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Molecular Diversity of Wheat (*Triticum aestivum* L.) Genotypes Resistance to Rice Weevil (*Sitophilus oryzae* L.) Revealed by SSR Markers

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A B S T R A C T

The present investigation was carried out to analyse for the presence of α-amylase inhibitor gene which is responsible for resistance to rice weevil. A total of 30 simple sequence repeat (SSR) markers were used to study the genetic diversity among 30 wheat genotypes resistant to rice weevil. Among them, seventeen primers were found polymorphic on agarose gel and depicted the significant diversity among susceptible and resistant genotypes while rest thirteen primers were found monomorphic. The number of polymorphic bands per loci ranged from 1 to 3 with an average of 1.6 alleles per locus. A clear cut differentiation was exhibited among the genotypes. The average PIC value was 0.37 ranging from 0.17 (WMC120/ WMC76/WMC245) to 0.67 (WMC267), indicating diverse nature of the wheat genotypes and/or highly informative SSR markers used in this study. The analyzed wheat genotypes showed a good level of genetic variability for assessed quantitative, physio-chemical and molecular characters. Molecular markers linked with major genes for traits of interest which have been amplified by the primer set UCW108, a genic marker for GPC-B1. The range of similarity coefficient varied from 54% (PBN-51) to 74% (K 20). SAHN cluster analysis using UPGMA method separated the parental genotypes into four cluster groups, PBN 51 was positioned as single genotype in separate groups i.e., in cluster-I, K 50, K 76 and K 77 in cluster-II, K 2I and K 50 in cluster-III and K 20 in cluster-IV. In dendrogram, based on results of markers validated were able to diversify the resistant and susceptible parents, first group member PBN51 and fourth group member K20 found on the two distant groups, which were observed as most susceptible and resistant genotypes respectively, according to our controlled experiment on weevil infestation and molecular analysis done and hence, the study supports and correlates the result, obtained by these analysis. The present study also indicates that microsatellite markers able to access the genetic diversity among studied genotypes of wheat for weevil resistance.

Introduction

Wheat is second most important cereal crop after rice and is widely grown almost all over the world. India ranks second in production after China with a production of 97.44 million tonnes from an area 30.72 million hectare during 2016-17 (Anonymous, 2017). Phenomenal increase in wheat production and productivity has been achieved with the advent of high yielding improved varieties. One of the most important reasons for this success has been the relatively pest free field conditions of wheat. Producers should consider varietal suitability for long term storage when selecting crop varieties. Farmers keep their produce in homemade storage primarily to increase the net value of the crop selling when prices are more favourable. However, grain price is determined in part by test weight, absence of insects and damage caused by insects. Wheat is quite susceptible to storage pests which cause substantial qualitative (nutritional) and quantitative losses of various magnitudes depending on the pest species and duration of storage (Singhamony *et al.*1985). In Indian subcontinent, however wheat is heavily infested by a number of insect pests in storage, among these, *Sitophilus oryzae* (L.), *Rhizopertha dominica* (F.) and *Trogoderma granarium* Everts. are most important. Insect-pests which feed mainly on crops causes 10 to 30% damage in both traded and elevator stored grains every year (Karunakaran *et al.,* 2004). It is estimated that 5-10% of world's grain production is lost due to ravages of insects. The losses may reach up to 50% in tropical countries where summer is hot and humid and storage facilities are improper and inadequate (Ahmad and Ahmad, 2002). The rice weevil is a pest of economic importance causing losses in weight, deterioration of quality and facilitating the development of micro-organisms in stored cereals.

Unfortunately, wheat varieties are not developed for their ability to resist insect attack at postharvest. Yield, adaptability to specific growing conditions, quality parameters and resistance to diseases and post-harvest insect-pest infestation are the main breeding objectives in wheat. Due to increasing threat of insect-pests in storage particularly rice weevil, there is a need for understanding the various dimensions of its resistance to breed the resistant genotypes. However, the major hindrance in utilizing the resistance against the pest is that resistant source is scanty. Also the nature of resistance is still not very clear. Therefore, the success of the breeding programme lies in the identification of source of resistant genes and sound knowledge of genetic behaviour of the resistant genes. In this concern knowledge of genetic diversity in a crop species is fundamental to its improvement. The Polymerase Chain Reaction **(**PCR) based molecular markers such as RAPD, SSR and ISSR have successfully been utilized to assess the genetic diversity among the genotypes of several crop plants. Out of these, SSR are highly informative and locus specific genetic markers which are co-dominant in nature with high information content (Danin-Poleg *et al.,* 2000). SSR has facilitated the studies of genetic diversity (Plaschke *et al.,* 1995), gene mapping (Pestsova *et al.,* 2002) and testing of authenticity of genetic stocks (Pestsova *et al.,* 2002). Microsatellite markers have high potential for use in studies regarding genetic diversity and relationships. Keeping the usefulness of resistant genes in view, the present investigation was carried out with microsatellite (SSR) markers for analysis of genetic diversity of 30 genotype of wheat for rice weevil resistance.

