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Research Article

Genetic diversity analysis of fertility restorer and CMS lines of wheat (*Triticum aestivum* L.) by using SSR markers

Letngam Touthang[1*] and Vinod[2]

[1]ICAR Research Complex for NEH Region, Arunachal Pradesh Centre, India
[2]Indian Agricultural Research Institute, New Delhi, India
***E-Mail:** letngam111@gmail.com

Abstract
Genetic diversity of 92 fertility restorer and four CMS lines were analyzed using 38 polymorphic SSR markers. A total of 102 alleles were detected which are ranged from 2 to 4 with an average of 2.68 alleles per locus. Polymorphism information content (PIC) of SSR markers ranged from 0.22 to 0.81 with an average of 0.53 per locus. The cluster analysis based on 38 polymorphic markers produced seven main clusters with 0.36 to 0.97 similarity coefficient. The highest similarity was observed between the restorer lines 919R and 920R and three restorer lines 907R, 923R, and 922R were found to be most distinct from the rest. Analysis of molecular variance revealed within and among populations variance of 97 and 3 per cent, respectively. Cluster analysis revealed the genetically distinct restorer and CMS lines that would serve as diverse parents to be exploited in future breeding programme to broaden the genetic base of wheat cultivars.

Key words
Genetic diversity, Restorer line, CMS line, SSR markers, *Triticum aestivum.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the world's largest cereal crop and has been described as the 'King of cereals'. To meet the world's growing demand for food, it is necessary to develop high-yielding varieties with good end-product (Curtis *et al.,* 2013). The narrow genetic base of germplasm is highly vulnerable to biotic and abiotic stresses (Khan 2015). Thus, the availability of genetic variability is a pre-requisite for genetic improvement of wheat (Drikvand 2013; Tyrka *et al.*,2021) and the knowledge on germplasm diversity has a significant impact on crop improvement (Hanaa *et al*., 2013*).* However, the genomic research in bread wheat remains a major challenge due to its huge genome size and complex hexaploid genome structure (Gupta *et al.,* 2008; Spanic *et al.,* 2012).

Although hybrid breeding may increase yields by 10 per cent (Longin *et al.,* 2013, Muhleisen *et al.,* 2014), it also requires technological advances that can regulate male sterility and fertility restoration system as a viable option to augment yield (Tucker *et al.,* 2017). Irrespective of the end product, wheat breeding through hybridization also requires the selection of diverse genotypes (Prasad *et al.,* 2000). Thus, various strategies have been developed for hybrid wheat production (Ni *et al.,* 2017), such as chemically induced male sterility (Longin *et al.,* 2013) and the application of the tight linkage between the dominant dwarfism gene Rht-D1c and Ms2 (Ni *et al.,* 2017). The Ms1 and Ms2 genes, which were recently sequenced, are useful for the large-scale, low-cost production of male-sterile female lines necessary for hybrid wheat seed production (Tucker *et al.,* 2017; Xia *et al.,* 2017). Among the various breeding systems available for producing hybrid cultivar, the involvement of cytoplasmic male sterility (CMS) is one of the most promising which is based on the

interaction between nuclear and mitochondrial genes, and the method has been widely used for breeding various crops (Bohra *et al.,* 2016).

Molecular markers established a principal way to improve the selection efficiency (Ciucă and Petcu 2009), provide information about genetic diversity, and helps in understanding the genetic control of quantitative characters (Zhang, 2009). Various marker systems have been used to study the genetic diversity of wheat and generate useful information for hybrid breeding programs (Tyrka *et al.,* 2021). Among these different molecular markers, Simple Sequence Repeat (SSR) marker has been widely used and ideal because of its multi-allelic nature, reproducibility, codominant inheritance, high abundance and extensive genome coverage (Gupta *et al*., 2000; Parker *et al.,* 2002) in many crops. Thus, the objective of this study is to analyze the genetic diversity within and across 92 fertility restorer lines and 4 CMS lines to enable the breeder to develop wheat cultivars with a broader genetic base.

MATERIAL AND METHODS

Plant material: The study was carried out at Indian Agricultural Research Institute (IARI), New Delhi. The experimental material comprised of 92 fertility restorer lines and four CMS lines of wheat (**Table1**).

