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STUDY OF GENETIC DIVERSITY IN SYNTHETIC HEXAPLOID WHEATS USING RANDOM AMPLIFIED POLYMORPHIC DNA

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ABSTRACT

Ten Synthetic Hexaploid Wheats (SHWs) (2n = 6x = 42, AABBDD) were analyzed using 7 Randomly Amplified Polymorphic DNA (RAPD) primers to investigate the potential genetic diversity resided within the D-genome of common wheat. Total 190 fragments were amplified in which 141 were polymorphic showing 74 % of polymorphism. Polymorphism Information Content (PIC), Genetic Similarity (GS) Coefficient, and Principle Component Analysis (PCA) showed that D-genome of wheat contain sufficient genetic diversity. Cluster analysis based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) grouped the genotypes into two main clusters with similarity of 0.44 to 0.80 with an average of 0.62. Analysis of variance (ANOVA) exhibited ample amount of variability among the genotypes. The results indicated that synthetic hexaploid wheats are an efficient way to use the genetic variation of D-genome from *Aegilops Squarrosa* for wheat improvement. The results further established the efficacy and usefulness of RAPD marker for evaluation of genetic polymorphism and diversity present within Synthetic Hexaploid Wheats.

Key words: Genetic diversity; D-genome; Synthetic hexaploid wheat; Polymorphism.

INTRODUCTION

The presence of sufficient genetic diversity in wheat germplasm is an important prerequisite for use in plant breeding and varieties development. Genetic and phenotypic diversity is indispensable for assessment, conservation and development of genetic resources. It is also helpful to determine genetic composition of genotypes and to analyze diversity of pre-breeding and breeding germlines. For selection purpose, identification of various ancestral combinations and production of segregating progeny, high genetic diversity is crucial. Analysis of wheat's genomic diversity is significant to identify parental combinations and create individuals containing high genomic diversity (Cox *et al.,* 1985).

Molecular markers based novel genetic tools have evolved which permit precise genotyping to investigate polymorphism in different crops (Varshney *et al.,* 2005; Khlestkina and Salina, 2006). Morphological traits are not suitable to evaluate germplasm as they are influenced by environmental factors. To assess genetic diversity, molecular markers are used preferably as compared to morphological traits as they are not influenced by environmental factors. Therefore, molecular markers can be used to characterize a large amount of germplasm in a short period of time (Perry and Mclntosh, 1991; Masood *et al.,* 2000).

RAPD has been used successfully to measure genetic diversity within germplasm collections through DNA profiling of various cultivars (Hernández *et al.,* 2001). They are dominant markers and have been successfully used for gene mapping (Chalmers *et al.,* 2001) and detection of specific traits linked loci (Sun *et al.,* 2003). However, it faced problems of reproducibility yet its simplicity and rapidity make it useful for evaluation of diversity and identification of genotypes in different crops (Abdellatif and Khidar, 2010). RAPD has reported to be used for finding variations in different varieties of *Triticum* (Gupta *et al.,* 2000) and to identify cultivars (Malik *et al.,* 1996).

Common wheat has low level of genetic polymorphism and hence limited genetic diversity. In order to broaden its genetic base, identification and use of wild relative for crossing is indispensable. SHWs produced by inter-specific hybridization of *T. turgidum* and *Ae. tauschii* followed by chromosome doubling, provided an effective means to transfer genes across ploidy levels, particularly from the wild diploid D-genome progenitor *Ae. Tauschii* accessions (Mujeeb-Kazi *et al.,* 1996). These are now well recognized sources of enhanced diversity for resistance/tolerance to many biotic and abiotic stresses, which can be used for improvement of common wheat (Mujeeb-Kazi *et al.,* 2008). Estimation of genetic diversity in SHWs is of paramount importance for improvement of common wheat. SHWs has already been investigated for its genetic diversity using RAPD (Saffdar *et al.,* 2009; Shah *et al.,* 2009; Tariq *et al.,* 2012; Shakeel *et al.,* 2013), SSR (Pestsova *et al.,* 2000; Roder *et al.,* 2002; Chen *et al.,* 2007), EST-SSR (Zhang *et al.,* 2005) and AFLP (Lage *et al.,* 2003). The present study aims to evaluate genetic diversity within ten SHWs derived from the same durum parent 'Decoy' using 7 RAPD primers.

MATERIALS AND METHODS

Plant Materials: Seeds of 10 different SHWs were obtained from National Agriculture Research Center (NARC) Islamabad (Table 1).

