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Research Article

DIVERSITY ANALYSIS IN THE SELECTED F_2 POPULATIONS OF WHEAT USING MOLECULAR MARKERS

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Abstract- The present investigations were conducted to study the gene effects and molecular analysis for spike morphology in *Triticum aestivum* L.em Thell during two successive winter growing seasons (2008-2009 and 2009-2010). The phenotypic coefficients of variability (PCV) values were higher than GCV in the F_2 population of five crosses for all the fifteen characters coupled with considerable differences between them indicating that the expression of these traits are more influenced or sensitive to the environmental conditions. Out of five crosses one diverse F_2 populations were selected for molecular analysis. Fifty SSR primers were used for the present investigation, out of which only 42 primers showed amplification. Using a total of 42 SSR primers, 118 bands with the number of band per primer varying from 1 to 6 with an average 2.81 bands per primer. Average polymorphism across all the 100 F_2 individuals was found to be 73.8%. Thus, a wide range of genetic variability was revealed from the similarity indices and dendrogram.

Keywords- Genetic variability, SSR Marker, Spike morphology, F_2 population

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Introduction

There is an urgent need of systematic attempts for wheat improvements for widening the genetic variability in wheat germplasm to accomplishing the world demand which is expected to rise by 60% for wheat by 2050 [6]. The search for individual differences plays a significant role in improving yield and quality of the crop through searching of new and diverse genetic resources. Both genetic and environmental factors contribute towards variation among individuals. Phenotypic and genotypic variances have been used to assess the magnitude of variance in wheat breeding material. Khaled *et al*., (2015) evaluated variability parameters for different metric traits which showed highly significant differences.

Breeders attempt to produce the best combination of genes for the use and growing conditions they envisage. Genetic crossing and selection of offspring based on their performance is, however, a painstaking process; keeping pace with the rapid changes in environment and demand is a major challenge. What is needed is a way to help plant breeding be more efficient. The key tools for assessing and applying genetic diversity in breeding are molecular markers. Molecular or DNA markers, which in essence deliver a rapid and informative summary of the genotype, a genetic "fingerprint" of the germplasm or crossing parents and offspring, are increasing used by the breeding community in Marker Assisted Selection. Development and deployment of more rapid and robust, less expensive, and denser molecular markers is a continuing area of development and attracts great interest both for basic research, pre-competitive but applied work, and in practical breeding and germplasm management. There will be a continuing need both to sample the genetic diversity of the genome and have the ability to recognize specific allelic forms. The most commonly used methods currently are microsatellites, Microsatellite markers represent a new class of genetic markers in plants. Such markers reveal a high level of polymorphism even in species with a narrow genetic base, such as hexaploid wheat (*Triticum aesivum* L.) [11, 15, 16]. SSR markers are considered to be the markers of choice for assessment of genetic diversity among cultivars and their wild relatives [17]. SSR markers play an important role in cultivar identification and in the study of genetic diversity [5]. Thus, the present study planned to attempt for the effective management of germplasm and the identification of genetic diversity among F_2 population for spike morphology and grain yield component traits which can be used selecting genotypes for further breeding programs which is fully acknowledged to UGC, New Delhi for their financial support.

Material and Methods

Growing Genetic Material and Layout of Experiment

To achieve the objectives of present investigation, the seven genetically diverse homozygous genotypes of wheat (*T. aestivum* L. em. Thell) *viz.* PBW502, HS27, HS67, HJP81, HG2, Rm-Ts17 and $HD2009_M$ were selected to generate the genetic material. The genetic material comprised of F_2 population of each of the following five crosses: HJP81 x Rm-Ts17(C-I); HS27 x PBW502 (C-II); HJP81 x PBW502 (C-III); HS67 x PBW502 (C-IV); HG2 x $HD2009_M$ (C-V). The five different crosses (C-I, C-II, C-III, C-IV, C-V) developed as above were grown separately in randomized complete block design with three replications during two successive winter growing seasons i.e., 2008-2009 and 2009-2010 under two dates of sowing i.e. November 20, 2008 and December 3, 2009, respectively. The inter row and intra plant distance was 30 cm and 10 cm respectively. Agronomic management followed technical recommendations for wheat crops.