Table1. List of genotypes used in the study

S.No.	Genotypes	S.No.	Genotypes	S.No.	Genotypes
1	4099R	33	910R	65	943R
2	4101R	34	912R	66	944R
3	2988R	35	913R	67	945R
4	2995R	36	914R	68	946R
5	T282R	37	915R	69	947R
6	T2003R	38	916R	70	948R
7	1752R	39	917R	71	949R
8	1771R	40	918R	72	950R
9	888R	41	919R	73	951R
10	889R	42	920R	74	952R
11	890R	43	921R	75	953R
12	891R	44	922R	76	954R
13	892R	45	923R	77	955R
14	893R	46	924R	78	956R
15	894R	47	925R	79	957R
16	895R	48	926R	80	958R
17	896R	49	927R	81	959R
18	897R	50	928R	82	960R
19	898R	51	929R	83	961R
20	899R	52	930R	84	962R
21	900R	53	931R	85	963R
22	901R	54	932R	86	964R
23	902R	55	933R	87	965R
24	903R	56	934R	88	966R
25	904R	57	935R	89	967R
26	905R	58	936R	90	968R
27	906R	59	937R	91	969R
28	907R	60	938R	92	970R
29	908R-1	61	939R	93	CMS2041
30	908R-2	62	940R	94	CMS2019
31	908R-3	63	941R	95	CMS365
32	909R	64	942R	96	WR1923

Genomic DNA isolation: Genomic DNA was isolated from leaf tissue of 20-25 day old seedlings by cetyl trimethyl ammonium bromide (CTAB) method as described by Saghai-Maroof *et al.* (1984). DNA samples were quantified by comparison with 100 ng/200 ng of Lambda uncut DNA on 0.8% agarose gel and diluted to a final working concentration of 25-30 ng/µl for PCR amplification.

Polymerase Chain Reaction (PCR) amplification was performed in a reaction volume of 10 µl which consist of 2 µl of Template DNA, 4.74 µl of sterile distilled water, 1.0 µl PCR buffer, 0.1 µl 2.5 dNTP's, 1µl (for both forward and reverse primers) and 0.16 µl of 3U/ µl taq DNA polymerase(Bangalore Genei Pvt Ltd, India). PCR products were then resolved on 3.5% metaphor gel at 130 V for 3.0 h. Gels were visualized under UV and photographed using a gel documentation system (Syngene G-Box, U.K.).

Amplified products from SSR markers analysis were scored qualitatively for the presence or absence of the corresponding band among the genotypes. Only the clear and unambiguous amplified bands were scored. The presence or absence of each band in all genotypes was scored manually by binary data matrix with '1' for the presence of the band and '0' for the absence of a band in excel. To measure the informativeness of the markers, the polymorphism information content (PIC) for each SSR marker was calculated according to the formula suggested by (Powell *et al.*,1996). PIC=1-(ΣP_i^2) where '*i*' is the total number of alleles detected for the SSR marker and 'P_i' is the frequency of the i^{th} plus allele

Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering was performed on Squared Euclidean distance matrix and similarity matrix using Jacquard's coefficient through the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. Data analyses was done using the software NTSYSpc version 2.1 and excel software (Rohlf FJ. NTSYS-PC 2004). Details of SSR primers were available in Grain Genes (http://wheat.pw.usda.gov/ggpages/SSR/WMC/). Principal Coordinate Analysis (PCA) was performed using the DARwin software version 6.0.12 (Perrier X, Jacquemoud-Collet JP (2006) DARwin software (http://darwin.cirad.fr/). Analysis of molecular variance (AMOVA) for genetic variance was estimated to study the genetic variance within and among inferred populations using GenAlex 6.5 software (Peakall ROD, Smouse PE 2006).

RESULTS AND DISCUSSION

A total of 103 SSR markers which include xgwm, wmc, xbarc, cfa and cfd dispersed throughout the 7 chromosomes of hexaploid wheat were used to assess the extent of genetic diversity within and among 92 fertility restorer lines and four CMS lines of wheat (**Table 2**). Out of 103 SSR markers tested, 38 were found to be polymorphic. A total of 102 alleles were detected across 96 genotypes by 38 polymorphic SSR markers. The number of alleles generated by each marker ranged from 2 (xbarc83, cfd59, xgwm122, xgwm630, xgwm340, WMC48, cfd18, xbarc171, xgwm219, cfd60 on chromosome 1A, 1B, 2A, 2B, 3B, 4B, 5D, 6A, 6B and 6D, respectively) to 4 (WMC415, WMC364 and xgwm296 on chromosome 5B,7B and 7D, respectively) with an average of 2.68 allele per locus (Table 3). A representative polymorphic banding pattern with one primer (xgwm344) is shown in (**Fig. 1**). The mean of alleles in each detected genome were 2.67, 2.59, and 2.89 for A, B, and D genome respectively. The PIC values varied widely among SSR loci tested which ranged from 0.22 (WMC397) detected on chromosome 6 of genome B to 0.81 (WMC419) detected on chromosome 4 of genome B, with an average of 0.53 per locus (**Table 3**).

