome A with highest number of alleles per locus (2.95), PIC (0.31), RP (1.33) and MI (0.90). A comparative study on the basis of homeologous chromosomes revealed homeologous chromosome 1 had highest number of alleles per locus (3.0), PIC value (0.32), RP (1.40) and MI (0.92). The homeologous chromosomes 5 and 7 were found to be most conserved with low PIC, RP and MI. The dendrogram, prepared on the basis of similarity matrix using the UPGMA algorithm, delineated the all genotypes into three clusters with 13, 11 and 11 genotypes in cluster I, II and III, respectively. The results demonstrate the utility of microsatellite markers for detecting polymorphism and for estimating genetic diversity in wheat.

Introduction

Wheat is a key global commodity in terms of acreage that fetches maximum tradable value in global market. It is an important component of staple in household diet and fulfills a major portion of total global calories, proteins and micronutrients that support growth and development. As a staple food, it is used in a variety of products that ranged from multipurpose flour used to make freshly baked breads or cookies to processed products such as canned soups that may contain wheat starch as a thickener (www.statistica.com). Wheat is being grown in diverse climatic regimes and can withstand wide temperature range as well as moisture availability. But with an increase in global average temperature wheat crop are being exposed to high temperature stresses during flowering and grain filling stage that

significantly reduces its yield. So, in this era of greater climate concern, the major focus lies on sustainable wheat production and genetic manipulation is the best way to boost up wheat production. Therefore, assessment of genetic diversity is the pre-requisite for any breeding strategy aimed towards the improvement of wheat productivity.

Genetic diversity is the key pillar of biodiversity and diversity within species, between species and of ecosystem and, from the very beginning of agriculture, this natural genetic variability within crop species has been exploited to meet subsistence food requirement. Evaluation of genetic diversity levels among adapted, elite germplasm can provide predictive estimates of genetic variation among segregating progeny for pure-line cultivar development (Salem *et al.,* 2008).

Molecular markers technology is the one possible approach to understand the genetic diversity which is independent of environment effects. Molecular markers based on polymerase chain reaction (PCR) methods, such as simple sequence repeats (SSRs) or microsatellites, have become important genetic markers in a wide range of crop species, including wheat. SSR markers are abundant, dispersed throughout the genome, and show higher levels of polymorphism than other genetic markers (Roder *et al.,* 1995, Al Khanjari *et al.,* 2007). These features, coupled with their ease of detection, make them ideal for identifying and distinguishing between accessions that are genetically very similar (Joshi *et al.,* 1993).

Various studies have used SSR markers to investigate genetic diversity in cultivated hexaploid wheat genotypes of *T. aestivum* L (Devos and Gale, 1992; Chao *et al.,* 1989; Plaschke *et al.,* 1995; Powell *et al.,* 1996; Schloetterer *et al.,* 1991). The present investigation was designed to use genomic SSR markers in assessing level of genetic diversity in bread wheat.

Materials and Methods

Plant material

The present investigation was carried out using 35 wheat genotypes representing a range of phenotypic variation. The experimental material included released varieties for different production conditions of various zones and pre-released advance lines developed at Indian Agricultural Research Institute, New Delhi and other esteemed institutes of India (Table 1). Molecular work was undertaken in Molecular Lab at NRCPB, Pusa, New Delhi.

SSR marker and PCR analysis

Total genomic DNA was isolated from 5 gm of fresh young leaf tissue, collected from five one-month old bulked leaf samples following the CTAB procedure (Plaschke *et al.,* 1995). A set of 77 Simple Sequence Repeats (SSR) markers, representing all three genomes, were used for molecular characterization of wheat genotypes. In order to ensure whole genome coverage microsatellite repeats were selected from each arm of 21 chromosomes, including 'Xgwm', 'Xwmc' and 'Xbarc' markers which represent the organization responsible for identification and characterization of these markers. The PCR amplification was performed with a final volume of 15 µl containing 50 ng DNA of genomic DNA. Amplification products were resolved on 3% metaphor agarose gel and the electrophoresed DNA samples were visualized, photographed and documented using a UV Trans-illuminator geldoc system. The allele sizes were determined by comparing with 50 bp ladder. Out of 77 SSRs, 45 markers (26 'Xgwm', 14 'Xwmc' and 5 'Xbarc' markers) showed polymorphism and were subjected to further

analysis. The list of 45 polymorphic markers has been presented in Table 2.