Diversity Analysis

Estimation of genotypic coefficient of variability: The PCV and GCV were calculated as follows:

$$PCV = \frac{\sqrt{V\bar{F}_2}}{\bar{F}2} \quad GCV = \frac{\sqrt{V\bar{F}_2 - VE}}{\bar{F}2}$$

SSR Marker Methodology

Two wheat crosses are grown in the crop seasons 2009-10 studied for genetic polymorphism using chromosome specific SSR markers. Parentage and pedigree of the varieties is presented in [Table-1]. Genomic DNA was isolated using CTAB method [13] from a small amount of fresh leaf tissue (5.0 g) from 50 plants of each of cross. Agarose gel electrophoresis (0.8%) was used to check quality and quantity of genomic DNA. The DNA concentrations were estimated by visual assessment of band intensity in comparison with Lambda (λ) DNA of known concentration. Parental genotypes were screened using a total of fifty SSR markers for the molecular characterization, out of these 41 were polymorphic and used for diversity analysis. The PCR amplification conditions were optimized. The PCR reaction was conducted in a reaction volume of 20 µl containing 2ul of 1X PCR buffer, 100 µM dNTPs, 0.5 µl of each primer, 1.5 unit Taq DNA polymerase and 50 ng template DNA. The thermocycling program consisting of an initial denaturation at 95°C for 4 minutes followed by 40 cycles of 95°C for 1 minute, 1 minute and 20 second at annealing temperature (55-63 °C), 1 minute at 72°C and a final cycle of 72C for 10 minutes was used. Amplified products were resolved on 4% polyacrylamide gels using Amersham Biosciences system as described by Chen *et al.* (1997). Gels were pre-run until an adequate temperature (50-600C) was reached. DNA bands were visualized by using silver staining protocol (SILVER SEQUENCETM DNA Sequencing System, Promega Inc., Madison, WI, USA) after completion of electrophoresis. The frequency of polymorphism between different varieties of wheat for each type of marker was calculated based on presence (taken as 1) or absence (taken as 0) of bands. The 0/1 matrix was used to calculate similarity genetic distance using 'simqual' sub-program of software NTSYSPC (numerical taxonomy and multivariate analysis system programme) [9, 12]. The resultant distance matrix was employed to construct dendrograms by the un-weighted pair-group method with arithmetic average (UPGMA) subprogram of NTSYS-PC.

Table-1 *Estimation of genetic variability for spike morphology and grain yield component traits in F_2 population of five wheat crosses under two growing seasons 2008-09 (WS_1) and 2009-10 (WS_2)*