RAPD Analysis: Genomic DNA was isolated from 2 weak old seedling using CTAB method (Richard, 1997) and its quality was confirmed using 1 % agarose gel. PCR was carried out using 7 RAPD primers of OPC series (Table 2). PCR conditions used were initial denaturation at 94 °C for 5 minute followed by 44 cycles of denaturation at 94 °C for 30 seconds, annealing for 30 seconds at 55 °C, extension at 72 °C for two minutes and then final extension at 72°C for 20 minutes. The PCR mixture contained 1 µl of template, 1 µl (50 pM) of primer, 10.5 µl of nuclease free water and 12.5 µl of Master Mix (MBI Fermentas). Temperature cycling was performed using a thermal cycler (Multigene of Labanet international), amplified products were analyzed through gel electrophoresis on 1.5 % agarose gel stained with Ethidium Bromide and observed under Dolphin Doc ^{plus} gel documentation system.

Data Analysis: RAPD data was scored as 1 for presence and 0 absence of band. The coefficient of similarity among SHWs was determined according to Nei and Li's (1979) method. Dendrogram based on similarity index was constructed using Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) (Rohlf, 1997). The PIC value was calculated as:

$$PIC = 1-\sum P_{ij}^2$$

Where P_{ij} is the frequency of i^{th} allele in j^{th} population for each locus (Bostein *et al.,* 1980). Principle Component Analysis was performed using software Past. ANOVA was determined to calculate variability using Statistical Package for the Social Sciences (SPSS 16.0).

RESULTS AND DISCUSSION

RAPD Analysis: In RAPD analysis, 10 primers of OPC series were screened, only 7 primers gave reproducible and countable amplification. Total number of bands, number of monomorphic and polymorphic bands, polymorphism, molecular size and number of amplified fragments along with their average were calculated for each primer across ten SHWs (Table 2). Average value of polymorphic band per primer was 20 which is accordance to the earlier result of 24.35 by Maric *et al.* (2004). Average polymorphism in this study was 73.68 %. Accordingly, Naghavi *et al*. (2004) revealed a polymorphism of 88 %, while a high degree of polymorphism of 93.5 % was reported by Cenkci *et al*. (2007). Moreover, Bhutta (2006) and Shoaib and Arabi (2006) reported a low level of polymorphism showing 46.97 % and 46.67 % respectively. Difference in level of polymorphism may be due to difference in number of germplasm and primer used.

Dendrogram Analysis: Similarity indices were constructed based on amplified products of 10 SHWs using 7 RAPD primers (Table 3). The genetic similarity values ranged from 0.44 to 0.80 with an average of 0.62. These results are in close agreement to that of Mahmood *et al*. (2011) who investigated that similarity coefficient ranged from 0.36-0.93 with a mean of 0.64 across 15 Pakistani wheat cultivars and dendrogram contained two main clusters. Dendrogram divided the SHWs into two main cluster 1 with six genotypes which show 63 % similarity and cluster 2 with only one genotype showing similarity of 54 %. It was observed that SH-381 have shown more diversity among all studied accessions based on OPC analysis (Fig 1). This study showed overall genetic diversity of 38 %. Previously, Saffdar *et al*. (2009) reported genetic diversity of 46 % in 33 elite SHWs using 10 RAPD primers, Shah *et al*. (2009) described genetic diversity of 69 % in 53 SHW derived genotypes of Richard's selection, Tariq *et al*. (2012) reported genetic diversity of 83 % in 33 SHWs using 15 RAPD primers and Shakeel *et al*. (2013) reported genetic diversity of 48 % in 24 SHWs using 10 RAPD primers. The variation in polymorphism may be due to difference in number of germplasm, primer and diverse origin of SHWs that were used in different studies.

Polymorphic information Content: PIC analysis was performed to determine Allelic Frequency, number of amplified alleles per primer and Gene diversity for each primer as given in Table 4. Its values ranged from 0.59 (OPC-2) to 0.74 (OPC-4, OP-6 and OPC-9) with an average of 0.69 in comparison to the result investigated by Cifci and Yagdi (2012) who determined PIC values ranging from 0.11 to 0.93 with an average of 0.59. Low range of PIC value may be due to low number of studied germplasm and used primers.

Principle Component Analysis: It divided the genotypes into four groups in contrast to cluster analysis which grouped genotypes into two main clusters (Fig 2) which is in contrast to the Previous study of Cifci and Yagdi (2012) that studied 16 wheat genotypes and reported that all genotypes under their study grouped into two clusters based on cluster analysis whereas PCA analysis grouped them into five groups. In comparison PCA analysis and cluster analysis have shown nearly similar results except PCA analysis grouped the genotype SH-381 together with SH-323 whereas cluster analysis grouped SH-381 separately. This variation may be due to the fact that bands of similar molecular sizes may have different origin.

Analysis of Variance: It was performed for SHWs in terms of amplified fragments by RAPD primers showed significant values for SH-907, SH-401, SH-323 and SH-

618 (Table 5). However, a low variability of band distribution was observed in SH-131 and SH-678. Previously similar results were found by Ciulca *et al*. (2010) while studying genetic variations in some winter barley cultivars using RAPD analysis.

