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Research Article

ASSESSMENT OF PHYLOGENTIC RELATIONSHIP OF WHEAT CULTIVARS USING MICROSATELLITE MARKERS

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Abstract- In present study Genetic diversity of the ten wheat varieties were analyzed at the DNA level using 14 wheat specific microsatellite markers. 12 markers out of 14 had detected 58 alleles with an average of 4.8 alleles per primer. The resolving power (Rp) of each primer ranged from 1.6 for the SSRW3 to 7.2 for SSRW2 primer. The gene diversity based on microsatellite markers was ranged from 0.24 to 0.97 with an average value of 0.66. The genotypes V-23 and VWTH-08-07 showed the maximum genetic similarity 0.66 out of all 10 wheat varieties. The genotypes HUW-312 shows maximum genetic diversity with HD-2133 and HUW-825. The range of genetic diversity values broadly indicates the degree of heterogeneity or homogeneity in different genotypes of the plant species. As a result of this study, genetically diverse parents can be identified and also indicates that microsatellite markers could characterize and discriminate varieties from each other.

Keywords- Wheat, Genetic Diversity, Resolving Power, Alleles, Microsatellite Marker.

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Introduction

Now-a-days all world scientific researchers facing the biggest problem to develop better major cereal crops like wheat, rice etc. due to limited resources and loss of genetic diversity. The loss of genetic diversity retards the better improvement with respect to quality and quantity in wheat. The stagnant yield of wheat in different countries is due to limited genetic diversity in the germplasm used in breeding programmes. Through breeding and selection, great numbers of alleles have been deleted because of which more difficulties have emerged for researchers for wheat improvement in modern agriculture systems [1,2]. A closely related wheat species to hexaploid wheat, are the valuable source of genes for disease, drought, salinity resistant and high grain quality [3,4]. Therefore, wheat and related species are the excellent source of genes for crop improvement [5].Consequently, the gene pool of hexaploid wheat is much narrowed as compared to its progenitors which are confirmed by molecular genetic analysis of bread wheat and its evolutionary parents [6]. However, today`s bread wheat is greatly affected by various biotic and abiotic stresses. A number of vital traits have been transferred into most cultivated wheat species from wild relatives [7]. Hexaploid wheat have been found to have greater genetic diversity and desirable traits with more adaptability to abiotic stresses [8-10]. The development and improvement of plant varieties can be made successfully by using molecular tools. For selecting and evolving better varieties there must be high genetic diversity among the varieties. The conventional breeding methods are not highly reliable as they are affected by environmental conditions [11]. However, molecular markers methods are more stable and they can provide detailed characterization of genetic resources. DNA based molecular markers are the direct source to measure the genetic diversity and go beyond the indirect diversity measures based on agronomic traits or geographic origin. The most widely used molecular markers include restriction fragment length polymorphism [12], amplified fragment length polymorphism [13], and simple sequence repeats [14]. Microsatellite markers are short repeating nucleotide DNA sequences [15]. They have high reproducibility, are multi-allelic, specific to genes, co-dominant and highly polymorphic [16]. SSR markers have been used to characterize genetic diversity in wild relatives [17], determination of quantitative trait loci [18] and in a seed bank collection of improved wheat germplasm [19]. This study was conducted to estimate the genetic divergence among ten wheat genotypes as well as cultivated varieties with the help of 14SSR markers.

MaterialsandMethods

Plant Material

This investigation was carried out at the experimental field station of the Department of Biotechnology, SVPUA&T, Meerut, during the rabi season year 2011-12 and 2012-13. Twenty wheat varieties were collected *viz.* HD-2133, HUW-825, R-54, K-9533, V-110, V-70, HUW-312, V-23, VWTH-08-07, HUW-54 from Genetics and Plant Breeding Department, SVPUA&T, Meerut for this investigation.

DNA Isolation

Total genomic DNA was extracted from leaf tissue of each variety. Young leaves from eight weeks old plants were cut as tissue samples for DNA extraction. DNA was isolated as described by [20], with slight modification. In this method, Cetyl Trimethyl Ammonium Bromide (CTAB) was used as a detergent to lyse the cell wall for release of DNA.