Traits*		PH	NTP	NSPS	NSSP	FSSP	SL	GYP	HKW	NKS	KWS	PL	G:SB	SC	SB	DH
Mean																
Cross-I HJP81 x Rm-Ts 17	WS_1	103.00	9.48	23.66	2.71	20.88	13.34	16.44	5.81	59.55	3.41	41.91	72.48	1.79	4.75	92.67
	WS_2	104.86	10.44	23.42	2.55	20.90	13.16	16.88	3.12	58.88	2.4	43.36	71.99	1.81	4.74	93.11
Cross-II HS27 x PBW502	WS_1	94.34	12.83	22.45	1.21	22.24	13.01	15.25	3.22	50.54	2.93	35.66	78.52	1.8	3.69	94.28
	WS_2	86.97	10.40	21.34	1.21	20.13	12.74	10.92	3.19	53.04	1.76	35.74	73.15	1.69	2.41	93.42
Cross -III HJP81 x PBW502	WS_1	104.86	10.44	23.22	2.1	21.12	13.4	11.64	3.09	55.38	2.13	49.48	70.95	1.75	3.03	93.11
	WS_2	101.35	9.48	22.64	1.77	20.88	13.5	11.79	4.7	47.26	1.38	38.00	60.92	1.69	2.38	91.63
Cross-IV HS67 x PBW 502	WS_1	102.00	13.72	28.81	2.52	21.93	13.9	16.43	4.5	55.67	2.52	43.51	78.26	1.73	3.22	92.26
	WS_2	105.17	12.22	22.18	2.06	20.12	12.51	13.28	5.67	53.52	1.57	40.13	69.5	1.78	2.3	90.18
Cross -V HG2 x $HD2009_M$	WS_1	115.35	9.93	23.28	2.58	20.69	15.61	10.56	4.15	52.01	3.34	45.92	71.88	1.51	4.67	93.74
	WS_2	106.76	9.74	24.77	1.88	22.48	14.33	14.85	4.08	56.23	2.17	43.57	72.99	1.71	2.9	93.76
Phenotypic Coefficient of Variation																
Cross-I HJP81 x Rm-Ts 17	WS_1	13.773	30.666	9.434	48.604	12.221	13.078	24.719	27.422	20.672	26.254	29.20	20.91	15.87	18.12	4.973
	WS_2	13.76	32.74	12.07	45.05	14.56	17.56	28.54	21.87	16.95	25.48	34.39	19.76	16.89	17.77	5.26
Cross -II HS27 x PBW502	WS_1	17.349	35.421	10.784	66.59	12.276	13.65	28.963	39.838	17.635	35	17.78	13.53	11.53	27.65	9.622
	WS_2	13.398	32.74	10.12	8.43	66.59	10	27.08	35.94	18.73	26.01	17.98	14.98	11.55	20.79	3.71
Cross -III HJP81 x PBW502	WS_1	13.784	32.741	12.434	54.705	14.537	15.914	33.691	28.673	21.135	21.287	28.48	17.54	12.05	15.51	5.267
	WS_2	13.26	30.66	8.52	66.72	10.96	12.4	25.98	34.76	20.72	30.29	13.85	17.58	12	22.18	3.66
Cross -IV HS67 x PBW 502	WS_1	13.592	29.334	10.87	52.097	11.781	15.839	68.526	20.565	17.54	27.664	16.59	13.65	9.802	22.07	6.412
	WS_2	15.36	33.18	7.64	56.59	9.75	9.12	32.68	36.02	18.9	33.75	22.43	77.01	9.55	30.33	4.94
Cross -V HG2 x $HD2009_M$	WS_1	14.293	31.728	13.172	42.022	15.933	13.056	30.127	13.24	22.626	25.63	28.74	16.99	18.96	21.13	6.465
	WS_2	16.07	29.54	8.36	64.65	9.6	8.83	20.68	44.83	25.95	27.87	35.28	14.62	11.31	22.03	6.69
Genotypic Coefficient of Variation																
Cross-I HJP81 x Rm-Ts 17	WS_1	4.369	4.438	2.505	12.832	4.051	3.595	3.783	7.662	3.516	11.307	6.673	3.967	4.032	2.31	1.292
	WS_2	1.82	11.02	1.14	6.74	3.82	7.6	8.89	4.57	3.61	9.09	1.89	0.75	7.37	3.91	1.01
Cross-II HS27 x PBW502	WS_1	2.773	8.824	3.45	9.175	4.067	4.299	3.046	1.327	7.768	9.47	2.016	6.051	4.582	8.273	4.324
	WS_2	2.06	9.39	2.67	30.01	2.92	1.05	5.92	10.57	5.63	4.16	7.6	2.49	2.67	5.08	1.75
Cross -III HJP81 x PBW502	WS_1	4.246	8.85	0.752	25.871	1.553	2.873	6.441	1.097	2.371	7.391	2.406	3.861	1.373	7.589	2.23
	WS_2	3.777	10.79	2.28	8.5	3.65	2.49	9.38	5.97	4.78	8.84	8.35	17.86	2.25	10.37	1.7
Cross -IV HS67 x PBW 502	WS_1	1.822	11.024	0.91	5.282	4.42	5.447	15.295	8.964	5.122	7.212	10.04	5.68	1.187	4.11	1.013
	WS_2	0.83	4.43	3.11	18.33	3.22	2.32	4.33	5.43	4.19	2.85	1.77	2.61	3.48	5.5	1.3
Cross-V HG2 x $HD2009_M$	WS_1	6.59	6.878	2.546	10.128	2.892	0.882	7.617	5.352	6.171	9.091	1.155	3.352	2.641	7.046	3.15
	WS_2	3.07	11.02	1.76	2.16	10.12	1.9	5.07	7.36	5.49	7.27	2.54	4.02	3.25	3.54	0.91

