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

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**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 prerequisite 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.

**MATERIALS 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). 

| 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    |

Table1. List of genotypes used in the study

https://doi.org/10.37992/2021.1202.063
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-Maroo 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-(ΣP²)/P where 'i' is the total number of alleles detected for the SSR marker and 'P' is the frequency of the 'i'th 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).](https://doi.org/10.37992/2021.1202.063)
## Table 2. List of polymorphic SSR markers

| S.No | Primer | Chromosome | Forward sequence | Reverse sequence | AT Motif |
|------|--------|------------|-----------------|------------------|---------|
| 1    | xbarc83 | 1A         | AAGCAAGGAAAGCAGGACGAGCAGTAG | TGGATTTCGAGCGAGCATGAGATG | (CAT)9  |
| 2    | WMC24  | 1A         | GTGAGCATTATTTGATATTACTG | TACCCGTAGCTGTAATAGTGT | (GT)28  |
| 3    | xgwm413 | 1B         | TGCTTGTCTTAGTTGCGGGG | GATGTCGTGCTCGTGGCAGCA | (GA)18  |
| 4    | cfd59  | 1B         | TCACTGTGAGCTGATGCACTCACA | AAGAGGCGCTAGCCTGAGGCCG | (GC)6(GA)23 |
| 5    | xgwm33  | 1D       | GGAGTCACAATGGTGTGGCC | CACCTGCAACACTATCGGCACT | (GA)19  |
| 6    | xgwm122 | 2A       | GGTGGGAGGAAAGGAGATG | AAACCATCCTCCATCTGG | (CT)11(CA)31 |
| 7    | xgwm630 | 2B       | GTGCTTGTCTACATGGGAGG | GATCCTTCTGCTGACATGAGC | (GA)20  |
| 8    | xgwm148 | 2B       | GTGAGGCGCAAGGAAAGCACAA | AGAGCAGGGAAGCAGACACATG | (CA)22  |
| 9    | xgwm210 | 2B       | TGTATAGAGCGAGTGGGAGG | TGAGAGAACGCTACACATG | (GA)16  |
| 10   | xgwm608 | 2D       | ACATTGTGTGGCGCC | GATCCTTGTGTTTACAGCT | (GA)45  |
| 11   | WMC503  | 2D       | GGAGGCTTCTCTACAGGGAGG | GATCCTTATCTGCTGACAG | (GA)16  |
| 12   | xgwm2  | 3A       | CTGCAAGCCTGTGATCAACT | CATTCTCATAAGCTGACA | (GA)26  |
| 13   | xgwm340 | 3B       | GCAATTTTCTGTGAACGACAC | AGAGGAGGAAAGACACTACATG | (GA)26  |
| 14   | xgwm108 | 3B       | GCAACTGAGGCTGCTATCATG | TGCACCAATTTACACTCGC | (GA)26  |
| 15   | xgwm149 | 3B       | GATGCTTCTGCTCAGGGG | GATCCTATGCTGCTGACAG | (GA)26  |
| 16   | xgwm2117 | 3D      | GGGGCTTGTCTCTACAGGG | GATCCTGCTGCTGACAG | (GA)26  |
| 17   | WMC617  | 4A       | CGACGCATGCTGCTGCTATA | GATGCTGCTGCTGACAG | (GA)26  |
| 18   | WMC419  | 4A       | ATCTGAATGCTGCTGCTGCTA | GATGCTGCTGCTGACAG | (GA)26  |
| 19   | WMC48   | 4B       | GAGGGTTCTGAAATGTTTGCAG | AGGTAGCAGGGAGGTACATG | (GA)26  |
| 20   | xgwm149 | 4B       | CATTATGTGCTGCTGCTGCTA | TGCACCAATTTACACTCGC | (GA)26  |
| 21   | xgwm205 | 5A       | CTGACGCGCTGCTGCTGCTA | GATGCTGCTGCTGACAG | (GA)26  |
| 22   | xgwm1217 | 5A      | CATTATGTGCTGCTGCTGCTA | TGCACCAATTTACACTCGC | (GA)26  |
| 23   | WMC375  | 5B       | GTCCGCCTGCACACATCTCTACTA | GTGTTGCTGCTGCTGACAG | (GA)26  |
| 24   | WMC415  | 5B       | AATGAGCGATACCTCTCAGTGAC | GAAGGAGAGGAAAGGAGACACATG | (GA)26  |
| 25   | cfd18   | 5D       | CATTGAACGACCAAGGCGAAGAAGAAGAAG | AGGTAGCAGGGAGGTACATG | (GA)26  |
| 26   | xgwm276 | 6A       | ATTTGGCGCTGCTAGCATGTTTCTTGGT | CATTCTAGCTGCTGACTGACAG | (GA)26  |
| 27   | xgwm1217 | 6A      | CATTATGTGCTGCTGCTGCTA | TGCACCAATTTACACTCGC | (GA)26  |
| 28   | cfd459  | 6A       | ATGAGATGTGCTGCTGCTGCTA | GATGCTGCTGCTGACAG | (GA)26  |
| 29   | xgwm2117 | 6D      | GATGAGCGACACTGACCTGCTC | GGGGCTGCTGCTGACAG | (GA)26  |
| 30   | WMC397  | 7A       | ATGAGATGTGCTGCTGCTGCTA | GATGCTGCTGCTGACAG | (GA)26  |
| 31   | xgwm1217 | 7B      | CATTATGTGCTGCTGCTGCTA | TGCACCAATTTACACTCGC | (GA)26  |
| 32   | xgwm2117 | 7B      | CATTATGTGCTGCTGCTGCTA | TGCACCAATTTACACTCGC | (GA)26  |
| 33   | xgwm296 | 7D       | TGGAGGCGCTGCTGCTGCTGCTA | GATGCTGCTGCTGACAG | (GA)26  |
| 34   | xgwm296 | 7D       | TGGAGGCGCTGCTGCTGCTGCTA | GATGCTGCTGCTGACAG | (GA)26  |

https://doi.org/10.37992/2021.1202.063
Table 3. Allelic variation and PIC values for SSR loci identified among 96 genotypes

| S.No. | Primers   | Chromosomes | AT | Motif       | Allele Value | 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)(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)(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)(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)(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)(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 comprised of three distinct restorer lines (907R, 923R, 907R and 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; Malik et al., 2013; Sarkar et al., 2014). But it was found to
### 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, 965R, 970R, 967R, 927R, 928R, 929R, 930R, 933R, 934R, 951R, 952R | 31    |
|          | 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, 956R, 894R, 897R, 898R, 900R, 901R, 895R, 896R, 899R, 910R, 913R, 914R, 915R | 41    |
| IB       | 932R                                                                 | 1     |
| II       | 906R, 924R, 925R, 908R-1, 908R-3, 942R 926R, 957R, 950R               | 9     |
| III      | CMS2041, WR1923                                                        | 2     |
| IV       | 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
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 Grattapaglia, 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.

ACKNOWLEDGEMENT
The authors thankfully acknowledge the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi for providing all the facilities for conducting this experiment.

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