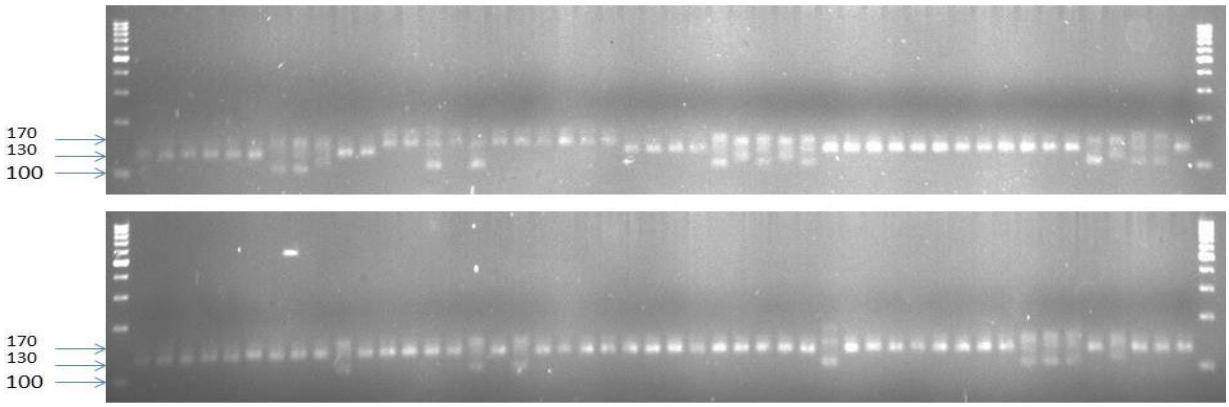

Fig. 1. Polymorphic banding patterns of 96 wheat genotypes (92 restorer lines and CMS lines) generated by xgwm 344 on 3.5 % metaphor gel with 100bp DNA ladder (Refer Table 1 for genotype details).

