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Study of genetic diversity in wheat genotypes using microsatelite markers

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Abstract

Molecular analysis for advance lines of hexaploid wheat (*Triticum aestvium* L.) cultivars was investigated by applying microsatellite markers. The plant materials consisted of 30 advance lines of *Triticum aestivum* obtained from Aldan, HD 29, WH 542 and WH 711. The advanced lines and their parents were classified in 7 clusters on the basis of Tocher's method with cluster-I containing the maximum of 11 genotypes. Clustering pattern revealed that variable number of advance lines were included into different clusters and also indicated the presence of considerable amount of genetic diversity in this material. Maximum intra-cluster distance (3.95) was observed in cluster-III indicating greater genetic divergence between the genotypes belonging to this cluster. Maximum inter-cluster distance (7.87) was recorded between cluster-V and III followed by cluster-III and VII (7.54) indicating wide genetic diversity and it may be used in wheat hybridization programme for improving grain yield. Seven primers out of 35 SSR primers showed polymorphism in banding pattern. A total of 15 alleles were detected. The number of alleles per locus ranged from 1-3 with an average of 2.14 alleles per locus. The overall size of PCR products amplified ranged from 100-300 bp.

Key words: Genetic divergence, SSR, wheat.

Introduction

Wheat is the second most important cereal in India after rice, grown in an area of 29.8 million hectares during 2011-12 (Anonymous, 2012). Due to its adaptation to wide agro-climatic conditions, it is most widely cultivated cereal crop and is staple food for a large proportion of world population, in more than 40 countries (Gupta *et al.*, 2009). The population of India is increasing tremendously and the country would require about 109 million tonnes of wheat by the year 2025 (DWR, 2007). The option for increasing wheat production by expanding area under cultivation has already been exploited to almost its maximum. So, continuous efforts are required to develop high yielding and disease resistant wheat genotypes. Genetic variability is the basic requirement for making progress in crop breeding. Inclusion of genetically divergent parents in any breeding programme is essential to create new genetic stocks. Genetic diversity is the most important tool in the hands of plant breeder in choosing the right type of parents for hybridization programme. Knowledge of genetic diversity among these has a considerable impact on the improvement of crop plants. It can be obtained from pedigree analysis, morphological traits or using molecular markers (Mohammadi and Prasanna, 2003). The divergence can be studied by technique using D^2 statistics developed by Mahalanobis (1936). It is based on multivariate analysis and grouped into various cluster as given by Tocher's method. Molecular markers offer the best estimate of genetic diversity since they are independent of the perplexing effects of environmental factors. In recent years, several molecular assays have been applied to assess genetic diversity among wheat cultivars. Several molecular markers like random amplified polymorphic DNAs (RAPD), inter simple sequence repeats (ISSR) and simple sequence repeats (SSRs) are presently available to assess the variability and diversity at molecular level (Palombi and Damiano, 2002). Hence, keeping in view the importance of these aspects the present study has been planned. This is considered as the most effective method for qualifying the degree of genetic diversity among the genotypes included in the study. The present investigation aimed to estimate the magnitude of genetic divergence present in the 30 wheat advance lines and to identify the diverse genotypes for future breeding programme.

Materials and Methods

The present investigation was conducted to evaluate

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30 advance wheat lines and their parents (Aldan, HD 29, WH 542, WH 711) in the RBD with 3 replications at the Experimental Farm of the Department of Genetics and Plant Breeding, CCS HAU, Hisar, during *Rabi* 2011 for genetic diversity study and to characterize these lines using molecular markers. A total of 35 SSR markers widely distributed on different wheat chromosomes were used in this study. SSR amplification profiles were scored visually, based on presence (taken as 1) or absence (taken as 0) of bands. The 0/1 matrix was used to calculate similarity index, genetic distance using 'simqual' sub-program of software NTSYS-PC (Rohlf, 1990). Dendrogram was constructed by using distance matrix by the unweighted pair-group method with arithmetic average (UPGMA) sub-programme of NTSYS-PC. Principal component analysis (PCA) was done using the 'CPCA' sub-programme of NTSYS-PC software.