SSR data analysis

Fragments amplified by microsatellite primers were scored as presence (1) or absence (0) relative to the molecular weight standard (100bp DNA ladder). Resolving Power (RP) of a primer was calculated as the sum of "band informativeness" of all bands produced by a primer (Prevost and Wilkinson 1999).

$$Band\ informativeness\ (Ib) = 1 - (2x\ |0.5 - p|)$$

Where, 'p' is the proportion of accession containing the band.

Resolving Power of the primer (RP) is represented as RP = ∑Ib.

The polymorphic information content, generally refers to the ability of a marker to establish polymorphism in the population based on the number of alleles detected and their distribution frequency, of each primer was determined according to the marker index of Powell *et al.*, (1996).

$$PIC = 1 - \sum (pi^2)$$

Where, 'pi' is the frequency of i^{th} allele and 'n' is the number of genotypes.

Marker index (MI) is a statistical parameter used to estimate the total utility of the maker system and is estimated as the product of polymorphic information content (PIC) and effective multiplex ratio (EMR) (Cheknoskov and Artemyeva, 2015) as:

$$MI = PIC\ x\ EMR$$

Where,

$$EMR = n_p\ (n_p/n)$$

Genetic dissimilarity indices were calculated by using simple matching coefficients. Principle coordinate analysis (PCoA) and the cluster analysis based on unweighted neighbor-joining method were undertaken using DARwin5.0 (Perrier *et al.*, 2003).

Results and Discussion

Assessment of genetic diversity

Genetic diversity of 35 wheat genotypes was carried out with 77 SSR markers of which 45 markers (26 'Xgwm', 14 'Xwmc' and 5 'Xbarc' markers) showed polymorphism. The 45 markers were selected from all seven linkage group that ensures the uniform coverage for whole wheat genome. The 45 polymorphic markers identified a total of 127 alleles. The number of alleles per SSR marker ranged from 2 to 6 with an average of 2.82. Out of 45 microsatellites, 21 were biallelic, 15 were triallelic, 7 were tertrallelic and 2 markers *viz.*, Xwmc684 and Xgwm264, were hexallelic. The 45 polymorphic markers identified 35 rare (frequency < 20%) and 9 unique alleles. Information regarding the number of alleles detected per SSR locus, polymorphism information content (PIC), resolving power (RP), and marker index (MI) is given in Table 2.

This revealed the occurrence of many alleles with frequencies less than 0.20 there by implying that these alleles have occurred in less than seven genotypes out of the 35 genotypes. One marker (Xgwm 130) revealed one allele, 17 markers revealed two alleles, 19 markers revealed three alleles, two markers revealed four alleles and one marker (Xgwm 264) detected five alleles. Number of alleles per marker ranged from 1 to 5 with an average value of 2.67 alleles per marker revealing the polymorphic ability of SSR markers. Salem *et al.*, (2008) also reported 2 to 7 with an average of 3.2 alleles per marker with 48 SSR markers

in wheat. The level of polymorphism observed in this study is lower than reported by other research workers in case wild wheat lines or landraces (Buchanan *et al.,* 1994; Rohlf, 2000; Schloetterer, 1991) due to the considerable amount of natural out crossing that occurs in those genotypes. However, Salem *et al.,* (2008) opined that the cultivars developed through repeated inbreeding are likely to show lower diversity. In the present investigation also majority of the genotypes developed through pedigree method.