Table 1. Detail of accession number and genetic background of ten SHWs.

S. No	Name	Pedigree
1	SH-401	DOY1 / AE. SQUARROSA (372) CIGM 93.229
2	SH-907	DOY1 / AE. SQUARROSA (1067) CASS 02B00008S
3	SH-381	DOY1 / AE. SQUARROSA (258) CIGM 93.207
4	SH-323	DOY1 / AE. SQUARROSA (333) CIGM 92.168
5	SH-373	DOY1 / AE. SQUARROSA (177) CIGM 93.187
6	SH-349	DOY1 / AE. SQUARROSA (458) CIGM 92.1727
7	SH-131	DOY1 / AE. SQUARROSA (1024) CIGM 92.173
8	SH-403	DOY1 / AE. SQUARROS (390) CIGM 93.385
9	SH-618	DOY1 / AE. SQUARROSA (172) CASW 98B00004S
10	SH-678	DOY1 / AE. SQUARROSA (1010) CIGM 93.208

Table 2. List of primers along with their sequences, obtained amplified band size, total bands, polymorphic and monomorphic bands.

Primers	Sequence(5'→3')	Band Size bp	Total Bands	Monomorphic Bands	Polymorphic Bands	Polymorphism %
OPC-1	TTCGAGCCAG	350-1350	27	10	17	63
OPC-2	GTGAGGCGTC	300-800	15	0	15	100
OPC-4	CCGCATCTAC	280-850	21	0	21	100
OPC-5	GATGACCGCC	350-1250	37	20	17	46
OPC-6	GAACGGACTC	380-1300	34	10	24	70
OPC-9	CTCACCCTCC	500-1500	34	10	24	70
OPC-10	TGTCTGGGTG	300-1600	22	0	22	100
Total			**190**	**50**	**140**	**73.68**
Mean			**27.14**	**7.14**	**20**	**78.42**

Table 3. Genetic Similarity Coefficient of SHWs.

G	1	2	3	4	5	6	7	8	9	10
1	1									
2	0.76	1								
3	0.49	0.51	1							
4	0.62	0.6	0.69	1						
5	0.69	0.58	0.44	0.58	1					
6	0.67	0.64	0.51	0.64	0.67	1				
7	0.51	0.53	0.53	0.67	0.64	0.67	1			
8	0.67	0.64	0.6	0.69	0.8	0.69	0.71	1		
9	0.76	0.73	0.47	0.73	0.8	0.64	0.62	0.78	1	
10	0.51	0.53	0.62	0.8	0.6	0.67	0.73	0.71	0.71	1

1: SH-401, 2: SH-907, 3: SH-381, 4: SH-323, SH-373, 6: SH-349, 7: SH-131, 8: SH-403,
10: SH-678

Table 4. List of RAPD primers, Major Allele Frequency, Allele number, Gene Diversity and Polymorphic Information Contents.

Primer	Major allele Frequency	Allele Number	Gene diversity	PIC
OPC-1	0.5	4	0.66	0.61
OPC-2	0.4	4	0.66	0.59
OPC-4	0.3	5	0.78	0.74
OPC-5	0.4	5	0.74	0.70
OPC-6	0.3	5	0.78	0.74
OPC-9	0.3	5	0.78	0.74
OPC-10	0.4	5	0.74	0.70
Mean	0.37	4.7	0.73	0.69

Table 5. Analysis of Variance for SHWs concerning different bands produced by RAPD Primers.

No.	Cultivar	Between groups		Within groups		F-test	*P*-value
		SS	DF	SS	DF		
1	SH-401	3.103	6	5.208	38	3.77	0.0001
2	SH-907	4.886	6	5.425	38	5.70	0.0000
3	SH-381	2.194	6	7.050	38	1.97	0.0244
4	SH-323	2.903	6	8.208	38	2.24	0.0000
5	SH-373	2.092	6	7.908	38	1.68	0.0464
6	SH-349	1.403	6	8.908	38	1.00	0.1587
7	SH-131	0.600	6	10.200	38	0.37	0.3557
8	SH-403	2.117	6	8.683	38	1.54	0.0618
9	SH-618	2.350	6	6.450	38	2.31	0.0000
10	SH-678	1.361	6	9.883	38	0.87	0.1922

(SS-sum of square, DF-degree of freedom, *p*- value of significance)