Results and Discussion

Genetic diversity existing with in and between groups of germplasm is important and particularly useful in proper choice of parents for realising higher heterosis and obtaining useful recombinants. D^2 statistic is a useful tool for estimating the genetic divergence in plant breeding experiments. The advanced lines and their parents involved in the present investigation were classified in 7 clusters (Table 1). Clustering pattern revealed that variable number of advance lines were included into different clusters and also indicated the presence of considerable amount of genetic diversity in this material. In general intra-cluster distances were relatively lower than inter-cluster distances showing that genotypes included within a cluster were genetically closer to each other than the genotypes included in different clusters. Intra-cluster distance was maximum for cluster-VII (3.95) and minimum for cluster-III (0.00). Cluster-V and III showed maximum inter-cluster distance (7.87) suggesting wide diversity between these clusters (Table 2). Therefore, genotypes belonging to these clusters may be used in hybridization programme for improvement of wheat. The least inter-cluster distance was observed between cluster-II and VI (4.22) followed by cluster-I and IV (4.26) indicating close relationship between the genotypes of these clusters and hence, may not be emphasized upon to be used in hybridization programme (Table 2). The higher inter-cluster distances exhibited the presence of more diversity among the genotypes involving these clusters. Dwivedi and Pawar (2004) and Chapla *et al*. (2011) assessed the genetic diversity among lines of bread wheat for yield and its component traits using Mahalanobis D^2 analysis and grouped them into different number of clusters. The most divergent clusters could be selected as parents in hybridization programme for obtaining a wide range of variation. The characters grain weight per spike, number of grains per spike, length of main spike, days to 50% flowering and peduncle length contributed maximum towards total genetic divergence. Talebi *et al.* (2012) studied sixty land races of bread wheat to assess mutual genetic distances by use of morphometric Markers.

Table 1: Classification of advance lines of bread wheat and their parents in different clusters

Cluster No.	Lines in cluster	Total no. of lines in cluster
I	L_1, L_{19}, L_{21}, L_{31}, L_{33}, L_{34}, L_8, L_{18}, L_9 ,L_{20}, L_{10}	11
II	L_3, L_{23}, L_4, L_{12}, L_{29}	05
III	L_5	01
IV	L_7, L_{16}	02
V	L_{15}, L_{30}	02
VI	L_{13}, L_{17}, L_{14}, L_{22}, L_{11}, L_{24}, L_{28}, L_{26}, L_{32}, L_2	10
VII	L_{25}, L_{27}, L_6	03

Table 2:Average intra (diagonal) and inter (above diagonal) cluster D^2 values in advance lines of bread wheat

Cluster No.	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
I	3.38	4.73	6.09	4.26	5.56	4.72	4.96
II		3.11	5.74	4.55	4.86	4.22	4.96
III			0.00	5.75	7.87	7.16	7.54
IV				2.52	4.84	5.62	6.31
V					3.40	4.56	5.62
VI						3.75	4.49
VII							3.94

The diversity was also supported by the appreciable amount of variation among the cluster means for different characters (Table 3). Number of tillers per plant had highest mean value in cluster-III (15.00) and lowest mean value in cluster-V (9.00). For flag leaf length cluster-V (29.19 cm) exhibited the highest mean value and cluster-III (16.79 cm) showed the lowest mean value. Protein content exhibited its highest and lowest mean values in cluster-VII (13.27%) and cluster-IV (7.50%), respectively. Highest yield per plant was recorded in cluster-III (15.87) and lowest in cluster-V (10.87) for mean values. Comparative evaluation of cluster means suggested that for improving specific characters the lines should be taken from cluster having high mean value for that character. Cluster-VII had the highest mean value for three characters. Therefore, cluster-VII was considered most desirable for selecting genotypes. Almost similar result were reported by Nimbalkar *et al*. (2002) and Dwivedi and Pawar (2004). Molecular diversity is a pre-requisite for making any crop improvement programme successful.

Table 3: Cluster mean values of different characters in advance lines of bread wheat

Cluster No.	Days to 50% flowering	Days to maturity	Flag leaf length (cm)	Plant height (cm)	Tillers per plant	100–grain weight (g)	Gluten content (%)	Hecto - litre weight (g)	Sedimen-tation value (ml)	Protein content (%)	Yield per plant (g)
I	93.97	129.69	22.03	86.07	9.78	3.41	26.81	73.39	49.51	9.04	12.64
II	84.73	130.66	26.34	106.48	12.13	4.32	25.12	77.37	50.26	9.86	14.90
III	103.33	132.33	16.79	129.90	15.00	3.38	22.05	79.26	46.00	8.09	15.87
IV	91.50	129.67	24.99	85.96	11.00	3.49	13.12	75.58	41.33	7.50	13.95
V	83.33	125.33	29.19	114.15	9.00	4.55	17.20	78.50	45.00	9.04	10.87
VI	84.70	125.53	24.74	104.53	10.20	4.56	28.95	75.58	53.63	10.16	12.43
VII	90.67	127.55	25.66	92.12	10.55	3.84	30.58	79.94	55.22	13.27	11.39