The PIC value for 45 SSR primer pairs ranged from 0.08 in xgwm595 to 0.50 in wmc169 and barc146. The wide range of PIC value indicated that the locus specific PCR based microsatellite markers were quite informative. The average PIC value was recorded to be 0.30. However, this observed PIC range is lower than the range reported by Prevost and Wilkinson (1999), Kara *et al.,* (2017) but comparable with the findings of Tomar *et al.,* (2009). The mean PIC value estimated across all SSR loci was 0.30. Twenty one primers (8 Xwmc, 10 Xgwm and 3 Xbarc markers) showed greater PIC value than mean suggesting the suitability of these markers in wheat diversity assessment. The RP values of the markers ranged from 0.17 (Xgwm595) to 2.82 (Xbarc146) with an average RP value across the loci 1.28. The Marker index (MI) value was calculated for all markers which ranged from 0.21 in Xgwm205 to 1.52 in Xgwm340 with an average MI value of 0.86 across the loci.

Genome wise genetic diversity assessment with 21, 26 and 13 markers from A, B and D genome identified highest number of alleles per locus in case of A genome (2.95) followed by D genome (2.62) and B genome (2.27) respectively (Table 3). F found highest number of allele per locus in A genome (7.17) followed by B genome (5.86) and least by D genome. The PIC value was also recorded to be highest in A genome (0.31). However, B and D genome had 0.25 and 0.30 PIC. RP also showed similar trend. The highest RP of 1.33 was recorded in A genome followed by 1.21 and 1.02 in case of D and B genome, respectively. Genome A also had highest MI (0.90) followed by D genome (0.79), whereas B genome shown lowest MI value of 0.69.

The Homoeologous chromosomes 1 showed highest number of allele per marker (3.0) followed by chromosome 6 (2.33) and 4 (2.29), respectively. The PIC value (0.32), RP (1.40) and MI (0.92) were also highest in case of homeologous chromosome 1. However, the PIC value, RP and MI value were low for homeologous chromosome 5 and 7 suggesting that these two chromosomes are either highly conserved or they may carry factors for important characters and any occurrence of any large variation or mutation occurring in these chromosomes might lead to the death of plants.

Genetic similarity analysis using UPGMA and principle co-ordinate analysis

Unweighted Pair Group method with Arthmetic Averages (UPGMA) cluster analysis was performed based on Jaccard"s similarity coefficient matrices derived from SSR markers and a denodgram of 35 wheat genotypes was constructed (Fig. 1). The 35 genotypes were grouped into three clusters with 13, 11 and 11 genotypes in cluster I, II and III, respectively. Based on similarity coefficient each cluster is further divided into two major sub-clusters. The two subclusters of cluster I contain 4 and 9 genotypes. Likewise, the two subclusters of cluster II had 3 and 8 genotypes and in case of cluster III the number was 5 and 6. The Jaccard's dissimilarity coefficient varied from 0.24 to 0.66 exhibiting the presence of wide amount genetic variability among the genotypes. The dissimilarity coefficient was highest between CL3183 and HD2733 however, it was lowest between DBW14 and DL788-2 (Fig. 3).