Polymerase chain reaction

For SSR analysis 14 wheat specific SSR primers [Table-1]] were used. PCR reactions were carried out according to standard procedure in a 20µl volume. For SSR-PCR, the reaction profile consisted of an initial denaturation step of 4 min at 95ºC, followed by a 1 min denaturation step at 95ºC, annealing for 1 min 40 sec. at 40ºC and extension for 2 min at 72ºC. A total of 35 cycles were performed followed by 10 min extra extension step after the last cycle in order to allow

completion of incomplete reactions. Amplified PCR products were separated on 1.5 % (w/v) agarose gel in standard 1 X TBE (pH 8).

Table-1 *The polymorphic SSR primers corresponding to genetic diversity and resolving power*

S.N.	Primer code	Primer Sequence	Total no. of band	polymorphic band	Expected gene diversity	Resolving power
1	SSRW1-F	ATGCAGCAATCCC CTCCC	3	3	0.97	3.5
	SSRW1-R	CCAGTCCCGAGCTTGTAAAA				
2	SSRW2-F	GCTGAAGCCATGCATAATAGT	11	11	0.77	7.2
	SSRW2-R	CCAGGGGTTTTCCATCTCC				
3	SSRW3-F	GCCGGCTCGCCATGTTCTCCA	3	3	0.91	1.6
	SSRW3-R	CTCATCATCTCGACTCGCCCT				
4	SSRW4-F	TCAGGGAAGCAGCGTGTAGA	6	6	0.96	1.8
	SSRW4-R	CGGCCAGTCAGCGCGGGTAAT				
5	SSRW5-F	TGCAGCCACAAAATCCATC	4	4	0.51	5.8
	SSRW5-R	TGCTGCAATACAACATCCAT				
6	SSRW6-F	CCTTTCTCATCCTTGCCATCC	6	6	0.79	3.8
	SSRW6-R	GTTGTTGTGGAAATGGTT				
7	SSRW7-F	GTCAACAACAACGCCTGG	3	3	0.88	2.0
	SSRW7-R	TAAGCGGAAGAAAGATG				
8	SSRW8-F	GGATAGTCAGACAATTCTTGT	2	2	0.42	2.2
	SSRW8-R	GTGAATTGTGTCTTGTATGCTT				
9	SSRW9-F	GCCCCCTTGCACAATC	4	4	0.24	4.0
	SSRW9-R	CGCAGCTACAGGAGGCC				
10	SSRW10-F	TACTGGTTCACATTGGTGCG	3	3	0.44	4.4
	SSRW10-R	TCGCCATCACTCGTTCAAG				
11	SSRW11-F	AGCCAGCAAGTCACCAAAAC	8	8	0.49	6.6
	SSRW11-R	AGTGCTGGAAAGAGTAGTGA				
12	SSRW12-F	ACCTCATCCACATGTTCTACG	3	3	0.56	3.4
	SSRW12-R	GCATGTATAGGACGCC				

Analysis of SSR Marker Data:

The ability of primers to differentiate between closely related varieties the resolving power (Rp) for each primer was calculated following [21] method as Rp = Ib (band information). Resolving Power is calculated as 1-[2 x (0.5-p)], p being the proportion of the 20 varieties containing the bands and Gene Diversity is calculated as 1-Σ pi2 [22]. The bands were calculated as present (1) or absent (0) for each DNA sample with the all 14 SSR primer. Only reproducible amplifications products were included in the data analysis. Similarity matrix using the similarity coefficient of [23] was constructed from the whole data. Pair wise distances between DNA accessions were calculated and analysed using the Un-weighted Pair Group Method Arithmetic average (UPGMA) [24]. Clusters were analysed using the computer program NTSYS-PC, version 2.11s [25]. In some cases no band were observed, possibly due to insufficient homology between the primer and DNA template. There is also the possibility that this situation might have occurred by failure of the PCR caused by some other region as well.