Materials and Methods

PCR based Molecular diversity work was carried out at Wheat Grain Quality

Laboratory, Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar in the year 2011-12 using microsatellite markers. Parents and their progenies were subjected to molecular marker analysis for the presence of α-amylase inhibitor gene information on the basis of *in vitro* experiments.

Plant materials

The materials used in the investigation comprised of 30 accessions of wheat genotypes resistant to *Sitophilus oryzae* based on *invitro* experiment have been for depicted in Table 1. Each accession was sown in pots and leaf sample from 2-3 week old seedlings were collected for genomic DNA isolation.

Genomic DNA extraction and quantification

Freshly collected leaf sample of each genotype was ground to fine powder in mortar and pestle using liquid nitrogen and immediately it was processed using the Plant Genomic DNA isolation kit (HiMedia Laboratories Pvt. Ltd, India). The kit was especially designed for plant genomic DNA isolation and based on Cetyl Trimethyl Ammonium Bromide (CTAB) method. The RNA content in genomic DNA isolated using the kit was removed using RNase A. Quantity and quality of the genomic DNA was determined using dual beam spectrophotometer (Systronics, India) and Agarose gel electrophoresis (Sambrook and Russel, 2001).

DNA markers

Polymerase chain reaction (PCR) based marker SSR was used to quantify the genetic diversity of the wheat. A total of 30 SSRs were used in the investigations which have been depicted in Table 2.

PCR amplification

PCR amplification was performed in a volume of 25 µl. Each 25µl reaction mixture consisted of 100 ηg of DNA, 200 µM dNTPs, 1U of Taq polymerase, 1X Taq buffer and 1.2 µl primer of SSR each of forward and reverse primers were used. Amplication reaction was performed with initial denaturation at 94 ºC for 5 min followed by 35 cycles of denaturation at 94 ºC and 30 second, annealing of primers for 1 min at 45-60 ºC and polymerization for 1 min at 72 ºC. One cycle of final extension was maintained for 7 min at 72 ºC. Amplification reaction was performed in dual block DNA engine (Bio-Rad, USA). PCR amplified DNA fragments were resolved using 3% Agarose gel for electrophoresis to resolve the fragments generated using SSR primers. The gels were stained with Ethidium bromide (0.5mg/ml) and documented using Gel Documentation system (Alpha Imager EC).

Scoring of gel and analysis of data

Each genotype was scored as presence (1) or absence (0) of respective allele against each primer. Pair wise Jaccard's similarity coefficients (1908) were calculated to determine the similarity of wheat genotypes. Unweighted pair group method with arithmetic average (UPGMA) based dendrogram was generated to determine marker based genetic relationship amongst the 30 genotypes. The data were analyzed using the NTSYS-pc software (Rohlf, 2000).

Results and Discussion

SSR markers are the markers of choice for variability studies in many crops as they are transferable, highly polymorphic, simple to interpret and they are multi-allelic and co-dominant (Rafaleski *et al.,* 1996; Agrama and Tuinstra, 2003). This high level of

polymorphism associated with SSR markers, is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage. Replication slippage is thought to occur more frequently than single nucleotide mutations and insertion/deletion events.

In wheat, abundant wheat genomic SSR markers are now available and have been mapped (Roder *et al.,* 1998), 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 (Gupta and Varshney, 2000) and are used to evaluate genetic diversity of hexaploid wheat (*Triticum aestivum* L.)

Besides above weevil resistant specific primers additional thirty SSR primers were used for the analysis of genetic diversity of parental genotypes, in which some of the primers were tagged with particular traits. Among them seventeen primers were found polymorphic on agarose gel and depicted the significant diversity among susceptible and resistant genotypes while rest thirteen primers were found monomorphic. The number of polymorphic bands per loci ranged from 1 to 3 with an average of 1.6 alleles per locus. Similar type of result were also reported by Dong and Zeng (2003), Ravi *et al.,* (2003), ZhongFu *et al.,* (2003), Mahmood *et al.,* (2004) and Medini *et al.,* (2005).

Number of alleles/locus and PIC values for each SSR marker were calculated. The average PIC value was 0.37 ranging from 0.17 (WMC120/ WMC76/WMC245) to 0.67 (WMC267), indicating diverse nature of the wheat parental genotypes and/or highly informative SSR markers used in this study. PIC values suggest that SSRs employed in the present study resulted adequate and efficient. The analyzed wheat genotypes showed a good level of genetic variability for assessed quantitative, physio-chemical and molecular characters.