Table.1 Details of experimental materials

S.N.	Genotype	Pedigree/Parentage	Developing center
1	HD 2997	BOW//HD 2285//HD2444	IARI, New Delhi
2	CL 3104	HD 2883/ CL 1603	IARI, New Delhi
3	CL 3119	HD 2878/ HD 2891	IARI, New Delhi
4	C 306	REGENT19473*CHZ//2*C591/3/P19/C281	HAU, Hisar
5	MP 4010	ANGOSTURA 88	Gwalior
6	CL 3198	SUNSU/CHIBIA	IARI, New Delhi
7	CL3108	DW 1272/HD 2307	IARI, New Delhi
8	HD 2643	VEE `S'/HD 2407/HD 2329	IARI, New Delhi
9	CL 3178	DW 1293/CL 1497	IARI, New Delhi
10	HD 2733	ATILLA/3/TUI/CARC//CHEN/CHTO/4/ATILLA	IARI, New Delhi
11	CL 3176	HD 2877/CL1465	IARI, New Delhi
12	PBW 343	ND/VG/9144//KAL/BB/3/YACO`S'/4/VEE#5 `S'	PAU, Ludhiana
13	KUNDAN	TANORI71/NP890	IARI, New Delhi
14	DL 788-2	K7537/HD2160/HD2278//L24/K4.14	IARI, New Delhi
15	CL 3146	HW 1085/ DW 1302	IARI, New Delhi
16	DBW 17	CMH 79A.95/3*CNO 79/RAJ 3777	IIWBR, Karnal
17	HD 3016	PBW65/2*PASTER	IARI, New Delhi
18	HD 2932	KAUZ/STAR/HD 2643	IARI, New Delhi
19	CL 3190	HD 2824/DW 1285	IARI, New Delhi
20	HD 2864	K7537/HD2160/HD2278//L24/K4.14	IARI, New Delhi
21	PBW 590	WH594/RAJ3814//W485	PAU, Ludhiana
22	HD 2894	TURACO/PRINIA/DL 788-2/DW 871	IARI, New Delhi
23	CL 3153	HW 5015/ CL 1556	IARI, New Delhi
24	PBW 373	ND/VG9144//KAL/BB/3/YACO "S"/4/VEE # 5 'S'	PAU, Ludhiana
25	CL 3144	CL 1509/ UP 2571	IARI, New Delhi
26	CL3106	NG 8675/ METSO	IARI, New Delhi
27	WR 544	K. SONA/HD1999//HD2204/3 /DW38	IARI, New Delhi
28	HD 2967	ALD/COC//URES/HD 2160 M/HD 2278	IARI, New Delhi
29	CL 3125	HD 2687/ CL 1525	IARI, New Delhi
30	HD 2189	HD 1963/HD1931	IARI, New Delhi
31	CL 3171	HD 2643/ CL 1556	IARI, New Delhi
32	L 512	HD 2877/CL 1567	IARI, New Delhi
33	CL 3183	CL 1449/CL 1603	IARI, New Delhi
34	DBW 14	RAJ3765/PBW343	IIWBR, Karnal
35	CL3118	HD 2824/DW 1299	IARI, New Delhi

Table.2 Number of alleles detected PIC, RP, HI and MI of all SSR markers

S.N.	SSR Marker	Chromosome	Alleles	Average PIC	RP	MI
1	xgwm282	7A	3	0.23	0.83	0.70
2	barc59	2D, 5B	3	0.49	2.63	1.48
3	barc70	4A, 7A, 7D	3	0.37	1.44	1.10
4	barc105	3A, 4D, 7A, 7D	4	0.25	1.31	0.99
5	wmc216	1D	2	0.26	0.83	0.53
6	wmc597	1B, 2B, 3B, 4A, 6B, 7B	2	0.28	0.74	0.56
7	wmc59	1A, 1B, 6A	3	0.31	1.35	0.93
8	wmc169	3A	3	0.50	2.72	1.49
9	barc146	6A, 6B, 6D	3	0.50	2.82	1.49
10	barc101	2B, 3B, 6B	3	0.30	1.35	0.90
11	xgwm335	5B	4	0.29	1.52	1.15
12	xgwm130	2B, 7A, 7B	3	0.35	1.61	1.04
13	xgwm132	6B, 6D	3	0.29	1.22	0.88
14	xgwm614	2A, 2B	4	0.19	1.03	0.75
15	xgwm369	3A	4	0.29	1.77	1.16
16	xgwm205	5D	2	0.10	0.22	0.21
17	xgwm121	5D, 7D	2	0.13	0.28	0.26
18	xgwm120	2B	2	0.47	1.65	0.94
19	xgwm131	7B	2	0.47	1.64	0.94
20	xgwm349	2D	2	0.24	0.56	0.48
21	xgwm285	3B	3	0.28	1.22	0.84
22	xgwm459	6A	4	0.31	1.72	1.25
23	xgwm337	1B, 1D	2	0.48	1.61	0.96
24	xgwm469	6D	3	0.09	0.28	0.26
25	wmc215	3A, 5A, 5D	2	0.31	0.78	0.62
26	xgwm526	2A, 2B, 7A, 7B	2	0.13	0.28	0.25
27	wmc737	6B	2	0.29	0.72	0.57
28	wmc219	4A	2	0.24	0.56	0.48
29	xgwm340	3B	4	0.38	2.11	1.52
30	wmc684	6A	6	0.22	1.83	1.33
31	xgwm372	2A	3	0.43	2.11	1.29
32	wmc497	4A, 7A	2	0.42	1.22	0.84
33	wmc85	3B	3	0.41	1.78	1.24
34	xgwm297	7B	2	0.20	0.44	0.39
35	wmc11	3A	3	0.45	2.11	1.35
36	wmc258	4A, 5B	2	0.13	0.28	0.26
37	xgwm400	7B	2	0.13	0.28	0.26
38	xgwm304	5A	2	0.43	1.28	0.85
39	wmc238	4B	4	0.36	2.32	1.45
40	xgwm595	5A	2	0.08	0.17	0.16
41	xgwm566	3B	2	0.47	1.50	0.93
42	xgwm264	1B, 3B	6	0.25	2.12	1.49
43	xgwm533	3B	2	0.31	0.89	0.61
44	xgwm389	3B	2	0.24	0.61	0.48
45	wmc631	1B, 3D	3	0.34	1.72	1.02
Mean			**2.82**	**0.30**	**1.28**	**0.86**