Table 2. List of polymorphic SSR markers

S.No	Primer	Chromo-some	Forward sequence	Reverse sequence	AT	Motif
1	xbarc83	1A	AAGCAAGGAACGAGCAAGAGCAGTAG	TGGATTTACGACGACGATGAAGATGA	58	(CAT)9
2	WMC24	1A	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	51	(GT)28
3	Xgwm413	1B	TGCTTGTCTAGATTGCTTGGG	GATCGTCTCGTCCTTGGCA	60	(GA)18
4	cfd59	1B	TCACCTGGAAAATGGTCACA	AAGAAGGCTAGGGTTCAGGC	60	(GC)6(GA)23
5	xgwm33	1D	GGAGTCACACTTGTTTGTGCA	CACTGCACACCTAACTACCTGC	60	(GA)19
6	xgwm122	2A	GGGTGGGAGAAAGGAGATG	AAACCATCCTCCATCCTGG	60	(CT)11(CA)31
7	xgwm630	2B	GTGCCTGTGCCATCGTC	CGAAAGTAACAGCGCAGTGA	60	(GT)16
8	xgwm148	2B	GTGAGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	60	(CA)22
9	xgwm210	2B	TGCATCAAGAATAGTGTGGAAG	TGAGAGGAAGGCTCACACCT	60	(GA)20
10	xgwm608	2D	ACATTGTGTGTGCGGCC	GATCCCTCTCCGCTAGAAGC	60	(GA)16
11	cfd51	2D	GGAGGCTTCTCTATGGGAGG	TGCATCTTATCCTGTGCAGC	60	(GA)45
12	WMC503	2D	GCAATAGTTCCCGCAAGAAAAG	ATCAACTACCTCCAGATCCCGT	61	(GT)11
13	xgwm2	3A	CTGCAAGCCTGTGATCAACT	CATTCTCAAATGATCGAACA	50	(CA)18
14	xgwm340	3B	GCAATCTTTTTTCTGACCACG	ACGAGGCAAGAACACACATG	60	(GA)26
15	xgwm108	3B	CGACAATGGGGTCTTAGCAT	TGCACACTTAAATTACATCCGC	60	(GT)35imp
16	xbarc71	3D	GCGCTTGTTCCTCACCTGCTCATA	CGTATATTCTCTCGTCTTCTTGTTGGTT	55	(TAGA)7(TA)2
17	WMC617	4A	CCACTAGGAAGAAGGGGAAACT	ATCTGGATTACTGGCCAACTGT	61	-
18	WMC419	4B	GTTTCGGATAAAACCGGAGTGC	ACTACTTGTGGGTTATCACCAGCC	61	(GA)16
19	WMC48	4B	GAGGGTTCTGAAATGTTTTGCC	ACGTGCTAGGGAGGTATCTTGC	61	(GA)9
20	xgwm149	4B	CATTGTTTTCTGCCTCTAGCC	CTAGCATCGAACCTGAACAAG	55	(GA)23imp
21	xgwm205	5A	CGACCCGGTTCACTTCAG	AGTCGCCGTTGTATAGTGCC	60	(CT)21
22	xgwm617	5A	GATCTTGGCGCTGAGAGAGA	CTCCGATGGATTACTCGCAC	60	(GA)43
23	WMC75	5B	GTCCGCCGCACACATCTTACTA	GTTTGATCCTGCGACTCCCTTG	61	(GT)13
24	WMC415	5B	AATTCGATACCTCTCACTCACG	TCAACTGCTACAACCTAGACCC	61	(CA)23
25	cfd18	5D	CATCCAACAGCACCAAGAGA	GCTACTACTATTTCATTGCGACCA	60	(GA)25
26	xbarc171	6A	GCGGGGTCATCTTAGTAACTCAAATA	ACTGTCAACGTTGGTTCACATTCA	50	(ATT)27
27	cfa2114	6A	ATTGGAAGGCCACGATACAC	CCCGTCGGGTTTTATCTAGC	60	(CA)32
28	xgwm459	6A	ATGGAGTGGTCACACTTTGAA	AGCTTCTCTGACCAACTTCTCG	55	(GA)>28
29	xgwm219	6B	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	60	(GA)35imp
30	WMC397	6B	AGTCGTGCACCTCCATTTTG	CATTGGACATCGGAGACCTG	61	
31	cfd13	6B	CCACTAACCAAGCTGCCATT	TTTTTGGCATTGATCTGCTG	60	(CT)20(TGTA)3
32	cfd60	6D	TGACCGGCATTCAGTATCAA	TGGTCACTTTGATGAGCAGG	60	(CA)25
33	xgwm276	7A	ATTTGCCTGAAGAAAATATT	AATTTCACTGCATACACAAG	55	CT)24
34	xgwm332	7A	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	60	(GA)36
35	xgwm344	7B	CAAGGAAATAGGCGGTAACT	ATTTGAGTCTGAAGTTTGCA	55	(GT)24
36	WMC364	7B	ATCACAATGCTGGCCCTAAAAC	CAGTGCCAAAATGTCGAAAGTC	61	(CA)18
37	xgwm121	7D	TCCTCTACAAACAAACACAC	CTCGCAACTAGAGGTGTATG	50	(CAAA)2(CA)28
38	xgwm296	7D	AATTCAACCTACCAATCTCTG	GCCTAATAAACTGAAAACGAG	55	(CT)28

Table 3. Allelic variation and PIC values for SSR loci identified among 96 genotypes