*Abbreviations: PH: Plant Height; NTP: Number of Tillers; NSPS: Number of Spikelet Per Spike; NSSP: Number of Sterile Spikelet Per Spike; FSSP: Number of Fertile Spikelet Per Spike; SL: Spike Length; GYP: Grain Yield Per Plant; HKW:Hundred Grain Weight; NKS: Number of Kernels Per Spike; KWS: Kernel Weight Per Spike; PL: Peduncle Length; G:SB: Grain Weight: Spike Biomass Ratio; SC: Spike compactness; SB: Spike Biomass; DH: Days to Heading

Result and Discussion

In order to meet these challenges, genetic improvement based on exploitation of genetic resources is required. Nevertheless, investigation of the wheat genome has faced difficulties due to the large genome size of bread wheat (17,000 Mb) and the high proportion (80%) of repetitive sequences. Therefore, adequate tools for the investigation of the bread wheat genome are essential. The characterization of genetic diversity within a closely related crop germplasm is an essential tool for rational use of genetic resources. The analysis of genetic variation in breeding materials is of fundamental interest to plant breeders, as it contributes to selection, monitoring of germplasm and prediction of potential genetic gain [8, 14, 15, 18]. Molecular markers have the potential to detect genetic diversity and to aid in the management of plant genetic resources [5]. Among various molecular markers currently available, SSR or microsatellite markers are often chosen as the preferred markers for a variety of applications in breeding

because of their multi-allelic nature, co-dominant inheritance, relative abundance, and extensive genome coverage [1]. Microsatellite markers are becoming the markers of choice due to the level of polymorphism, as well as higher reliability. In wheat, abundant wheat genomic SSR markers are now available and have been mapped [11], making them a useful resource for further studies. Microsatellite markers are useful and becoming popular for different applications in wheat breeding due to their high level of polymorphism and easy handling [5, 10, 11] In order to impart meaning to the results obtained and fully comprehend their implications, a statistical analysis is of utmost importance. Based on data on presence/absence of bands, genetic similarity was calculated to estimate all pair wise differences in the amplification product for all genotypes. Based on this data, cluster analysis was done to estimate relationship among genotypes.

The average linkage between wheat genotypes was used for constructing phylogenetic tree depicting the relationship among the 7 parental wheat genotypes and the selected F_2 populations of both crosses. The results of genetic similarity coefficient analysis showed that extensive genetic diversity (from 53% to 92%) was present among parental genotypes which are used for making five different crosses.

Morphological Diversity:

Estimates of phenotypic and genotypic coefficients of variability (GCV) values are presented in [Table-1]. The phenotypic coefficients of variability (PCV) values were higher than GCV in the F_2 population of five crosses for all the fifteen characters coupled with considerable differences between them indicating that the expression of these traits are more influenced or sensitive to the environmental conditions. Days to heading registered low value of GCV and PCV in all the crosses whereas the plant height exhibited low values in cross-III (WS_2) and cross-V (WS_2). Number of tillers per plant, number of sterile spikelet per spike, grain yield per plant, kernel weight per spike, 100 kernel weight exhibited high values of GCV and PCV in all the five crosses. In general, considerable differences exist between both PCV and GCV which indicate greater influence of environment in shaping these traits. A moderate to high level of variability for number of spikelet per spike, number of sterile spikelet per spike, fertile spikelet per spike, spike length and peduncle length at both levels was recorded. Significant differences between PCV and GCV were moderate to high in all five crosses, which indicated the major effect of environment on these characters.

Number of kernels, grain: spike biomass ratio, spike compactness and spike biomass exhibited moderate PCV and GCV values across all five crosses. The overview indicated that a high magnitude of variability was created for majority of characters and cross-I produced higher magnitude of variation followed by cross IV, III and V.