Table.3 Genetic diversity in different genome and homoeologous chromosome

Genome	No. of markers used	Number of alleles detected	Average allele per locus	PIC	Resolving Power	Marker Index
Genome						
A	21	62	2.95	0.31	1.33	0.90
B	26	59	2.27	0.25	1.02	0.69
D	13	34	2.62	0.30	1.21	0.79
Homeologous chromosome						
1	6	18	3.00	0.32	1.40	0.92
2	9	16	1.78	0.20	0.80	0.53
3	15	18	1.20	0.14	0.60	0.39
4	7	16	2.29	0.24	0.92	0.65
5	8	12	1.50	0.15	0.38	0.30
6	9	21	2.33	0.17	0.79	0.58
7	10	12	1.20	0.15	0.41	0.29

Fig.1 Cluster dendogram depicting genetic divergence among 35 wheat genotypes based on SSR markers

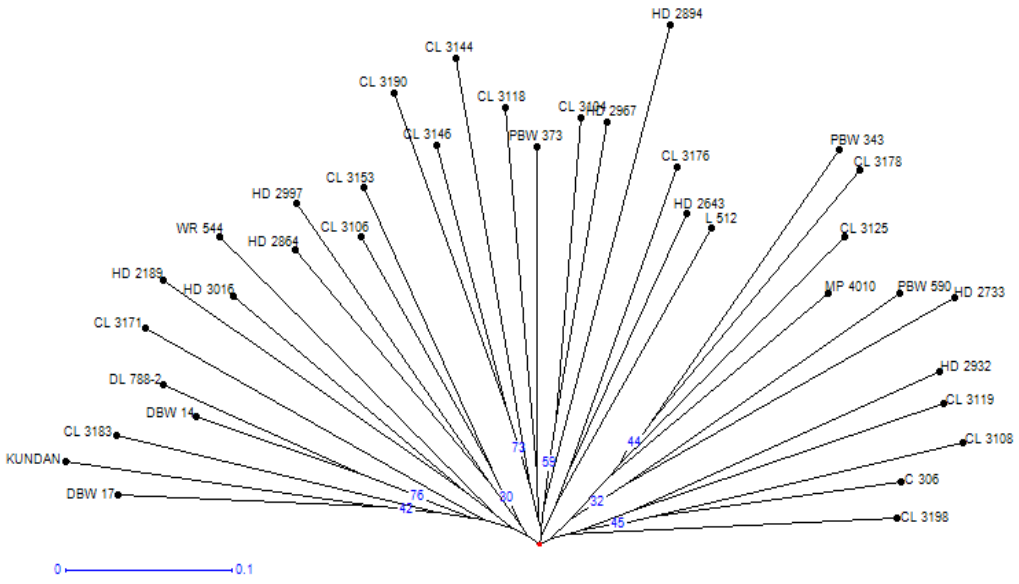


Fig.2 Principle coordinate analysis based on molecular data depicting genetic divergence of 35 wheat genotypes