S.No.	Primers	Chromosomes	AT	Motif	Allele	PIC
1	xbarc83	1A	58	(CAT)9	2	0.52
2	WMC24	1A	51	(GT)28	3	0.41
3	Xgwm413	1B	60	(GA)18	3	0.77
4	cfd59	1B	60	(GC)6(GA)23	2	0.63
5	xgwm33	1D	60	(GA)19	3	0.35
6	xgwm608	1D	60	(GA)16	3	0.67
7	xgwm122	2A	60	(CT)11(CA)31	2	0.62
8	xgwm630	2B	60	(GT)16	2	0.67
9	xgwm148	2B	60	(CA)22	3	0.36
10	xgwm210	2B	60	(GA)20	2	0.55
11	cfd51	2D	60	(GA)45	3	0.52
12	WMC503	2D	61	(GT)11	3	0.71
13	xgwm2	3A	50	(CA)18	3	0.35
14	xgwm340	3B	60	(GA)26	2	0.55
15	xgwm108	3B	60	(GT)35	2	0.75
16	xbarc71	3D	55	(TAGA)7(TA)2	3	0.42
17	WMC617	4A	61	-	3	0.35
18	WMC48	4B	61	(GA)9	2	0.53
19	WMC419	4B	61	(GA)16	2	0.81
20	xgwm149	4B	55	(GA)23	2	0.51
21	xgwm205	5A	60	(CT)21	3	0.42
22	xgwm617	5A	60	(GA)43	3	0.61
23	WMC75	5B	61	(GT)13	3	0.37
24	WMC415	5B	61	(CA)23	4	0.47
25	cfd18	5D	60	(GA)25	2	0.63
26	xbarc171	6A	50	(ATT)27	2	0.54
27	cfa2114	6A	60	(CA)32	3	0.65
28	xgwm459	6A	55	(GA)28	2	0.58
29	xgwm219	6B	60	(GA)35	2	0.42
30	WMC397	6B	61	-	3	0.22
31	cfd13	6B	60	(CT)20(TGTA)3	3	0.49
32	cfd60	6D	60	(CA)25	2	0.67
33	xgwm276	7A	55	CT)24	3	0.63
34	xgwm332	7A	60	(GA)36	3	0.56
35	xgwm344	7B	55	(GT)24	3	0.53
36	WMC364	7B	61	(CA)18	4	0.23
37	xgwm121	7D	50	(CAAA)2(CA)28	3	0.46
38	xgwm296	7D	55	(CT)28	4	0.48
	Mean/locus				2.68	0.53

The cluster analysis performed by using Unweighted Pair Group Method with Arithmetic Averages (UPGMA) based on Jacquard's similarity co-efficient values between the various genotypes resolved 96 genotypes comprising of 92 fertility restorer lines and 4 CMS lines into seven main clusters with 0.42 similarity coefficient (**Fig.2**). Out of 92 restorer lines, 72 lines were grouped into Cluster-I which further sub-divided into two sub-clusters-IA and IB comprising of 31 and 41 genotypes, respectively. While cluster-II consists of single restorer line 932R and cluster-III consist of 9 genotypes. Cluster-IV consists of two CMS lines (CMS2041 and WR1923). Cluster-V consists of six genotypes. While cluster-VI was comprised of 3 genotypes with one restorer line (T2003R) and two CMS lines (CMS2019 and CMS365). Cluster-VII was