Similar types of results were revealed by XinMin *et al.,* (2003), Roussel *et al.,* (2004), Naghavi *et al.,* (2004), Jian Cheng *et al.,* (2007), Prasad *et al.,* (2009), Zeb *et al.,* (2009) and Najaphy *et al.,* (2012). The detected genetic diversity for the six parental genotypes was lower than that of earlier reported by Salem *et al.,* (2008) in wheat genotypes. Molecular markers linked with major genes for traits of interest are being routinely developed in several crops. Besides our specific objective, diversity showed by the parental lines might be due to others important morphological and physio-chemical tagged traits like protein content; which have been amplified by the primer set UCW108, a genic marker for GPC-B1 (Table 3–5).

Likewise the primer sets WMC 83 and WMC 149 were tagged to days to flowering, tillers number, grain yield, harvest index, spikelet per spike, days to flowering etc. Similar type of results in wheat have already been reported by earlier eminent workers, Huang *et al.,* (2002), Gupta *et al.,* (2003), Kuleung *et al.,* (2004), Nicot *et al.,* (2004), You *et al.,* (2004), Stodart *et al.,* (2005), Hayden *et al.,* (2006), Roy *et al.,* (2006), Malik *et al.,* (2008), Iqbal *et al.,* (2009) and Yildirim *et al.,* (2011).

Table.1 List of genotypes used for molecular study on the basis of *invitro* Experiment

S.No	Generations	Pedigree	Weevil Infestation Reaction
1	**P_1**	PBN51	**S**
2	**P_2**	K76	**S**
3	**P_3**	K77	**S**
4	**P_4**	K20	**R**
5	**P_5**	K21	**R**
6	**P_6**	K50	**R**
7	**F_1 1**	PBN51 x K20	**R**
8	**F_1 4**	PBN51 x K76	**S**
9	**F_1 5**	PBN51 x K77	**S**
10	**F_1 9**	K76 x K77	**S**
11	**F_1 13**	K20 x K21	**R**
12	**F_1 14**	K20 x K50	**R**
13	**F_1 15**	K21 x K50	**R**
14	**F_1 6**	K76 x K20	**R**
15	**F_1 7**	K76 x K21	**R**
16	**F_1 8**	K76 x K50	**R**
17	**F_2 1**	PBN51 x K20	**R**
18	**F_2 2**	PBN51 x K21	**R**
19	**F_2 7**	K76 x K21	**R**
20	**F_2 10**	K77 x K20	**R**
21	**BC_1 1**	(PBN51 x K20)/PBN51	**R**
22	**BC_1 11**	(K77 x K21)/K77	**R**
23	**BC_1 13**	(K20 x K21)/K20	**R**
24	**BC_1 14**	(K20 x K50)/K20	**R**
25	**BC_1 15**	(K21 x K50)/K21	**R**
26	**BC_2 3**	(PBN51 x K50)/K50	**R**
27	**BC_2 7**	(K76 x K21)/K 21	**R**
28	**BC_2 10**	(K77 x K20)/K 20	**R**
29	**BC_2 13**	(K20 x K21)/K 21	**R**
30	**BC_2 15**	(K21 x K50)/K 50	**R**