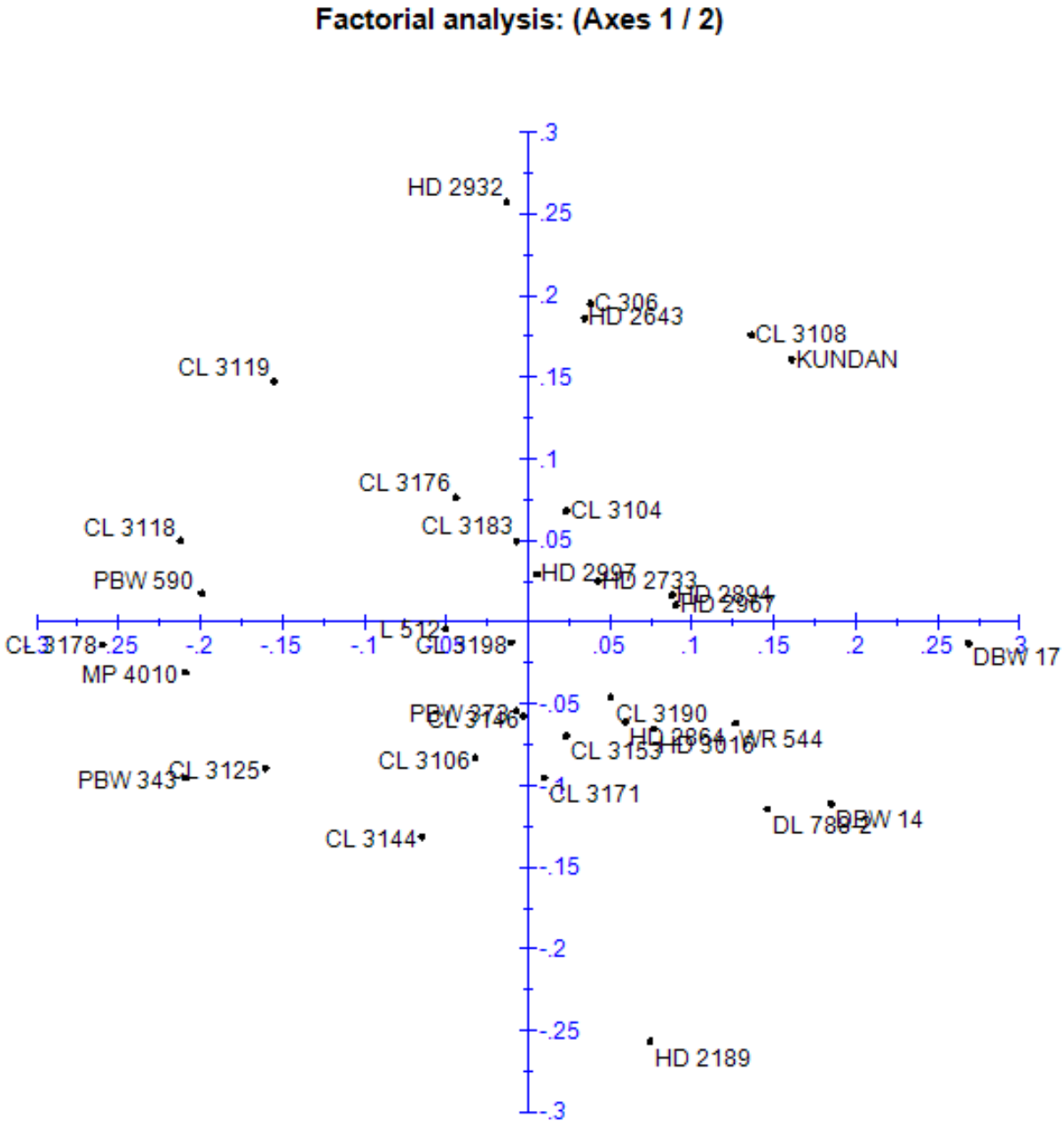
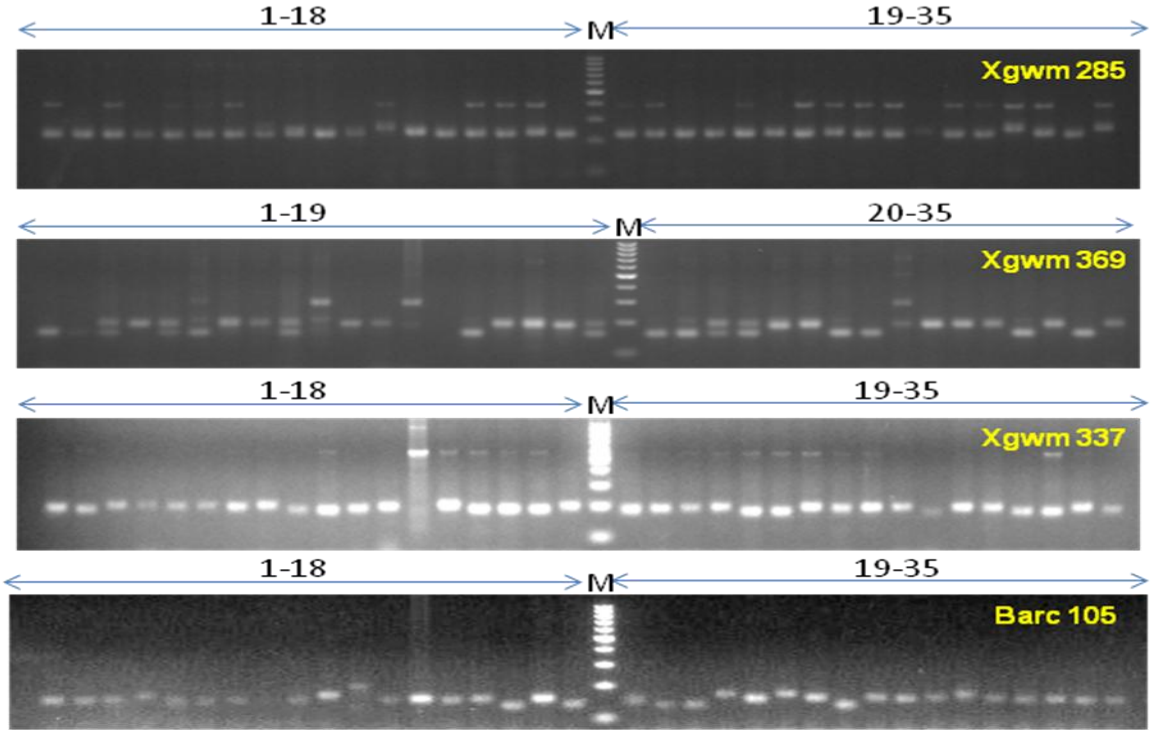