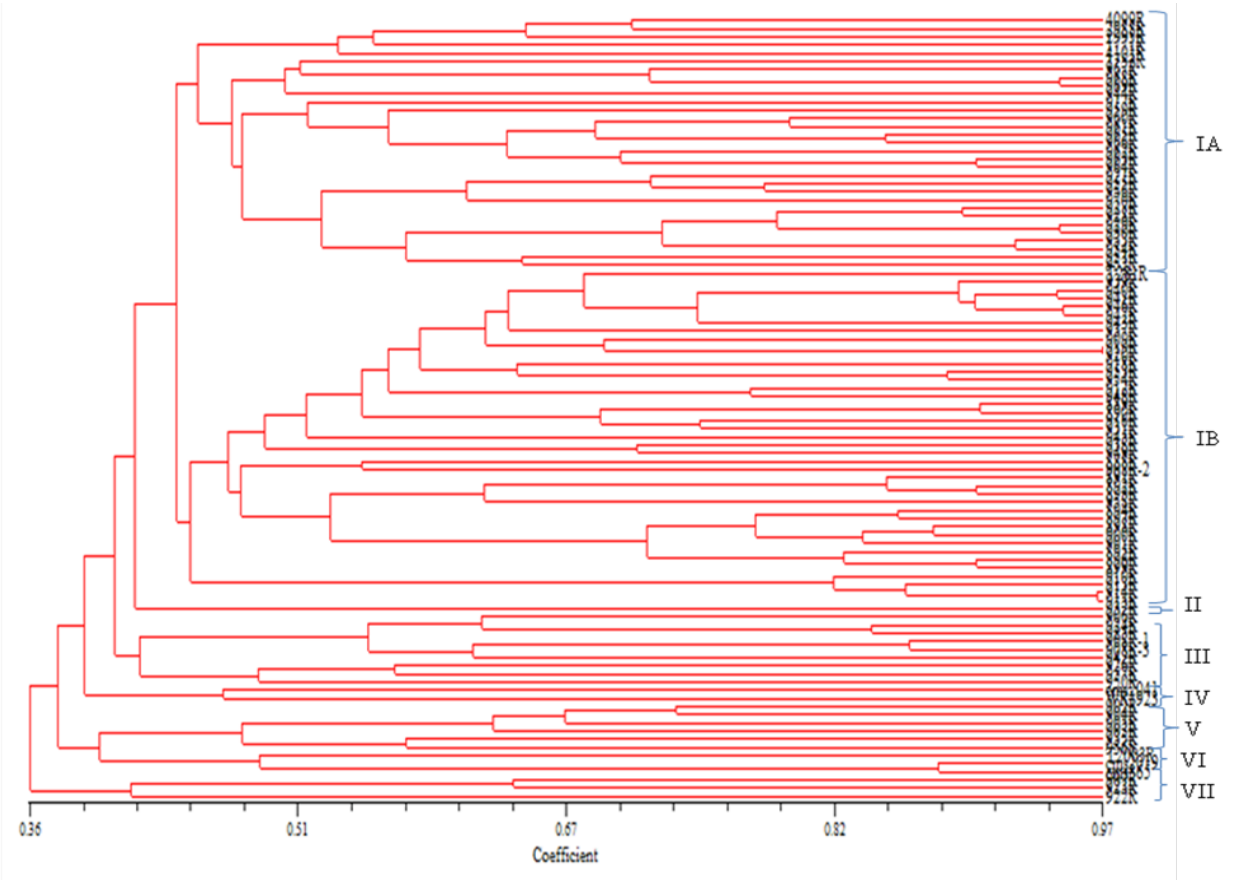

Fig. 2. Dendrogram resulting from UPGMA cluster analysis of 96 Wheat genotypes (92 restorer lines and 4 CMS lines) based on data derived from 38 polymorphic SSR markers.

comprised of three distinct restorer lines (907R, 923R, and 922R) (**Table 4**). Among the restorer lines involved in this study, 919R and 920R were found to be most similar with 0.97 similarity coefficient. The results obtained from cluster analysis were in accordance with Principal component analysis (**Fig. 3**) which scatters the genotypes into two principal coordinates. The similarity coefficient of 0.36 to 0.97 in the present study revealed an abundance of genetic variations among the genotypes under study. From the AMOVA, it was observed that 97% of variations were within the population and 3% among the population (**Fig.4**).

Among the classes of repetitive DNA sequences used for PCR amplification, SSRs remains the unsurpassed choice of markers (Jacob *et al*., 1991). SSR markers are valuable genetic markers because they detect high levels of allelic diversity, co-dominant, easy and economically assayed by PCR (Weber and May 1989), easily automated (Smith 1998), abundance and even genomic distribution (Weber and May 1989), high level of polymorphism (Saghai – Maroof *et al.,* 1994), high variability (Brown *et al.,* 1996), highly polymorphic even between closely related lines (Gupta *et al*., 1999).

The average number of alleles detected (2.68 per locus) was found to be higher than those reported by the earlier workers (Malik *et al.,* 2013; Islam *et al*., 2012, Sheoran *et al.,* 2015) in elite wheat genotypes. Whereas, the average number of alleles detected in the present study was found to be lower than the average of 3.2 (Salem *et al*., 2008), 3.2 (Schuster *et al.,* 2009), 5.7 (Spanic *et al*., 2012), 10 (Nasab *et al.,* 2013), 3.3 (Sarkar *et al*., 2014) and 5.89 (Abbasabad *et al.,* 2016) reported in the genetic diversity studies on bread wheat using microsatellite markers. This discrepancy might be related to the genotypes used and the selection of SSR primers with scorable alleles. Number of alleles per marker depends on the relative distance of the locus from the centromere (high genetic variation occurs in the non-centromeric regions compared to the centromeric regions of chromosomes.) and also it was related to the motif and repeat number of the allele frequencies (Huang *et al.,* 2009).