Estimates of phenotypic and genotypic coefficients of variability (GCV) values are presented in [Table-1]. The phenotypic coefficients of variability (PCV) values were higher than GCV in the F_2 population of five crosses for all the fifteen characters coupled with considerable differences between them indicating that the expression of these traits are more influenced or sensitive to the environmental conditions. Days to heading registered low value of GCV and PCV in all the crosses whereas the plant height exhibited low values in cross-III (WS_2) and cross-V (WS_2). Number of tillers per plant, number of sterile spikelet per spike, grain yield per plant, kernel weight per spike, 100 kernel weight exhibited high values of GCV and PCV in all the five crosses. In general considerable differences exist between both PCV and GCV which indicate greater influence of environment in shaping these traits. A moderate to high level of variability for number of spikelet per spike, number of sterile spikelet per spike, fertile spikelet per spike, spike length and peduncle length at both levels was recorded. Significant differences between PCV and GCV were moderate to high in all five crosses, which indicated the major effect of environment on these characters. Number of kernels, grain: spike biomass ratio, spike compactness and spike biomass exhibited moderate PCV and GCV values across all five crosses. The overview indicated that a high magnitude of variability was created for majority of characters and cross-I produced higher magnitude of variation followed by cross IV, III and V [1].

Estimates of PCV values were higher than GCV in the five crosses for all traits, indicating that these traits are more sensitive to the environmental conditions. The results revealed that the highest value of PCV is 66.72 for number of sterile spikelet per spike, whereas the lowest PCV was estimated for Day to heading. The PCV and GCV for Cross-I and cross were comparatively higher than the other crosses. Significant differences between PCV and GCV, which implies that the expressions of these morphological traits are influenced by environment. Earlier reports also in agreement of the present results [2, 6, 7, 16]. So, these crosses were further analysed by molecular markers (SSRs) for molecular diversity analysis.

Molecular Diversity

The diversity analysis in F2 population of wheat cross HJP81 x Rm-Ts17 (C-I), fifty SSR primers were used for out of which only 42 primers showed amplification. Using a total of 42 SSR primers, 118 amplified bands were obtained of which 89 bands were polymorphic [Table-2]. The DNA amplification and polymorphism generated among various individuals of F_2 population of Cross-I using these SSR primers are presented in [Table-1]. The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently. The total number of amplified bands varied between 1 (WMC500.1, CFD233 and WMC421) and 6 (primer Xgwm 149) with an average of 2.81 bands per primer. The polymorphism percentage ranged from as low as 33.3% to as high as 100%. Average polymorphism across all the 100 wheat genotypes was found to be 73.8%. Overall size of PCR amplified products ranged between 90bp and 320bp.

The PCR products were resolved by using PAGE used for electrophoretic separation of amplified product. DNA banding profile of individual plant DNA samples from F_2 genotypes of wheat after amplification with different SSR primers (Xbarc 133, Xwmc 413 and Xwmc 149) are depicted on PAGE in Plate 3 to Plate 5. SSR similarity matrices of 100 F_2 individuals of each cross along with their respective parental genotypes revealed the relationship among them. In cross HJP81 x Rm-Ts17 (C-I) the similarity indices between different F_2 individuals ranged from 0.237 to 0.797. Maximum similarity value of 0.0.79 was observed between genotypes 95th and 82th. The individual 45th was genetically most diverse with similarity value of 0.237. The average similarity across all the genotypes was found out to be 0.517 indicating a high level of genetic diversity among the F_2 individuals. The results of genetic similarity coefficient analysis showed that extensive genetic diversity (from 53% to 92%) was present among parental genotypes which are used for making five different crosses. The bivariate (1-0) data and similarity coefficient matrices of 100 individuals based on the data of 39 SSR primer sets were used to construct dendrogram using computer program "Simqual NTSYS PC- version 2.0 [Fig-1].