Fig.3

Fig 3: SSR allelic profile among the 35 wheat genotypes. Genotypes numbers are according to Table 1.



The results obtained from Principle coordinate analysis, as presented in Figure 2, classifies the 35 genotypes into four quadrates. A slight difference was observed between the results obtained through cluster diagram and principal co-ordinate analysis. The genotypes lying closer to each other in a co-ordinate are genetically more similar as compared to those located distantly.

The present study demonstrates the utility of SSR markers which can be profitably utilized in wheat for detecting polymorphism, tagging genes, (Prasad *et al.*, 2000; Roy *et al.*, 1999) and for estimation of genetic diversity. PIC values along with RP and MI of makers enabled finer distinction between wheat genotypes than routinely used PIC alone. Molecular characterization also facilitates selection of diverse genotypes and augment wheat improvement programme. Some genotypes possessing rare alleles for many different loci could be used for broadening the allelic diversity and the potential for cultivar improvement of wheat. Likewise, rare alleles can be identified using highly polymorphic single-locus SSR markers.

Acknowledgement

The first author is grateful to Indian Council of Agricultural Research (ICAR), New Delhi, for providing Junior Research Fellowship for Masters Degree programme.

References

Al Khanjari, S., Hammer, K., Buerkert, A. and Röder, M. S., 2007, Molecular diversity of Omani wheat revealed by

microsatellites: I. Tetraploid landraces. *Genetic Resources and Crop Evolution*. 54: 1291–1300.

Buchanan, F. C., Adams, L. J., Littlejohn, R. P., Maddox, J. F. and Crawford, A. M., 1994, Determination of evolutionary relationships among sheep breeds using microsatellites. *Genomics*. 22: 397–403.