The mean PIC value of (0.53) observed in the present study was found to be higher than those reported in earlier studies (Tomar *et al*., 2009; Islam *et al*., 2012;

Table 4. Clustering of genotypes

Clusters	Genotypes	Total
IA	1752R, 967R, 968R, 969R, 944R,4099R, 2988R, 2995R, 1771R and 4101R,917R, 959R, 960R, 961R, 965R, 966R, 962R, 963R, 964R,921R, 955R, 956R, 970R,927R, 928R, 929R, 930R, 933R, 934R, 951R, 952R	31
IB	T281R, 939R, 940R, 941R, 946R, 947R, 943R, 937R, 909R, 919R, 920R, 918R, 953R, 954R, 912R, 948R, 889R, 890R, 916R, 931R, 945R, 938R, 949R,988R and 908R,891R, 892R, 893R, 958R, 894R, 897R, 898R, 900R, 901R, 895R, 896R, 899R,910R, 913R, 914R, 915R	41
II	932R	1
III	906R, 924R, 925R, 908R-1, 908R-3, 942R 926R, 957R, 950R	9
IV	CMS2041, WR1923	2
V	902R, 904R, 905R, 903R, 935R, 936R,	6
VI	T2003R, CMS2019, CMS365	3
VII	907R, 923R, 922R	3

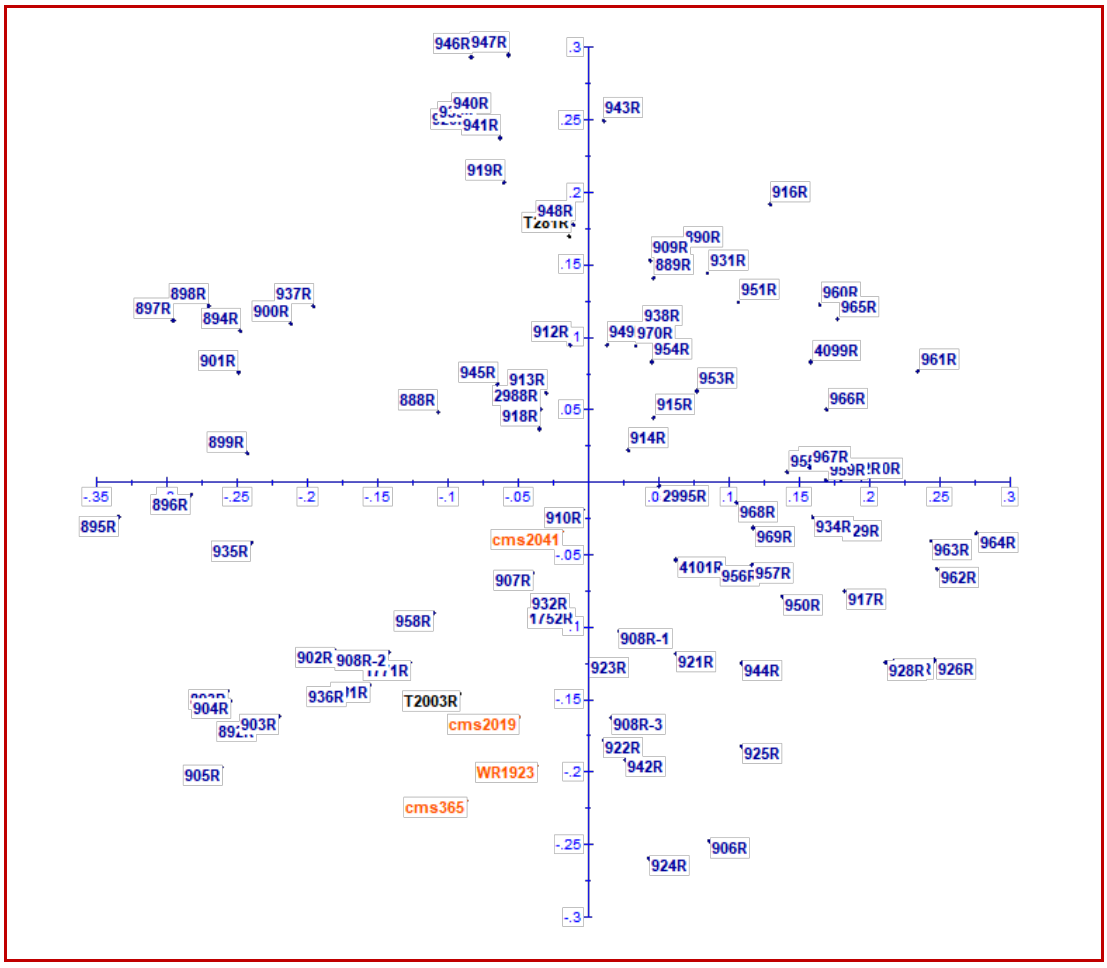

Fig.3. Illustration of genetic relationships among the 96 wheat genotypes based on Principal Coordinate Analysis of SSR data