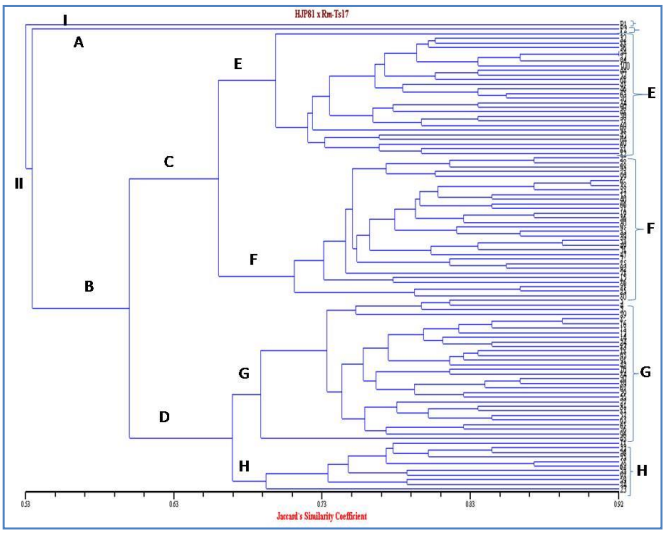

Fig-1 Dendrogram showing relationship among 100 individual plants of F_2 population of HJP81 x Rm-Ts17 along with both parents generated by UPGMA analysis based on single primers using polymorphic SSR primer pairs

As per the dendrogram constructed from SSR data, the F_2 individuals along with parents were clustered in two main clusters-I and II. The cluster-I compromised of parental genotype HJP81 (parent I) whereas cluster-II was further divided into two sub-clusters A and B at similarity coefficient of 0.53. The HJP81 parent (P-I) was present in sub-cluster A whereas the parent Rm-Ts17 was placed in cluster-I. The sub-cluster B was further divided into two major groups C and D at similarity coefficient of 0.6. The group C bifurcated into sub-groups E and F at the similarity coefficient of 0.67 and the sub-group E comprised of 23 F_2 individuals while subgroup F consists of 35 individuals. In-group D, the sub-group G and H were bifurcated at similarity coefficient of 0.67. The sub-group G consists of 31 F_2 individuals whereas the 11 individuals were clustered under sub-group H. Thus, a wide range of genetic variability was revealed from the similarity indices and dendrogram. Principal component analysis was used to derive two and three dimensional scatter plots of parents and F_2 population of both crosses. PCA results based on SSR data in F_2 population of C-I showed that 74.43% of the total variation can be explained by three principal components based on first, second & third eigen vectors which accounted for 65.16, 5.95 and 3.32% variation respectively. The grouping of the 100 F_2 plants is shown in the 2D and 3D scaling along with their two parents in Figures 1 and 2, respectively. It was evident from this analysis that almost all of the groups followed the same pattern as depicted in the dendrogram [Fig-1]. HJP81 and Rm-Ts17 genotypes, which were forming cluster A&B in the dendrogram, appeared to be present nearer to each other in PCA also. F_2 individuals forming cluster C, E, F, G in the dendrogram appeared to be present in PCA also. Similarly, the 2D explained the same pattern of dendrogram for F_2 population from this cross.

Table-2*DNA amplification profile and polymorphism generated in 100 F_2 population of wheat cross-I (HJP81 x Rm-Ts17) using 50 SSR primers.*