Chesnokov, Y. V. and Artemyeva, A. M., 2015, Evaluation of the measure of polymorphism information of genetic diversity. *Agric Biol*. 50(5): 571–578.

Chao, S., Sharp, P. J., Worland, A. J., Warham, E. J. and Koebne, R. M. D., 1989, RFLP-based genetic maps of wheat homoeologous Group 7 chromosomes. *Theor. Appl. Genet.*78: 495–504.

Devos, K. M. and Gale, M. D., 1992, The use of random amplified polymorphic DNA markers in wheat. *Theor. Appl. Genet.* 84: 567–572.

https://www.statistica.com

Joshi, C. P. and Nguyen H.T., 1993, RAPD (Random Amplified Polymorphic DNA) analysis based intervarietal genetic relationships among hexaploid wheats. *Plant Science.* 93: 95–103.

Kara, K., Rached-Kanouni, M., Debbabi, O. S. and Naceur, M. B., 2017, Genetic diversity of bread wheat genotypes (*Triticum aestivum* L.) revealed by agromorphological characteristics and microsatellite SSR markers. *Inter. Jour. of Eng. Res. & Tech.* 6 (1): 178–182.

Kim, H. S. and Ward, R. W., 2000, Patterns of RFLP-based genetic diversity in germplasm pools of common wheat with different geographical or breeding program origins. *Euphytica.* 115: 197–208.

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

Perrier, X., Flori, A. and Bonnot, F., 2003, Data analysis methods. In: Hamon, P., Seguin, M., Perrier, X. and Glaszmann, J. C., (eds) Genetic diversity of cultivated tropical plants. *Science Publishers Montpellier, Enfield*, pp 43–76.

Plaschke, J., Ganal, M. W., and Roder, M. S., 1995, Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theor. Appl. Genet.*91: 1001–1007.

Powell, W., Machray, G. and Provan, J., 1996, Polymorphism revealed by simple sequence repeats. *Trends in Pl. Sci.* 1: 215–222.

Prasad, M., Varshney, R.K., Roy, J.K., Balayan, H.S. and Gupta, P.K., 2000, The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. *Theor. Appl. Genet.* 100: 584–592.

Prevost, A. and Wilkinson, M. J., 1999, A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 98: 107–112.

Rohlf, F. J., 2000, NTSYS-pc: numerical taxonomy and multivariate analysis system, vers. 2.1. *Exeter Publications, New York, USA.*

Roy, J. K., Prasad, M., Varshney, R. K. and Balyan, H. S., 1999, Identification of a microsatellite on chromosomes 6B and a STS on 7D of bread wheat showing an association with preharvest sprouting tolerance. *Theor. Appl. Genet.*99: 336–340.

Salem, K. F. M., El-Zanaty, A. M. and Esmail, R. M., 2008, Assessing Wheat (*Triticum aestivum* L.) Genetic Diversity Using Morphological Characters and Microsatallite Markers. *World Journal of Agricultural Sciences*

4 (5): 538–544.

Schloetterer, C., Amos, B. and Tautz, D., 1991, Conservation of polymorphic simple sequence loci in cetacean species. *Nature*. 354: 63–65.

Siedler, H., Messmer, M. M., Schachermayr, G. M., Winzeler, H., Winzeler, M. and Keller, B., 1994, Genetic diversity in European wheat and spelt breeding material based on RFLP data. *Theor. Appl. Genet.* 88: 994–1003.

Spanic, V., Buerstmayr, H. and Drezner, G., 2012, Assessment of genetic diversity of wheat genotypes using microsatellite markers. *Periodicum Biologorum* 114 (1): 37–42.

Tomar, S. M. S. and Anabalgan, S., 2004, Characterization of cytoplasmic male sterile lines in wheat (*Triticum aestivum* L.). *Indian J. Genet*. 64: 189–195.

How to cite this article:

Monu Kumar, Sharma, R.K., Singh, G.P. and Anima Mahato. 2018. Microsatellite based Genetic Divergence Study in Bread Wheat (*Triticum aestivum* L. em. Thell.). *Int.J.Curr.Microbiol.App.Sci.* 7(12): 2222-2232. doi: https://doi.org/10.20546/ijcmas.2018.712.253