Table.2 Seuences of the SSR Primers

S. No.	Source Name	Forward primer Sequence (5′- 3′)	Reverse primer Sequence (5′- 3′)	Annealing Temperature
1	wmc25	TCTGGCCAGGATCAATATTACT	TAAGATACATAGATCCAACACC	45 ^{0}C
2	wmc35	GCAGAGAGGCACAACTAGCGAG	AACCTTAAGGGTCTGCCGGAAC	50 ^{0}C
3	wmc76	CTTCAGAGCCTCTTTCTCTACA	CTGCTTCACTTGCTGATCTTTG	45 ^{0}C
4	wmc83	TGGAGGAAACACAATGGATGCC	GAGTATCGCCGACGAAAGGGAA	55 ^{0}C
5	wmc120	GGAGATGAGAAGGGGGTCAGGA	CCAGGAGACCAGGTTGCAGAAG	55 ^{0}C
6	wmc149	ACAGACTTGGTTGGTGCCGAGC	ATGGGCGGGGGTGTAGAGTTTG	55 ^{0}C
7	wmc169	TACCCGAATCTGGAAAATCAAT	TGGAAGCTTGCTAACTTTGGAG	50 ^{0}C
8	wmc170	ACATCCACGTTTATGTTGTTGC	TTGGTTGCTCAACGTTTACTTC	45 ^{0}C
9	wmc177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	45 ^{0}C
10	wmc243	CGTCATTTCCTCAAACACACCT	ACCGGCAGATGTTGACAATAGT	50^{0}C
11	wmc245	GCTCAGATCATCCACCAACTTC	AGATGCTCTGGGAGAGTCCTTA	50 ^{0}C
12	wmc254	AGTAATCTGGTCCTCTCTTCTTCT	AGGTAATCTCCGAGTGCACTTCAT	45 ^{0}C
13	wmc267	TCTTCACCCCATAATTGGAGAAG CCT	TGCTTATTCTGCGCACTGGATGCCTA	55 ^{0}C
14	cfd15	CTCCCGTATTGAGCAGGAAG	GGCAGGTGTGGTGATGATCT	60 ^{0}C
15	cfd24	GGCGCAATCTGAAAGAAAAG	CCAGGTCCCACTTTCGTCT	60 ^{0}C
16	cfd26	TCAAGATCGTGCCAAATCAA	ACTCCAAGCTGAGCACGTTT	60 ^{0}C
17	cfd30	AATCGCACAACAATGGTTCA	GCCTCTCCTCTCTGCTCCTT	60 ^{0}C
18	UCW 108(GPC-B1)	AGCCAGGGATAGAGGAA	AGCTGTGAGCTGGTGTCCTT	60 ^{0}C
19	W24A	TCGTGGCGACACCCRTACCA	ACTCATTTGCTTGACTAGGC	63 ^{0}C
20	W47AT	AGTACGACGCATGGAGTAT	ACTCATTTGCTTGACTAGGC	57 ^{0}C
21	wmc167	AGTGGTAATGAGGTGAAAGAAG	TCGGTCGTATATGCATGTAAAG	45 ^{0}C
22	wmc44	GGTCTTCTGGGCTTTGATCCTG	TGTTGCTAGGGACCCGTAGTGG	50 ^{0}C
23	wmc47	GAAACAGGGTTAACCATGCCAA	ATGGTGCTGCCAACAACATACA	50 ^{0}C
24	wmc221	ACGATAATGCAGCGGGGAAT	GCTGGGATCAAGGGATCAAT	50 ^{0}C
25	wmc93	ACAACTTGCTGCAAAGTTGACG	CCAACTGAGCTGAGCAACGAAT	60 ^{0}C
26	wmc83	TGGAGGAAACACAATGGATGCC	GAGTATCGCCGACGAAAGGGAA	55 ^{0}C
27	cfd1	ACCAAAGAACTTGCCTGGTG	AAGCCTGACCTAGCCCAAAT	60 ^{0}C
28	cfd2	GGTTGCAGTTTCCACCTTGT	CATCTATTGCCAAAATCGCA	60 ^{0}C
29	cfd3	GCACCAACACACGGAGAAG	TTGAGAGGAGGGCTTGGTTA	60 ^{0}C
30	cfd5	TGCCCTGTCCACAGTGAAG	TTGCCAGTTCCAAGGAGAAT	60 ^{0}C

Table.3 Analysis of SSR marker

S. No.	Primers Code	% Polymorphism	Number of bands		
			Total bands	Monomorphic Bands	Polymorphic Bands
1	wmc25	**66.67**	12	4	8
2	wmc35	**50**	12	6	6
3	wmc76	**83.33**	12	2	10
4	wmc83	**50**	12	6	6
5	wmc120	**33.33**	12	8	4
6	wmc149	**66.67**	12	4	8
7	wmc169	**50**	12	6	6
8	wmc170	**66.67**	12	4	8
9	wmc177	**66.67**	12	4	8
10	wmc243	**66.67**	12	4	8
11	wmc245	**83.33**	12	2	10
12	wmc254	**50**	12	6	6
13	wmc267	**66.67**	12	4	8
14	cfd15	**100**	12	0	12
15	cfd24	**66.67**	12	4	8
16	cfd26	**50**	12	6	6
17	cfd30	**50**	12	6	6
18	UCW 108(GPC-B1)	**50**	12	6	6
19	W24A	**50**	12	6	6
20	W47AT	**50**	12	6	6
21	wmc167	**83.33**	12	2	10
22	wmc44	**50**	12	6	6
23	wmc47	**66.67**	12	4	8
24	wmc221	**50**	12	6	6
25	wmc93	**33.33**	12	8	4
26	wmc83	**83.33**	12	2	10
27	cfd1	**50**	12	6	6
28	cfd2	**66.67**	12	4	8
29	cfd3	**50**	12	6	6
30	cfd5	**50**	12	6	6

Table.4 Estimated Jaccard's similarity coefficient of 30 genotypes of wheat using SSR data