Result and Discussion

Molecular profiling using SSR

The new plant varieties having desired traits that help to increase crop production and thus improve the level of human nutrition can be developed using collected and preserved germplasms [26]. To assess the genetic diversity of 10 genotypes of wheat, 14 SSR wheat specific primers were used to amplify the genomic DNA of wheat. Out of 14 SSR wheat specific primers used, 12 (85.7%) primers resulted in polymorphic, scorable and reproducible results whereas the two primers did not amplify any of the wheat genotypes [Fig-1]. The total of 58 alleles had been amplified by 12 primers across the 10 genotypes. All of them were polymorphic and showed 100% polymorphism. The high polymorphic rate suggests that the SSR markers are the viable approach for the examination of genetic diversity of wheat genotypes. The amplified alleles ranged from minimum 2 to maximum 11 with primer SSRW8 and primer SSRW2 respectively. SSR gene specific primers were scored for presence (1) and absence (0) across all wheat genotypes for each primer. All of them were found to be polymorphic hence considered for genetic diversity analysis. Gene diversity was calculated for each primer, which varied from 0.24 to 0.97 values with a mean diversity of 0.66 [Table-1]. On comparison with other plant species, the gene diversity of wheat was comparatively in wide range, which can be explained as the genotypes of wheat are collected from various regions and diverse enough. This reflects that the broad area of sampling is supposed to be the major reason of wide range of gene diversity. Present results were supported by [27] that the use of few SSR markers can distinguish between closely related wheat genotypes. An average of 4.8 alleles per locus was detected for twenty wheat varieties. Similar, observation was reported by [28]. The present result is also in consistent with previous work done by [29] who observed that the genetic diversity at each SSR locus was significantly correlated with the number of alleles detected. Resolving power of the 12 SSR primers ranged from 1.6 to 7.2 with an average 3.85 [Table-1]. Based on resolving power and the ability of primers to differentiate all accessions, the primers SSRW11 and SSRW2 were found most informative with resolving power 6.6 and 7.2. Thus, the significant value of resolving power indicated the ability of primers to resolve the different closely related genotypes of wheat. Thus, the significant value of resolving power indicated the ability of primers to resolve the different closely related genotypes of wheat. Present results were in agreement with earlier reports of [30-32].

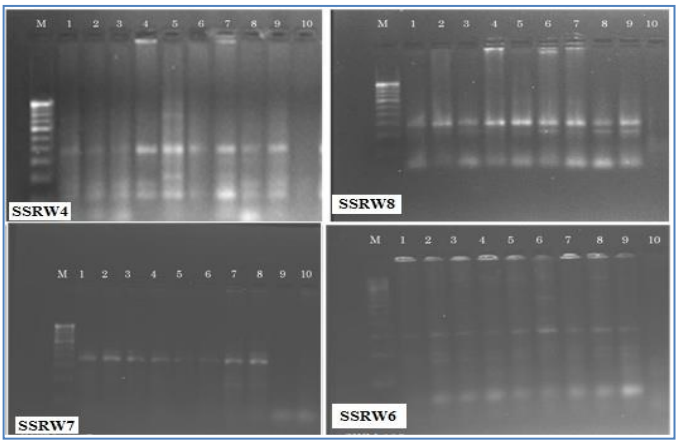

Fig-1 Amplification Profiling of 10 Genotypes of wheat using SSR primers

Genetic similarity matrix and cluster analysis

The pair wise comparison of the accessions based on shared and unique amplification products shown by SSR markers to generate a similarity matrix with NTSYS-PC (version 2.11s). Dendrogram was developed by using UPGMA method which was based on distance matrix. Similarity value

for all the 10 accessions ranged from 0.1 to 0.66. The lowest similarity displayed by HUW-312 and HD-2133. Of 10 samples analysed, the genotypes VWTH-08 and V-23 displayed the greatest genetic similarity [Table-2] & [Fig-2]. The resultant dendrogram grouped the 10 genotypes into two main clusters [Fig-3]. The cluster 1 grouped 3 genotypes *viz.* HD-2133, HUW-825 and HUW-54. The maximum genetic similarity within group was exhibited by HD-2133 and HUW-825. The main cluster 2 was subdivided into two sub clusters. The sub cluster 1 grouped 2 genotypes *viz.* R-54 and K-9533. The sub cluster 2 grouped 5 genotypes *viz.* V-110, V-70, HUW-312, V-23 and VWTH-08. The genotypes V-23 and VWTH-08-07 showed the maximum genetic similarity 0.66. The genotypes HUW-312 shows maximum genetic diversity with HD-2133 and HUW-825. This showed that climatic conditions may affect the plant genome as the plant is adapted and these changes are inherited through genome generation to generation. The wide range of genetic diversity values indicates the degree of heterogeneity or homogeneity among the diverse germplasm species [33]. The present study suggests that microsatellite markers are appropriate to study of genetic difference among the varieties. Moreover, SSR could able to amplify the different loci of all the 10 genotypes.