Microsatellite markers are useful and becoming popular for different applications in wheat breeding due to their high level of polymorphism and easy handling (Roder *et al*., 1995; Bryan *et al*., 1997; Roy *et al*., 1999; Lelley *et al*., 2000) and are used to evaluate genetic diversity of hexaploid wheat (Khanjari *et al*., 2007). In the present study SSR markers almost succeeded in discrimination wheat cultivars. A total of 35 SSR markers were used in study, out of these three markers did not show amplification, seven markers showed polymorphism in banding pattern (Fig. 1) and rest of them showed monomorphic band pattern. Database of SSRs was generated using above primers and a total of 15 alleles were detected. The number of alleles per locus ranged from 1-3 with an average of 2.14 alleles per locus. The overall size of PCR products amplified ranged from 100-300 bp (Table 4). Marmar *et al*. (2013) studied 12 wheat genotype using 184 SSRs and observed that of the SSRs used, 145 (79%) amplified DNA while 39 (21%) did not. A total of 104 of the amplified SSRs were polymorphic while 41 were monomorphic. Cluster tree analysis led to the grouping of 30 lines and four parents in seven different clusters in such a way that genotypes within each cluster showed high similarity than those between clusters. Cluster pattern revealed that, cluster-VII was the largest consisting of 17 lines followed by cluster-VI (6 lines), cluster V (5 lines), cluster-IV (3 lines) and cluster-I, II, III (1 line each) (Table 5)

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Table 4: DNA amplification profile of advance lines and parental genotypes of bread wheat

Number of markers used	35
Number of markers that did not show amplification	3
Number of polymorphic markers	7
Number of alleles detected using polymorphic markers	15
Range of alleles	1-3
Average number of alleles	2.14
Size of PCR products	100-300 bp

Table 5: Classification of advance lines and parental genotypes of bread wheat in different clusters using SSR marker data base

Cluster No.	No. of lines	Name of lines
I	1	L_{32}
II	1	L_{31}
III	1	L_{12}
IV	3	L_8, L_9, L_{21}
V	5	L_{19}, L_{20}, L_{34}, L_{11}, L_{33}
VI	6	L_2, L_3, L_{13}, L_{15}, L_{16}, L_{17}
VII	17	L_1,L_5,L_7,L_{27},L_{29},L_{30},L_4,L_{10},L_{26},L_{28},L_{14},L_{18},L_{22},L_{23},L_{24},L_{25},L_6

L_1-L_{30} represent the advance lines and L_{31}, L_{32}, L_{33} and L_{34} represent the parents Aldan, HD 29, WH 542 and WH 711 respectively.

First two clusters were formed at similarity index of 0.44 (Fig. 2). At similarity index 0.55 two parents, Aldan (L_{31}) and HD 29 (L_{32}) diverged into two different groups because during this study, some markers for karnal bunt resistance were used and these two parents were resistant to karnal bunt. Parent WH 542 and WH 711 were grouped into same cluster because these were susceptible to karnal bunt. Two-dimensional PCA analysis (Fig. 3) showed that the lines were scattered in two major groups which were further divided into different groups and parent Aldan was quite diverse from other parents. Though most of the lines overlapped with each other in 2D cluster diagram yet the diversity among the parents ($L_{31,}$ $L_{32,}$ L_{33} and L_{34}) was quite evident. Similar studies conducted by Hao *et al*. (2011) using SSR markers based principle coordinate analysis of 250 wheat genotypes indicated landraces and modern varieties were two relatively independent genetic sub-groups. Studies conducted by Mir *et al*. (2012) on 263 Indian bread wheat cultivars using 90 SSR markers grouped them into 4 subgroups.

In both the cases D^2 analysis and molecular analysis, these lines were included in cluster VI and cluster VII, so these clusters may be used for selecting parents in future breeding programmes due to presence of diversity among genotypes which were grouped in these clusters. Based on *per se* performance and genetic divergence some advance lines figured important such as L_3, L_6 and L_{25}. These genotypes can be used in future breeding programmes for improvement because the mean value of each of the 11 characters were different, in different clusters which represent the variability in respect of these characters.