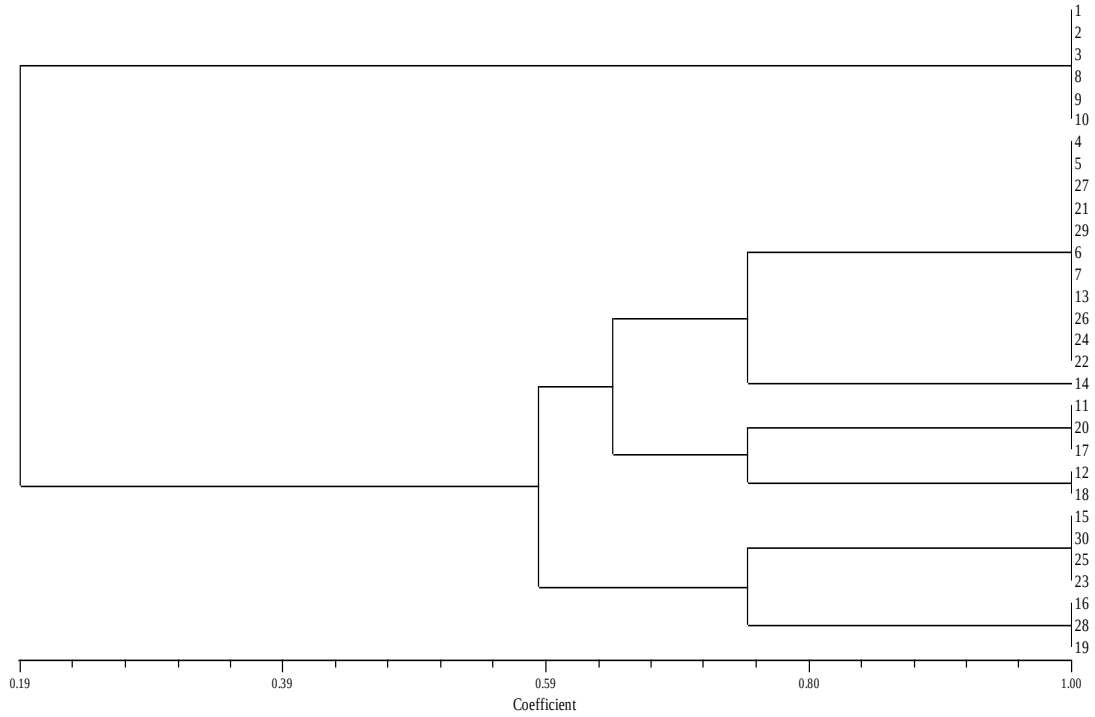
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1																													
2	1	1																												
3	1	1	1																											
4	0	0	0	1																										
5	0	0	0	1	1																									
6	0	0	0	1	1	1																								
7	0	0	0	1	1	1	1																							
8	1	1	1	0	0	0	0	1																						
9	1	1	1	0	0	0	0	1	1																					
10	1	1	1	0	0	0	0	1	1	1																				
11	0.25	0.25	0.25	0.75	0.75	0.75	0.75	0.25	0.25	0.25	1																			
12	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.75	1																		
13	0	0	0	1	1	1	1	0	0	0	0.75	0.5	1																	
14	0.25	0.25	0.25	0.75	0.75	0.75	0.75	0.25	0.25	0.25	0.5	0.75	0.75	1																
15	0.25	0.25	0.25	0.75	0.75	0.75	0.75	0.25	0.25	0.25	0.5	0.25	0.75	0.5	1															
16	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.75	0.5	0.5	0.25	0.75	1														
17	0.25	0.25	0.25	0.75	0.75	0.75	0.75	0.25	0.25	0.25	1	0.75	0.75	0.5	0.5	0.75	1													
18	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.75	1	0.5	0.75	0.25	0.5	0.75	1												
19	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.75	0.5	0.5	0.25	0.75	1	0.75	0.5	1											
20	0.25	0.25	0.25	0.75	0.75	0.75	0.75	0.25	0.25	0.25	1	0.75	0.75	0.5	0.5	0.75	1	0.75	0.75	1										
21	0	0	0	1	1	1	1	0	0	0	0.75	0.5	1	0.75	0.75	0.5	0.75	0.5	0.5	0.75	1									
22	0	0	0	1	1	1	1	0	0	0	0.75	0.5	1	0.75	0.75	0.5	0.75	0.5	0.5	0.75	1	1								
23	0.25	0.25	0.25	0.75	0.75	0.75	0.75	0.25	0.25	0.25	0.5	0.25	0.75	0.5	1	0.75	0.5	0.25	0.75	0.5	0.75	0.75	1							
24	0	0	0	1	1	1	1	0	0	0	0.75	0.5	1	0.75	0.75	0.5	0.75	0.5	0.5	0.75	1	1	0.75	1						
25	0.25	0.25	0.25	0.75	0.75	0.75	0.75	0.25	0.25	0.25	0.5	0.25	0.75	0.5	1	0.75	0.5	0.25	0.75	0.5	0.75	0.75	1	0.75	1					
26	0	0	0	1	1	1	1	0	0	0	0.75	0.5	1	0.75	0.75	0.5	0.75	0.5	0.5	0.75	1	1	0.75	1	0.75	1				
27	0	0	0	1	1	1	1	0	0	0	0.75	0.5	1	0.75	0.75	0.5	0.75	0.5	0.5	0.75	1	1	0.75	1	0.75	1	1			
28	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.75	0.5	0.5	0.25	0.75	1	0.75	0.5	1	0.75	0.5	0.5	0.75	0.5	0.75	0.5	0.5	1		
29	0	0	0	1	1	1	1	0	0	0	0.75	0.5	1	0.75	0.75	0.5	0.75	0.5	0.5	0.75	1	1	0.75	1	0.75	1	1	0.5	1	
30	0.25	0.25	0.25	0.75	0.75	0.75	0.75	0.25	0.25	0.25	0.5	0.25	0.75	0.5	1	0.75	0.5	0.25	0.75	0.5	0.75	0.75	1	0.75	1	0.75	0.75	0.75	0.75	1

