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MOLECULAR EVALUATION OF D GENOME BASED DOUBLE HAPLOID MAPPING POPULATION OF WHEAT USING SSR PRIMERS

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ABSTRACT

A molecular diversity analysis of 20 double haploid (DH) plants from a double haploid Mapping Population (MP, its parents, *i.e.*, Opata M-85 (drought susceptible, 2n=6x=42) and a Synthetic Hexaploid (SH-257, 2n=6x=42, drought tolerant) and local drought tolerant cultivars Nesser, Zarghoon and Margalla was carried out using simple sequence repeats (SSR) primers designed for D genome of Wheat. A total of 59 SSR primers yielded a total of 177 polymorphic bands in the size ranged from 50 to 900 bp. Cluster analyses demonstrated that the genotypes from MP were genetically distinct from local drought tolerant cultivars and its parents Opata M-85 and SH-257. The 7 best DHs of the mapping population with ample genetic distance were 2 (85.80%), 9 and 15 (74.19%), 18 and 20 (66.67%), 1 (63.00%) and 14 (62.31%) and the genetic distance of these DHs was almost similar or better than the genetic distance of SH-257 (65.20%). Genetic distance of local drought tolerant cultivars; Nesser (42.17%), Margalla-99 (42.17%) and Zarghoon (58.49%) was less than SH-257(65.20%). Opata M-85 with genetic distance of 83.49% was found to be distinct from SH-256 and its population. Ample diversity in double haploid mapping populations of wheat can be of tremendous use for wheat improvement.

Key words: SSRs, morphological and molecular diversity, synthetic wheats, double haploid` mapping population, drought tolerance.

INTRODUCTION

Wheat improvement relies mainly upon the genetic diversity which provides alleles that plant breeders incorporate into their recombination-breeding programs. A significant genetic diversity had been observed in the D genome of *Ae. Tauschii* populations compared with the D genome of *T. aestivum* through molecular markers (Dvorak *et al.*, 1998). The wild D genome has been exploited for wide crossing and applied to increase yield under drought (Trethowan *et al.*, 2003) and there is already evidence for impact in drier regions worldwide based on data from recent international drought trials (Trethowan & Reynolds 2006). In the field of plant biotechnology,

double haploid plant (DH) production is worthwhile because it permits breeders to collect homozygous lines from hybrid directly (Barakat *et al*., 2012). A double haploid Mapping Population (MP) has been developed by crossing drought susceptible *T. aestivum* (Opata M-85) with drought tolerant synthetic hexaploid (SH-257) yielding F_1 hybrids which are polymorphic. The F_1 is then crossed with maize to develop haploids. These haploids are then doubled with colchicine. These doubled haploid plants form double haploid mapping population with has the pedigree; D67.2/P66.270//Ae. Squarrosa (257)/ 3/Opata (Mujeeb- Kazi *et al.*, 2004).

Morphological traits based genetic diversity suffered from drawbacks that are influenced by climatic condition and traits number is quite limited (Maric *et al*., 2004). On other hand genetic diversity based on molecular markers do not require early pedigree information, abundant in number and cannot be influenced by environment (Bohn *et al*., 1999). Molecular markers are utilized for genetic diversity and genetic variation analysis (Baldwin, *et al.*, 2012; Park, *et al.*, 2009 and Dos Santos, *et al*., 2012), fingerprinting of genotype (Figueiredo, 2013 and Hameed, 2012), for genotypic identification (Yu, *et al*.,2013 and Zhang, 2012), for marker assisted selection, required mapping genes (20-23) and linkage maps construction (Zhang *et al.*, 2012 Schouten, *et al.*, 2012). Molecular markers provide an opportunity for scanning plant genotypes at molecular level and to pyramid genes of interest into breeding lines (Hussein, 2008, Ibrahim, 2009 & Bux *et al*., 2012). Among the markers, microsatellite or SSRs are broadly utilized for genetic diversity studies of wheat crop. These are evenly distributed in chromosomes, multiallelic, chromosome-specific and QTL mapping (Barakat *et al*., 2011). SSRs provide highly informative markers as they are co-dominant and have high polymorphic information content (Gupta *et al.*, 1996). The objective of current study was, therefore, to analyze the genetic diversity in a doubled-haploid (DH) population using SSR (microsatellite) markers.

MATERIALS AND METHODS

Plant materials: The germplasms were collected from Wheat Wide Crosses (WWC) and Cytogenetics programme, located at National Agricultural Research Centre (NARC), Islamabad. The double haploid (DH) mapping population was derived from a cross between the variety Opata 85 and the synthetic hexaploid wheat SH257. The parents of SH527 were *Aegilops squarrosa* accession 257 and durum (tetraploid) wheat variety D67.2/P66. 270. Drought tolerant cultivars Nesser, Zarghoon and Margalla-99 were also added in the set.