Sr. No.	Primer	Size of of bands	Total No. of bands	Number of polymorphic bands	Number of monomorphic bands	Percentage polymorphism
1	BARC19	110-140	2	2	0	100
2	BARC26	110-190	2	2	0	100
3	BARC28	165-205	3	2	1	66.67
4	BARC45	80-210	4	3	1	75
5	BARC48	115-250	2	1	1	50
6	BARC113	150-235	3	2	1	66.67
7	BARC133	170-260	4	3	1	75
8	BARC187	110-190	3	3	0	100
9	BARC236	120-200	2	2	0	100
10	BARC263	125-215	2	2	0	100
11	BARC275	110-140	2	2	0	100
12	BARC288	95-160	2	2	0	100
13	BARC297	No amplification				
14	BARC344	No amplification				
15	BARC350	130-210	3	3	0	100
16	BARC359	No amplification				
17	BARC1005	100-280	3	3	0	100
18	CFA2104	120-205	4	3	1	75
19	CFA2262	90-230	4	4	0	100
20	CFA2292	120-210	3	3	0	100
21	CFD233	110	1	0	1	--
22	CFD239	105-190	3	2	1	66.67
23	WMC41	140-175	2	1	1	50
24	wmc110	100-265	3	2	1	66.67
25	WMC134	No amplification				
26	WMC149	135-210	6	4	2	66.67
27	WMC 170	105-140	2	2	0	100
28	WMC232	185-230	3	2	1	----
29	WMC254	105-200	5	1	3	33.33
30	WMC296	115-210	2	1	1	50
31	WMC349	110-190	3	2	1	66.67
32	WMC407	100-150	2	1	1	50
33	WMC413	105-190	5	4	1	80
34	WMC416	90-150	5	3	2	60
35	WMC421	110	1	..	1	-
36	WMC475	185-220	2	1	1	50
37	WMC500.1	150	1	-	1	-
38	WMC601	160-200	2	2	0	100
39	WMC719	No amplification				
40	WMC727	120-320	2	2	0	100
41	WMC758	105-140	2	2	0	100
42	WMC766	No amplification				
43	WMC819	No amplification				
44	Wmc827	No amplification				
45	Xgwm219	160-230	5	3	2	60
46	Xgwm261	165-200	2	2	0	100
47	Xgwm443	150-200	4	4	0	100
48	Xgwm515	170-210	2	2	0	100
49.	*Xgwm642*	165-225	3	2	1	66.67
50	*Xcfd223*	130-240	3	3	0	100
Average			118/42= 2.81			73.80

As per the dendrogram constructed from SSR data, the F_2 individuals along with parents of cross-I were grouped into four major sub-groups E and F at the similarity coefficient of 0.67 and the sub-group E comprised of 23 F_2 individuals while subgroup F consists of 35 individuals. The sub-group G consists of 31 F_2

individuals whereas the 11 individuals were clustered under sub-group H. Thus, a wide range of genetic variability was revealed from the similarity indices and dendrogram.

It was evident from this analysis that all of the groups followed the same pattern as depicted in the dendrogram. Similar reports were recorded by using microsatellites markers for assessment of genetic diversity among cultivars and their wild relatives of wheat [8, 14, 15, 17, 18]. Principal component analysis was used to derive two and three dimensional scatter plots of parents and F_2 population [Fig-2]. Similar reports were recorded by using microsatellites markers for assessment of genetic diversity among cultivars and their wild relatives of wheat [3]. The present study addressed the utility of SSR markers in polymorphism study at the molecular level among F2 population of wheat cross wherein 42 screened polymorphic SSR primers were used, which were earlier identified in the genomic regions of A, B, and D genomes of wheat. The microsatellite or SSR primers generated 118 bands with the number of band per primer varying from 1 to 6 with an average 2.81 bands per primer. Similar, results have been reported by Salem *et al*., (2008) using SSR markers. The total number of amplified allele varied between 2 and 5 with an average of 3.2 allele per locus, which is close to results of present investigation.

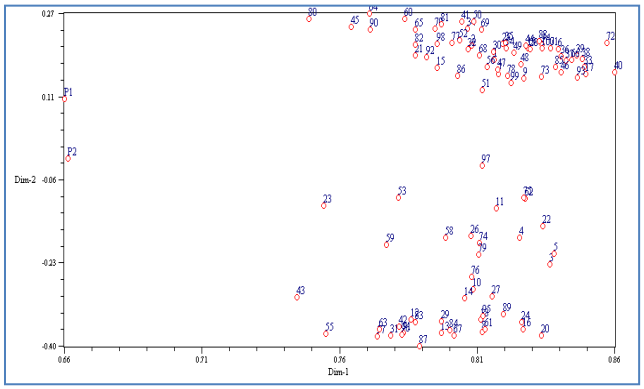

Fig-2 Two dimensional PCA (Principal component analysis) scaling of 100 F_2 individuals of HJP81 x Rm-Ts17 along with both parents using SSR markers