Where, 1: P_1, 2: P_2, 3: P_3, 4: P_4, 5: P_5, 6: P_6, 7: $F_1$1, 8: $F_1$4, 9: $F_1$5, 10: $F_1$9, 11: $F_1$13, 12: $F_1$14, 13: $F_1$15, 14: $F_1$6, 15: $F_1$7, 16: $F_1$8, 17: $F_2$1, 18: $F_2$2, 19: $F_2$7, 20: $F_2$10, 21: $B_1$1, 22: $B_1$11, 23: $B_1$13, 24: $B_1$14, 25: $B_1$15, 26: $B_2$3, 27: $B_2$7, 28: $B_2$10, 29: $B_2$13, 30: $B_2$15.

Table.5 Clustering group at 80 % similarity level based on SSR using UPGMA dendrogram

Cluster group	No. of genotypes	Name of genotypes
I	6	PBN51, K76, K77, PBN51 x K76, PBN51 x K77, K76 x K77
II	11	K20, K21, K50, PBN51 x K20, K21 x K50, (PBN51 x K20)/PBN51, (K77 x K21)/K77, (K20 x K50)/K20, (PBN51 x K50)/K50, (K76 x K21)/K21, (K20 x K21)K21
III	1	K76 x K20
IV	3	K20 x K21, PBN51 x K20, K77 x K20
V	2	K20 x K50, PBN51 x K21
VI	4	K76 x K21, (K20 x K21)/K20, (K21 x K50)/K21, (K21 x K50)/K50,
VII	3	K76 x K50, K76 x K21, (K77 x K20)/K20,

Fig.1 Dendrogram depicting the genetic relationship among 30 genotypes of different generations of wheat, constructed though UPGMA. Scale indicates the Jaccard's coefficient of genetic similarity. Where, 1: P_1, 2: P_2, 3: P_3, 4: P_4, 5: P_5,6: P_6, 7:$F_1$1, 8: $F_1$4, 9:$F_1$5, 10: $F_1$9, 11:$F_1$13, 12:$F_1$14, 13: $F_1$15, 14: $F_1$6, 15: $F_1$7, 16: $F_1$8, 17: $F_2$1, 18: $F_2$2, 19: $F_2$7, 20: $F_2$10, 21: $B_1$1, 22: $B_1$11, 23: $B_1$13, 24: $B_1$14, 25: $B_1$15, 26: $B_2$3, 27: $B_2$7, 28: $B_2$10, 29: $B_2$13, 30: $B_2$15



Jaccard's similarity coefficient

On the basis of SSR banding pattern provided by the 17 different primers, a dendrogram (Figure 1) based on Jaccard's similarity coefficient and UPGMA, was created. Range of coefficient varied from 0.54 to 0.74. First group at a similarity coefficient ranged from 0.56 to 0.74 comprised of one genotype PBN 51. Second group at a similarity coefficient ranged from 0.59 to 0.74 comprised of K21, K50, K76 and K77 while third group at a similarity coefficient of 0.54 to 0.74 consisted of K20. In the dendrogram, K76 and K50 were found to be closely related genotypes, followed by K21 and K77. First group genotype PBN51 was found different from second group having four genotypes followed by third group genotype K20, which was found quite different from the rest 5 genotypes. Markers were able to diversify the resistant and susceptible parents. In dendrogram based on results of seventeen markers, first group member PBN51 and third group member K20 found on the two distant groups, which were observed as most susceptible and resistant genotypes respectively according to our controlled experiment on weevil infestation and molecular analysis done and hence, the study supports and correlates the result, obtained by these analysis. The results observed in this research are in agreement with those reported by Eivazi *et al.,* (2008) and Izaj and Khan (2009).