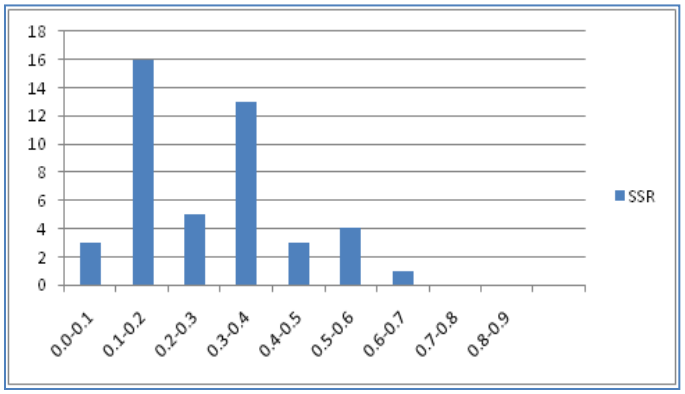

Fig-2 Histogram of 10 genotype of wheat showing the genetic diversity

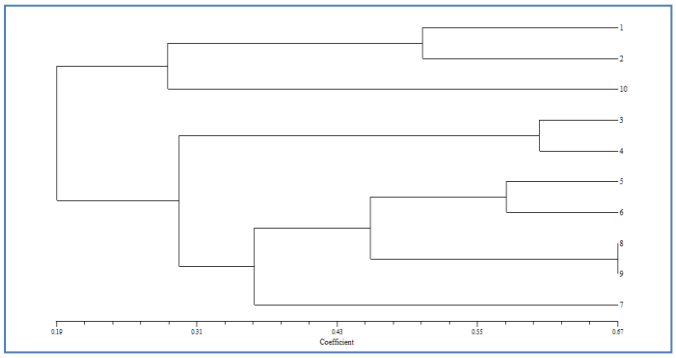

Fig-3 Grouping of 10 wheat genotypes (HD-2133, HUW-825, R-54, K-9533, V-110, V-70, HUW-213, V-23, VWTH-08-07, HUW-37)

Table-2 *Similarity matrix coefficient of 10 wheat genotypes*

	HD-2133	HUW-825	R-54	K-9533	V-110	V-70	HUW-213	V-23	VWTH-08-07	HUW-37
HD-2133	1									
HUW-825	0.5	1								
R-54	0.17	0.4	1							
K-9533	0.17	0.17	0.6	1						
V-110	0.17	0.17	0.33	0.33	1					
V-70	0.11	0.11	0.22	0.38	0.58	1				
HUW-213	0.1	0.1	0.2	0.33	0.33	0.5	1			
V-23	0.11	0.11	0.22	0.38	0.57	0.55	0.36	1		
VWTH-08	0.22	0.1	0.2	0.33	0.33	0.36	0.23	0.66	1	
HUW-37	0.4	0.16	0.14	0.33	0.14	0.22	0.2	0.22	0.5	1