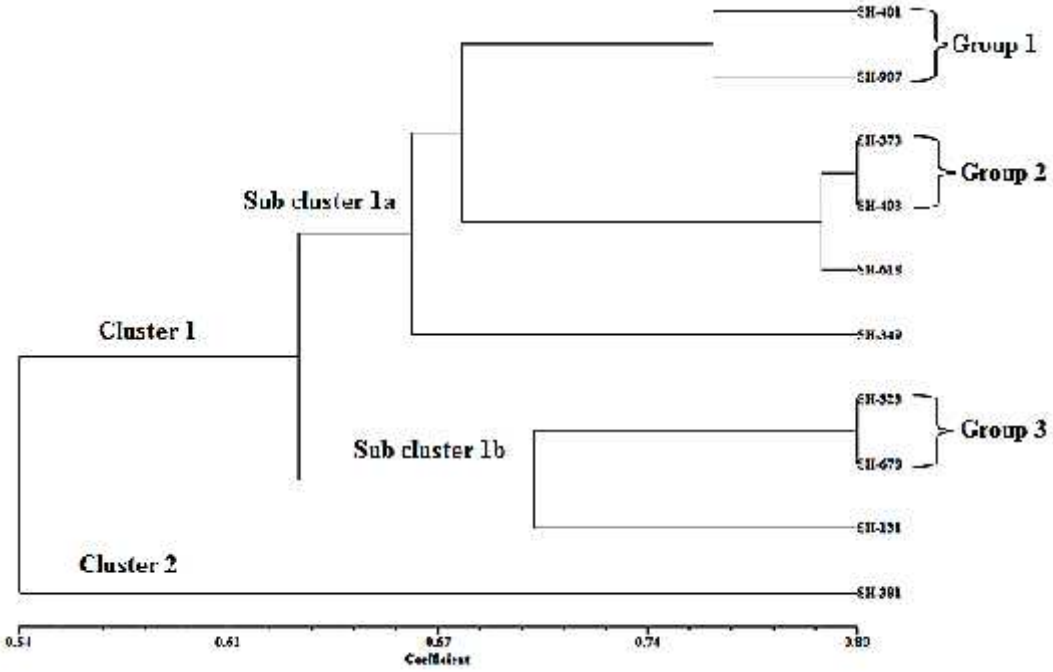

Figure. 1. Dendrogram showing phylogenetic relationship among 10 different SHWs

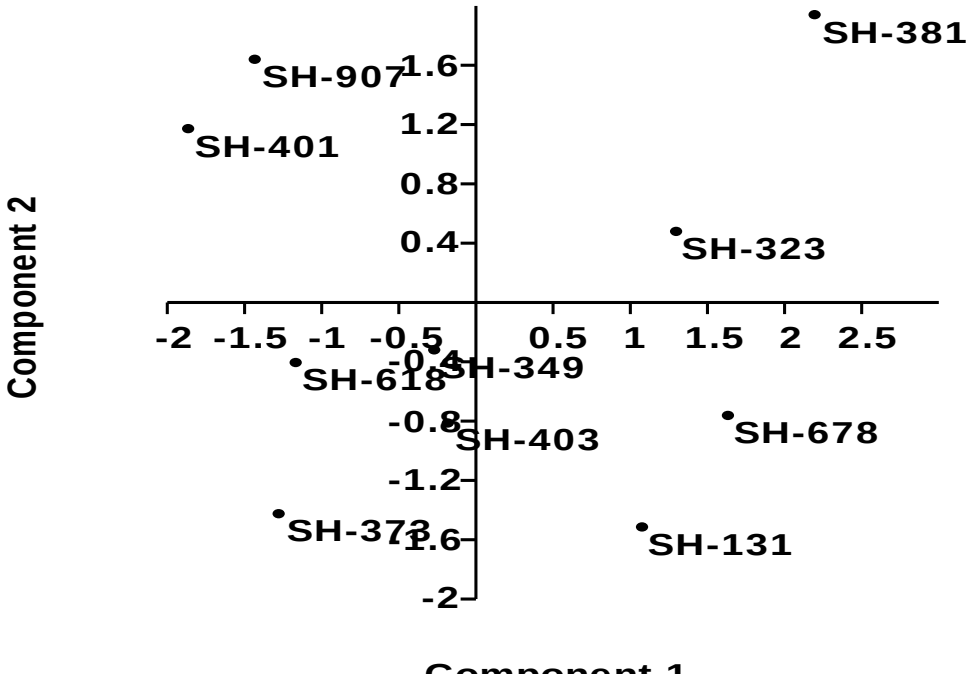

Figure. 2. Principle Component Analysis of RAPD for ten SHWs.

Conclusion: SHWs emerged as a novel source of genetic variation in wheat that can be successfully exploited in crop breeding system. Overall, 74% of polymorphisms were found in the D-genome of these genotype suggesting high genetic diversity. However some SHWs including SH-331, SH-349, SH-381, and SH-618 were genetically more diverse which can be used in breeding program for wheat improvement. Further the study suggested that RAPD along with some other useful analysis can be used as an effective strategy for estimation of genetic diversity.

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