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References

Ahmad, M. and Ahmad, A., 2002, Storage of food grains. *Farming Outlook* 1: 16-20.

Anonymous, 2017, Director's Report of AICRP on Wheat and Barley 2016-17. *Ed:* G.P. Singh. ICAR-Indian Institute of Wheat & Barley Research, Karnal, Haryana, India. 2017, 87.

Agrama, H. A. and Tuinstra, M. R., 2003, Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. *African J. Biotech*. 2: 334-340.

Danin-Poleg, Y., Reis, N., Baudracco-Arnas, S., Pitrat, M., Staub, J. E., Oliver, M., Arus, P., de Vicente, C.M. and Katzir, N., 2000, Simple sequence repeats in *Cucumis,* mapping and map merging. *Genome* 43: 963-974.

Dong, Z. Z. and Zeng, J. J., 2003,. Microsatellite marker development and applications in wheat genetics and breeding. *Yichuan*. 25(3): 355-360.

Eivazi, A. R., Naghavi, M. R., Hajheidari, M., Pirseyedi, S. M., Ghaffari. M. R., Mohammadi S. A., Majidi, I., Salekdeh, G. H. and Mardi, M., 2008, Assessing wheat (*Triticum aestivum* L.) genetic diversity using quality traits, amplified fragment length polymorphisms, simple sequence repeats and proteome analysis. *Ann Appl Biol*. 152: 81–91.

Gupta, P. K. and Varshney, R. K., 2000, The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113(3): 163-185.

Gupta, P. K., Rustgi, S., Sharma, S., Singh, R., Kumar, N. and Balyan, H. S., 2003, Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. *Molecular Genetics and Genomics* 270(4): 315-323.

Huang, Q., Borner, A., Roder, S. and Ganal, W., 2002, Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theor. Appl. Genet.* 105: 699-707.

Hayden, M. J., Stephenson, P., Logojan, A. M., Khatkar, D., Rogers, C., Elsden, J., Koebner, R. M. D., Snape, J. W. and Sharp, P. J., 2006, Development and genetic mapping of sequence-tagged microsatellites (STMs) in bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 113: 1271-1281.

Ijaz, S. and Khan, I. A., 2009, Molecular characterization of wheat germplasm using microsatellite markers. *Genetics and Molecular Research* 8(3): 809-815.

Iqbal, N., Tabasum, A., Sayed, H. and Hameed, A., 2009, Evaluation of genetic diversity among bread wheat varieties and landraces of Pakistan by SSR markers. *Cereal Research Communications* 37(4): 489-498.

Jaccard, P., 1908, Nouvelles researches sur la distribution florale. *Bul. Soc. Vaud. Sci. Nat*. 44: 223-270.

JianCheng, L., Like, L., Ning, H., AiMin, Z. and ChunGuang, L., 2007, Genetic diversity of wheat gene pool of recurrent selection assessed by microsatellite markers and morphological traits. *Euphytica* 155(1/2): 249-258.

Karunakaran C., Janas D. S. and White N. D.

G., 2004, Detection of internal wheat seed infestation by *Rhyzopertha dominica* F. using X-ray imaging. *J. Stored Products Res*. 40: 507-516.

Kuleung, C., Baenziger, P. S. and Dweikat, I., 2004, Transferability of SSR markers among wheat, rye and triticale. *Theor. Appl. Genet.* 108(6): 1147-1150.

Mahmood, A., Baenziger, P. S., Budak, H., Gill, K. S. and Dweikat, I., 2004, The use of microsatellite markers for the detection of genetic similarity among winter bread wheat lines for chromosome 3 A. *Theor. Appl. Genet.* 109: 1494-1503.

Malik, R., Chowdhury, V. K., Jain, S. and Jain, R. K., 2008, Microsatellites based genetic diversity analysis of Indian bread wheat genotypes. *Env. and Eco*. 26(1): 100-106.

Medini, M., Hamza, S., Rebai, A. and Baum, M., 2005, Analysis of genetic diversity in Tunisian durum wheat cultivars and related wild species by SSR and AFLP markers. *Genetic Resources and Crop Evolution* 52(1): 21-31.