Conflict of Interest: None declared

References

[1] Allard R.W. (1996) *Euphytica.,* 92, 1-11.
[2] Hoisington D., Khairallah M., Reeves T., Ribaut J.M., *et al.,* (1999) *Proc. Natl. Acad. Sci. U. S. A.,* 96, 5937-5943.
[3] Schachtman D.P., Schroeder J.I., Lucas W.J., Anderson J.A., Gaber R.F. (1992) *Science,* 258, 1654-1658
[4] Tariq-Khan M., Ul-Haque M.I., Kayani M.Z., Rattu A.U.R. and Mujeeb-Kazi A. (2012) *Pak. J. Bot.,* 44(3), 1147-1152.
[5] Talbert L.E., Smith L.Y. and Blake N.K. (1998) *Genome,* 41, 402-407.
[6] Nazem V. and Arzani A. (2013) *J. Appl. Environ. Biol. Sci.,* 3(7), 20-28.
[7] Khan S. and Khan J. (2010) *Pak. J. Agri. Sci.,* 47, 355-359.
[8] Imtiaz M., Ogbonnaya F.C., Oman J. and Van G.M. (2008) *Genet.,* 178, 1725-1736.
[9] Dreisigacker S., Kishii M., Lage J., Warburton M. (2008) *Aust. J. Agric. Res.,* 59, 413-420.
[10] Tang Y.L., Yang W.Y., Tian J.C., Li J. and Chen F. (2008) *Agric. Sci. China,* 7, 101-105.
[11] Marmar A., Dweikat I., Baenziger S., El-Hussein A.A., Elbasyoni I.S. (2013) *Middle-East J. of Sci. Res.,* 14(9), 1135-1142.
[12] Kim H.S. and Ward R.W. (2000) *Euphytica.,* 115, 197–208.
[13] Barrett B.A., Kidwell K.K. (1998) *Crop Sci.,* 38, 1261–1271.
[14] Masmoudi K., Rebai A., Ellouz R. (2006) *Cereal. Res. Commun.,* 34, 871–878.
[15] Varshney R.K., Graner A., and Sorrell A.E. (2005) *Trends Biotechnol.,* 23, 48-55.
[16] Röder M.S., Korzun V., Wendehake K., Plaschke J., Tixier M.H., Leroy P., Ganal M.W. (1998) *Genet.*, 149, 2007-2023.
[17] Hammer K., Filatenko A.A. and Korzun V. (2000) *Genet. Resour. Crop Evol.,* 47, 497-505.
[18] Ganal M.W. and Röder M.S. (2007) Microsatellite and SNP markers in wheat breeding. In Genomic assisted crop improvement: genomics applications in crops, R.K. Varshney and R. Tuberosa (eds.), 2: 1-24, The Netherlands: Springer
[19] Huang Q., Borner A., Roder S. and Ganal W. (2002) *Theor. Appl. Genet.,* 105, 699-707.
[20] Doyle J.J. and Doyle J.L. (1990) Focus, 12, 13-15.
[21] Prevost A. and Wilkinson M.J. (1999) *Theoretical and Applied Genetics,* 98, 107-112
[22] Anderson J.A., Churchill G.A., Autrique J.E., Tanksley S.D., Sorrells M.E. (1993) *Genome.,* 36, 181- 186
[23] Jaccard P. (1910) *Bulletin Society Vaudoise Sciences Naturelles,* 44, 223-270.
[24] Sneath P.H.A. and Sokal R.R. (1973) Numerical Taxonomy. WH Freeman, San Francisco, CA
[25] Rohlf F.J. (2000) Numerical Taxonomy and Multivariate Analysis System, version 2.11 manual. New York, Applied Biostatistics, Inc.
[26] Singh S.P., Gutierrez J.A., Molina A., Urrea C. and Gepts P. (1991) *Crop Science,* 31, 23-29.
[27] Plaschke J., Ganaland M.W. and Röder M.S. (1995) *Theor. Appl. Genet.,* 91, 1001-1007.
[28] Salem K.F.M., El-Zanaty A.M. and Esmail R.M. (2008) *World J. Agril. Sci.,* 4(5), 538-544.
[29] Herrera T.G., Duque D.P., Almeida I.P., Nunez G.T., Pieters A.J., Martinez C.P., Tohme J.M. (2008) *Electron. J. Biotechnol.,* 11(5), 215-226.
[30] Huang X.Q., Hsam S.L.K., Zeller F.J., Wenzel G. and Mohler V. (2000) *Theor. Appl. Genet.*, 101, 407-414
[31] Fahima T., Röder M., Grama A. and Nevo E. (1998) *Theor. Appl. Genet.,* 96, 187-195.
[32] Ablett G., Hilland H. and Henry R. (2006) *Molecular Breeding,* 17, 281-289.
[33] Goswami M. and Ranade S. A. (1999) *J. Genet,* 78,141-147.