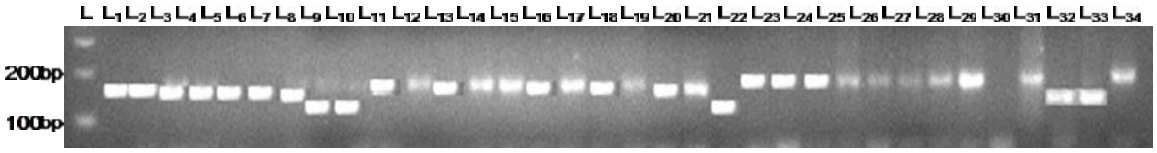

Fig. 1: Polymorphism in different advance lines (L_1-L_{30}) and parental genotypes (L_{31}=Aldan, L_{32}= HD 29, L_{33}= WH 542, L_{34} = WH 711) of bread wheat by using primer *Xwmc337-1D*

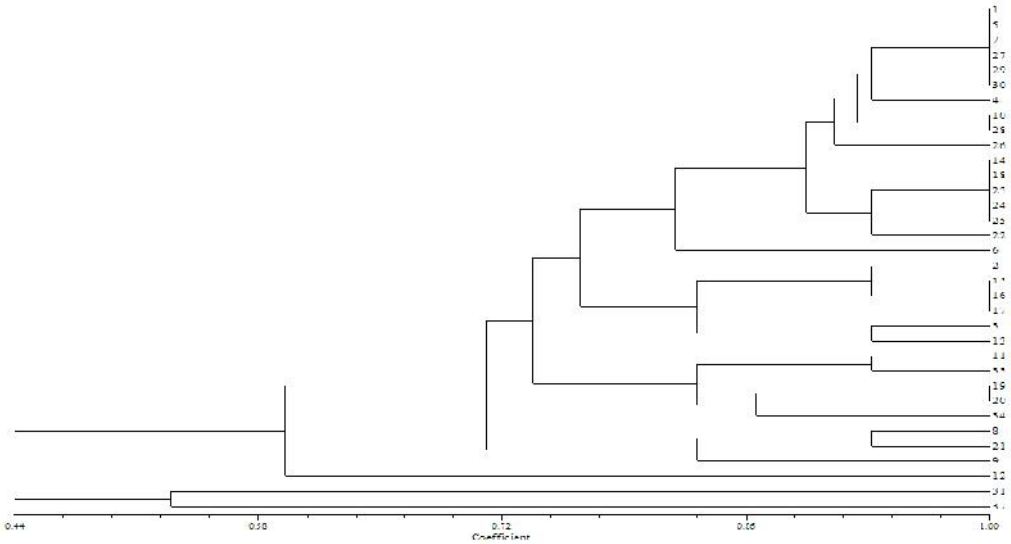

Fig. 2: Dendrogram showing genetic relationships among 30 advance lines and parental genotypes (Aldan, HD 29, WH 542, WH 711 based on similarity index data obtained using SSR markers.

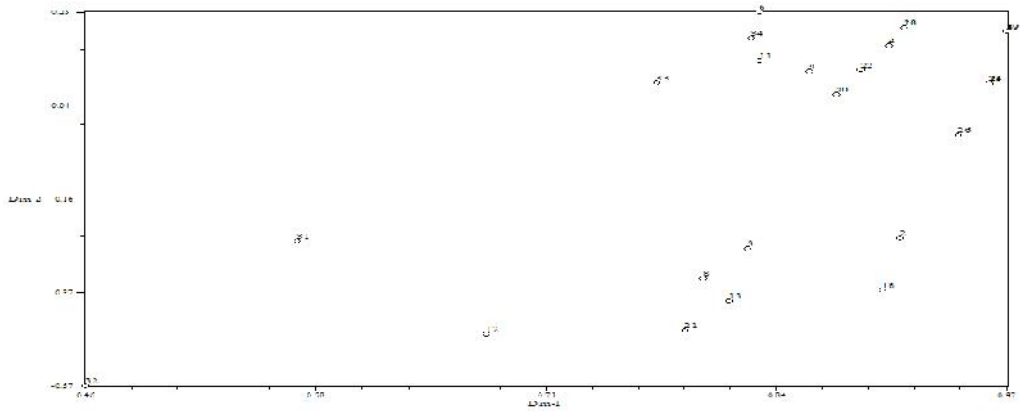

Fig. 3: 2-D cluster diagram based on PCA analysis.

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