Naghavi, M.R., Mardi, M., Ramshini, H. A. and Fazelinasab, B., 2004, Comparative analyses of the genetic diversity among bread wheat genotypes based on RAPD and SSR markers. *Iranian J. Biotech*., 2(3): 585-598.

Najaphy, A., Parchin, R. A. and Farshadfar, E. 2012. Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. *AJCS* 6(2): 326-332.

Nicot, N., Chiquet, V., Gandon, B., Amilhat, L., Legeai, F., Leroy, P., Bernard, M. and Sourdille, P., 2004, Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). *Theor. and Appl. Genet*. 109(4): 800-805.

Prasad, B., Babar M. A., Xu, X. Y., Bai, G. H. and Klatt, A. R., 2009, Genetic diversity in the U.S. hard red winter wheat cultivars as revealed by microsatellite markers. *Crop Pasture Science* 60: 16–24.

Pestsova, E., Ganal, M.W. and Roder, M. S., 2002, Isolation and mapping of microsatellite markers specified for the genome of bread wheat. *Genome* 43: 689-697.

Plaschke, J., Ganaland, M. W. and Röder, M. S., 1995, Detection of genetic diversity in closely related bread awheat using microsatellite markers. *Theor. Appl.Gent*. 91: 1001-1007.

Rafalski, J. A., Vogel, J. M., Morgante, M., Powell, W., Andre, C. and Tingey, S. V.. 1996, Generating and using DNA markers in plants. *In*: Birren B, Lai E (eds) *Nonmammalian genomic analysis. A practical guide.* Academic Press, San Diego, pp 75-134.

Ravi, M., Geethanjali, S., Sameeyafarheen, F. and Maheswaran, M., 2003, Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphy*. 133: 243-252.

Roder, M. S., Korsun, V., Wendehake, K., Plaschke, J., Tixier, M. H., Leroy, P. and Ganal, M. W., 1998, A microsatellite map of wheat. *Genetic*, 149: 2007–2023.

Rohlf, F. J., 2000, NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.1 Exceter Software, New York, USA.

Roussel, V., Koenig, J., Beckert, M. and Balfourier, F., 2004, Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes. *Theor. Appl. Genet*. 108: 920–930.

Roy, J. K., Bandopadhyay, Rustgi, R. S.,

Balyan, H. S. and. Gupta, P. K., 2006, Association analysis of agronomically important traits using SSR, SAMPL and AFLP markers in bread wheat. *Current Science* 90(5): 683-389.

Salem, K. F. M., El-Zanaty, A. M. and Esmail, R. M., 2008, Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatallite markers. *World J. Agril. Sci*. 4(5): 538-544.

Singhamony, S., I. Anees, T. Chandrakala and Osman, Z., 1985, Efficacy of certain indigenous plants products as grain protectants against *S. oryzae* (L.) and *R. dominica* (F). *Journal of Stored Products Research* 22(10): 21-23.

Sambrook, J. and Russel, D. W., 2001, Molecular cloning: A laboratory manual. *Cold Spring Harbor Laboratory Press*, Cold Spring Harbor, New York.

Stodart, B. J., Mackay, M. and Raman, H., 2005, AFLP and SSR analysis of genetic diversity among landraces of bread wheat (*Triticum aestivum* L. em. Thell) from different geographic regions. *Australian Journal of Agricultural Research* 56(7): 691-697.

XinMin, C., ZhongHu, He., JanRong, Shi., LanQin, Xia., Ward, R., Zhou, Yang and Jiang, GuoLiang, 2003, Genetic diversity of high quality winter wheat varieties (lines) based on SSR markers. *Acta Agronomica Sinica*, 29(1): 13-19.

You, G. X., Zhang, X. Y. and Wang, L. F., 2004, An estimation of the minimum number of SSR loci needed to reveal genetic relationships in wheat varieties: Information from 96 random accessions with maximized genetic diversity. *Molecular Breeding* 14(4): 397-406.

Yildirim, A., Sonmezoglu, O. A., Gokmen, S., Kandemir, N. and Aydin, N., 2011, Determination of genetic diversity among Turkish durum wheat landraces by microsatellites. *African Journal of Biotechnology* 10(19): 3915-3920.

Zeb, B., Khan, I. M., Ali, S., Bacha, S., Mumtaz, S. and Swati, Z. A., 2009, Study on genetic diversity in Pakistani wheat varieties using simple sequence repeat (SSR) markers. *African Journal of Biotechnology* 8(17): 4016-4019.

Zhong, F. N., Zhang, Y. R., Liang, R. Q., Liu, G. T. and Sun, Q. X., 2003, Genetic diversity of D-genome revealed by SSR markers in wheat (*Triticum aestivum* L.). *Acta Agronomica Sinica* 29(1): 145-151.

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