Molecular analysis: Genomic DNA was isolated from fresh leaf tissues of seedlings using the Cetyl-trimethyl Ammonium Bromide (CTAB) method with some modify-cations (Murray and Thompson, 1980). The

germplasm was evaluated for molecular diversity using 59 SSR primers (Table 1) (Roder *et al.*, 1998). PCR was performed in a Gene Amp (R) PCR System 9700 Thermo-cycler. PCR reaction was carried out in 25µl reaction mixture consisted of final concentration of 0.4µM each primer, 0.2mM each of dCTP, dGTP, dTTP and dATP (Sigma Chemical Co., St. Louis, MO, USA), 2.5mM $MgCl_2$, 10X PCR Buffer, 1unit/µl of *Taq* DNA polymerase (Promega Madison WI, USA), and 2ng/µl genomic DNA. PCR programme was followed by procedures as published earlier) (Roder *et al.*, 1998).

Table - 1: SSR primers used for genetic analysis.

S. No.	Locus/Primer	S. No.	Locus/primer	S. No.	Locus/ Primer
1	*Xgwm 2-3D*	21	*Xgwm194-4D*	41	*Xgwm358-5D*
2	*Xgwm3-3D*	22	*Xgwm205-5D*	42	*Xgwm383-3D*
3	*Xgwm16-5D*	23	*Xgwm210-2D*	43	*Xgwm428-7D*
4	*Xgwm30-2D*	24	*Xgwm212-5D*	44	*Xgwm437-7D*
5	*Xgwm33-1D*	25	*Xgwm232-1D*	45	*Xgwm455-2D*
6	*Xgwm37-7D*	26	*Xgwm249-2D*	46	*Xgwm456-3D*
7	*Xgwm52-3D*	27	*Xgwm261-2D*	47	*Xgwm458-1D*
8	*Xgwm55-6D*	28	*Xgwm269-5D*	48	*Xgwm497-3D*
9	*Xgwm71-3D*	29	*Xgwm271-5D*	49	*Xgwm539-2D*
10	*Xgwm102-2D*	30	*Xgwm272-5D*	50	*Xgwm565-5D*
11	*Xgwm111-7D*	31	*Xgwm292-5D*	51	*Xgwm583-5D*
12	*Xgwm121-7D*	32	*Xgwm295-7D*	52	*Xgwm608-2D*
13	*Xgwm157-2D*	33	*Xgwm296-2D*	53	*Xgwm608-4D*
14	*Xgwm161-3D*	34	*Xgwm314-3D*	54	*Xgwm624-4D*
15	*Xgwm165-4D*	35	*Xgwm320-2D*	55	*Xgwm635-7D*
16	*Xgwm174-5D*	36	*Xgwm325-6D*	56	*Xgwm642-1D*
17	*Xgwm182-5D*	37	*Xgwm337-1D*	57	*Xgwm645-3D*
18	*Xgwm183-3D*	38	*Xgwm341-3D*	58	*Xgwm654-5D*
19	*Xgwm190-5D*	39	*Xgwm349-2D*	59	*Xgwm664-3D*
20	*Xgwm192-5D*	40	*Xgwm350-7D*		

Data analysis: Clusters were constructed on molecular data using NTSYSpc software (version 2.02a, Applied Biostatistics Inc., New York, NY). Binary (0 or 1) data were generated to construct dendrogram.

RESULTS

The 59 SSR primers yielded a total of 177 polymorphic bands in the size range of 50 bp to 900 bp. The highest number of scorable bands was obtained with primer Xgwm 174-5D (40), while the lowest numbers were obtained with primer Xgwm 2-3D (2). Maximum genotypes (19) were amplified by both primers Xgwm 261-2D and Xgwm 271-5D and minimum (1) by Xgwm 2-3D. Different primers showed variation in their ability to detect polymorphism. Primer Xgwm 232-1D and 349-2D showed highest polymorphism with 5 polymorphic bands each.

The SSR amplification data was used to obtain a similarity matrix (Matrix not shown). The value of similarity coefficients ranged from 0 to 70 percent. Genotypes which showed zero percent similarity were; 1 with 2, 7, 19 and Opata M-85, 2 with 5, 6, 8, 14, 16, Nesser, Zarghoon, Margalla and Opata M-85, 3 with 7 and Opata M-85, 4 with 7, 5 with 7, 7 with 9, 11, 16, 17, 20, Opata M-85 and SH-257, 15 with 18 and 19, 19 with 20 and SH-257, 20 with Zarghoon. Genotypes showing 70 percent similarity were 5 and 6. The similarity of remaining genotypes lies between 0 to 70 percent.