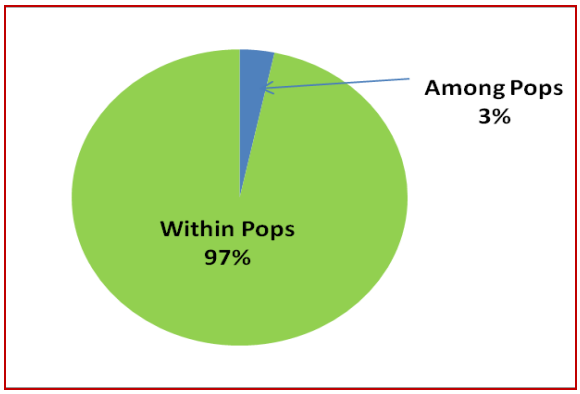

Fig. 4. Analysis of molecular variance depicting within and among populations genetic variations in 96 genotypes (92 restorer lines and 4 CMS lines) of bread wheat (*Triticum aestivum* L.)

Malik *et al.,* 2013; Sarkar *et al.,* 2014). But it was found to be lower than 0.76 reported in wild diploid wheat (Wang *et al.,* 2017), 0.6 in Iranian landraces (Abbasabad *et al.,* 2016), 0.55 among Egyptian wheat varieties (Salem *et al*., 2008), and 0.58 among the Indian wheat varieties (Arora *et al.,* 2014), respectively. PIC measures the informativeness of the DNA markers over a set of genotypes during gene mapping, molecular breeding, and germplasm evaluation (Varshney *et al.,* 2007; Wang *et al.*, 2007). Microsatellite markers exhibit high PIC value because of their codominance and multi-allelism (Ferreira and Grattapagl,ia 1998). The polymorphism in SSR could be due to a change in the SSR region itself caused by the expansion or contraction of SSR or interruption (Li *et al*., 2007). Markers with PIC values 0.5 or higher are considered as highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a specific locus (DeWoody *et al.*, 1995; Akkaya and Buyukunal Bal 2004). Out of 38 polymorphic SSR markers used in the present study, 22 markers have a PIC value ≥0.5. This indicates that the SSR markers used in the present study were highly informative and robust indicating their utility to study genetic diversity and molecular mapping in bread wheat.

From cluster analysis, the similarity coefficient among the 96 genotypes which are ranged from 0.36 to 0.97 revealed an abundance of genetic variations among the genotypes under study. In accordance with this, similarity coefficient of 0.05 to 0.75 (Ertugrul Filiz, 2012) in 56 CIMMYT inbred wheat lines from Russia, 0.42 to 0.74 (Sarkar *et al.,* 2014) in 35 Indian bread wheat cultivars, 0.03- 0.89 (Kumar *et al*., 2016), 0.03 to 0.97 (Wang *et al.,* 2017) in wild diploid wheat and 0.17 to 0.88 by (Drikvand, 2013) have been reported earlier respectively. Except for the restorer line (T2003R), all the restorer lines in this study were grouped in separate cluster distinct from the four CMS lines. Crossing of T2003R with two CMS lines (CMS2019 and CMS365) may not yield diverse hybrids as they are genetically similar. As revealed by clustering analysis, the three restorer lines 907R, 923R, and 922R which were distinct from the rest would be an ideal choice for the development of hybrids with a broader genetic base.

The analysis of molecular variance (AMOVA) revealed higher intra population variation (97%) indicating ample scope to exploit diversity with good combining ability. A similar variation of 87.59 and 12.41 per cent among and within the population in Indian bread wheat (*Triticum aestivum* L.) cultivars released during the last 100 years have been reported by (Mir *et al.,* 2011) and intensive breeding practices have led to the reduction of genetic variability among the varieties in post green revolution (Mir *et al*., 2011). Genetic studies of wheat in recent years have proven SSR markers to be an efficient molecular marker for diversity studies (Gupta *et al*., 2002; Song *et al.,* 2005; Periyannan *et al.,* 2013; Mir *et al*., 2011; Wang *et al*., 2017). The information related to the genetic diversity among adapted lines helps breeders in the selection of suitable parents for hybridization that maximize heterosis and combines useful genes in an adapted genetic background (Bohn *et al*., 1999). Heterosis enhances vigour in hybrid and is dependent on the genetic diversity of both the parents (Liu *et al.,* 2004). Therefore, the genetic diversity pattern observed in this set of restorer and male sterile wheat genotypes would be of immense help in the selection of parents for a hybrid breeding programme.

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