The present study also showed that primer pair Xwmc 421, CFD 233, WMC500.1 generated a minimum number of one band while primer pair Xgwm 149 produced maximum number of bands [6]. This revealed significant differences in allelic diversity among various microsatellite loci. Many studies have also reported remarkable differences in allelic diversity among various microsatellite loci [8]. The alleles revealed by markers showed a higher degree of polymorphism. The polymorphism percentage ranged from as low as 33% to as high as 100%, giving an average percentage polymorphism of 73.8% in present investigation. Similarly, Ijaz and Khan (2009) reported high level of polymorphism ranging from 10.52% to 98.42%. In the present investigation, size of amplified DNA fragments varied from approx. 90bp to 320bp. It is therefore suggested that a focused breeding scheme should be adopted while analyzing genome diversity estimates for parent selection to gain maximum value and practical impact on a breeding program.

Conflict of Interest: None declared

References

[1] Babay E., Chaabane R., Abdmouleh R.M., and Naceur M.B. (2015) *Biosci. J., Uberlândia,* 31(3), 701-708.

[2] Dwivedi A.N. and Pawar I.S. (2005) *Haryana Agric. Univ. J. Res*., 34(1), 35-39

[3] Hanif U., Rasheed A., Kazi A.G., Afza F., Khalid M., Muhammad M. and Kazi A.M. (2014) *Cytologia* 79(4), 485–500

[4] Ijaz S. & Khan I. A. (2009) *Genetics and Molecular Research*, 8(3), 809-815. http://dx.doi.org/10.4238/vol8-3gmr608

[5] Khaled F. M. Salem, Marion S. Röder and Andreas Börner (2015) *Genetic Resources and Crop Evolution,* 62(3), 377-385

[6] Muthamilarasan M., Parida S.K. and Prasad M. (2014) *Proc Indian Natn Sci Acad* 80(2) : 325-33

[7] Mohammadi S.A., Khodarahmi M., Jamalirad S. and Jalal Kamali M. R. (2009) *Annals of Applied Biology*, 154(1), 67-76. http://dx.doi.org/10.1111/j.1744-7348.2008.00273.x.

[8] Nefzaoui M., Udupa S.M., Gharbi M.S., Bouhadida M. and Iraqi D. (2014) *Romanian Agricultural Research*, 31, 33-39

[9] Nei M., Li W. (1979) *Proc. Natl. Acad. Sci.,* 76, 5269-5273.

[10] Nielsen N.H., Backes G., Stougaard J., Andersen S.U. and Jahoor A. (2014) *PLoS ONE,* 9(4), e94000.*doi:10.1371/journal.pone.0094000*

[11] Röder M.S., Plaschke J., Konig S.U., Börner A., Sorrells M.E., Tanksley S.D. and Ganal M.W. (1995) *Mol. Gen. Genet.,* 246, 327-333.

[12] Rohlf F J. (1993) NTSYS-PC numerical taxonomy and multivariate analysis system. *Exeter Software*.

[13] Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A. and Allard R.W. (1984) *Proceedings of National of Academy Science* (USA), 81, 8 014–8.

[14] Salem K.F.M., El-Zanaty A.M. and Esmail R.M. (2008) *World J. of Agri. Sci.* 4 (5), 538-544.

[15] Schuster I., Vieira E.S.N., Silva G.J., Franco F.A. and Marchioro V.S. (2009) *Genet. and Mol. Biol.,* 32(3), 557 563.

[16] Subhashchandra B., Lohithaswa H.C., Desai S.A., Hanchinal R.R., Kalappanavar I.K., Math K.K. and Salimath P.M. (2009) *Karnataka J. Agric. Sci.,* 22(1), 36-38.

[17] Wurschum T., Langer S.M., Longin C.F.H., Korzun V., Akhunov E., Ebmeyer E., Schachschneider R., Schacht J., Kazman E., and Reif J. C. (2013) *Theor Appl Genet,* 126,1477–1486 DOI 10.1007/s00122-013-2065-1.

[18] Zecevic V., Boskovoic J., Dimitrijevic M. and Petrovic S. (2010) *Bulgaria J. Agric. Sci*., 16, 422-428.