Dendrogram formulated on the basis of genetic distance was divided into two main clusters A and B (**Figure 1**). The cluster B was again divided into four sub clusters named as B1, B2, B3 and B4. Cluster A included five genotypes all of which belonged to MP. DH2 had maximum genetic distance of 85.80% with all other four genotypes of this cluster hence rated as most diverse. It was followed by sub-cluster B1 with three genotypes in which the DHs 9 and 15 with genetic distance of 74.19%, were genetically similar to each other. Opata M-85 with maximum genetic distance of 83.49% was most diverse in this sub cluster. In sub cluster B2 having four genotypes, the most diverse genotype was SH-257 showing maximum genetic distance of 65.20% with the rest of genotypes. In sub cluster B3 with nine genotypes, DHs 5 and 6 were genetically similar to each other. The most diverse double haploid was 14 with maximum genetic distance of 62.31%. The local drought tolerant cultivars Nesser and Margalla were genetically similar while Zarghoon was distinct from them.

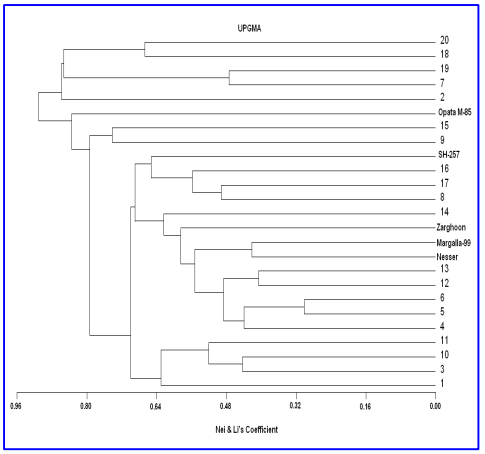

Figure-1: Dendrogram based on genetic distance in Double haploid mapping population of wheat

In sub cluster B4 with four genotypes, DH 1 with maximum genetic distance of 63.00% was most diverse from the rest of the genotypes. The DHs 10 and 3 were genetically similar to each other.

By observing the above mentioned data, it was established that the best DHs of the mapping population with high molecular diversity were 2 (85.80%), 9 and 15 (74.19%), 18 and 20 (66.67%), 1 (63.00%) and 14 (62.31%) and the genetic distance of these DHs was almost similar or better than the genetic distance of SH-257 (65.20%) parent. Genetic distance of local drought tolerant cultivars as Nesser (42.17%), Margalla-99 (42.17%) and Zarghoon (58.49%) was less than SH-257. Finally DHs 2, 9, 14 and 18 with good morphological traits (data not shown here) and molecular diversity are recommended for wheat yield improvement programs in Pakistan.

DISCUSSION

Food production of the world is limited primarily by environmental stresses (biotic and abiotic). Drought being one of the most common environmental stresses affects growth and development of plants through alterations in metabolism and gene expression (Leopold 1990). The arable lands for agriculture are lost annually and water is becoming a scarce (Barghouti, 1999). Domestication and modern plant breeding have presumably narrowed the genetic base of bread wheat (Tanksley & McCouch 1997). Cultivation of germplasm with a narrow genetic base entails a risk due to genetic vulnerability. Synthetic hexaploids wheats are useful for introducing not only disease and insect resistance, but also drought tolerance genes (Trethowan *et al.*, 2003).

Microsatellites have been considered to be the markers of choice for assessment of wheat genetic diversity among cultivars and their wild relative (Borislav *et al*., 2002). Zhang *et al*., (2002) also revealed high genetic diversity in wheat using SSRs. Likewise Dreisigacker *et al.,* (2004) also revealed that SSRs represent a powerful tool to quantify genetic diversity in wheat. Eujayl *et al*., (2001) set up similar results for genotypic variation in 64 durum lines, landraces and varieties using SSRs.

The dendrogram generated on the basis of SSR data revealed that there are 7 DHs of the mapping population having much molecular diversity. These were 2 (85.80%), 9 and 15 (74.19 %), 18 and 20 (66.67%), 1 (63.00%) and 14 (62.31%) and the genetic distance of these double haploids was almost similar or better than the genetic distance of SH-257 (65.20%). Genetic distance of local drought tolerant cultivars as Nesser (42.17%), Margalla-99 (42.17%) and Zarghoon (58.49%) was less than SH-257.

CONCLUSION

Conclusively, genotypes; DH1, DH 2, DH 9, DH 14, DH 18 and DH 20 possess sufficient molecular diversity and are recommended for wheat yield improvement programs in

Pakistan. The amount of genetic diversity in wheat genotypes is probably due to the D genome of synthetic wheat. The results suggested that the use of synthetic hexaploid wheat is an efficient way to enrich wheat for wheat improvement. The results also demonstrated the utility of microsatellite markers in detecting DNA polymorphism and estimating genetic diversity is promising.

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