Pak. J. Bot., 44(3): 1021-1028, 2012.

MOLECULAR AND MORPHOLOGICAL DIVERSITY WITH BIOTIC STRESS RESISTANCES OF HIGH 1000-GRAIN WEIGHT SYNTHETIC HEXAPLOID WHEATS

ALVINA GUL KAZI[1], AWAIS RASHEED[2*], TARIQ MAHMOOD[2], ABDUL MUJEEB-KAZI[3]

[1]*Atta-ur-Rehman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST), Islamabad, Pakistan*
[2]*Plant Biochemistry and Molecular Biology Lab, Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan*
[3]*National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan;*
**Corresponding author E-mail: awaispbg@gmail.com*

Abstract

Accumulation of favorable characteristics from the genetic resources of *Triticeae* and their exploitation for bread wheat improvement has always been the main thrust of wheat breeding programs. Harnessing the genetic diversity for yield contributing characteristics has recently diverted the attention of wheat breeders to utilize synthetic hexaploids (SHs) in their programs where one factor being exploited is the high thousand grain weight diversity present in SHs. This requires generating a wide array of data for economically important descriptors which will ultimately permit the acceptance of the synthetic hexaploids with favorable traits. In the present investigation synthetic hexaploids having higher grain weight are characterized and their genetic diversity elucidated to prove that synthetics with higher 1000-grain weight had different genetic makeup to substantiate the view that utilization of SHs for yield improvement will widen the genetic base of the bred derivatives leading to future varietal development. Of the morphological parameters days to flowering/days to physiological maturity and plant height are important for plant selection and progeny advance. Coupled with 1000 kernel weight diversity with these parameters synthetics have been identified for breeding utilization to cover both irrigated and rainfed (drought) cultivation areas. All the synthetics studied had karnal bunt resistance and 62% possessed resistance to prevailing strains of stripe rust which make them available to improve the yield potential of bread wheat in areas where these two biotic stresses are wheat production constraints. Further the utilization approach of synthetic hexaploids for wheat improvement is also discussed.

Introduction

Bread wheat is one of the most important global food crops. To meet the food demands of the increasing world population, it is estimated that about 40% increase in wheat production is required before 2020 (Rajaram, 2005). Therefore the achievement of higher yield is always a predominant objective of wheat breeding programs. The yield of wheat is determined by number of spikes per plant, number of grains per spike and grain weight. Grain weight is one of the most important yield contributing traits and has been an important selection criteria of higher yielding plants (Roder *et al*., 2008). Grain weight is usually represented in plant breeding programs by thousand grain weight (TGW) and is determined by grain length, grain width and grain thickness (Campbell, 1999). The physiological factors controlling grain weight in wheat were resolved to some extent by Brocklehurst (1977) who reported that grain weight was mainly dependent on the rate of accumulation of dry matter, which in turn was governed by the number of endosperm cells formed. These cell numbers in the endosperm seemed to be regulated by supply of assimilates available to the grain during the first two weeks after anthesis.

Synthetic hexaploid wheats (SHWs) are the products of artificial crossing between *Triticum turgidum* L. (2n=4x=28; AABB) and *Aegilops tauschii* Coss. (2n=2x=14; DD) accessions, the evolutionary progenitor of common bread wheat (Mujeeb-Kazi *et al*., 1996). Due to the same genomic constitution both synthetic hexaploids and bread wheats can be readily crossed. This makes synthetic hexaploids a unique germplasm resource for bread wheat breeding. Synthetic hexaploids can act as a vehicle for introducing specific characters from these numerous D-genome progenitor accessions into bread wheat backgrounds. The hybridization events that formed bread wheat are thought to be limited, thus the genetic diversity within the synthetic hexaploid wheats possesses novel alleles and genes for biotic, abiotic stresse tolerances and as well as for grain quality traits (Bibi *et al*., 2012; Rasheed *et al.*, 2012), not currently represented within the bread wheat gene pool. Increased grain size in bread wheat has a favorable effect on milling yield. Large grains have higher endosperm to surface area ratios, improving milling yields with reduced by-products. Increases in grain yield over the last 40 years have come partly from the use of gibberellic acid-sensitive dwarfing genes (*Rht-1* and *Rht-2*), which were globally distributed during the 'green revolution'. In addition to reducing plant height, these genes increase seed set and the number of kernels per m^2 compared to normal stature wheats. These changes have been accompanied by reductions in grain size. Synthetic hexaploids have been proposed as sources of genetic material for the improvement of thousand grain weight in bread wheat breeding (Calderini *et al*., 2003). Some synthetic hexaploids have also achieved yields similar to those of check cultivars under drought stress (Trethowan & Mujeeb-Kazi 2008). Backcrossing and top-crossing strategies have been employed to exploit primary synthetic hexaploids for these characters in both elite CIMMYT and local bread wheat backgrounds (Dreccer *et al*., 2007).

The objective of this study was to investigate the diversity in synthetic hexaploids with high grain weight and evaluate these SHs for karnal bunt and stripe rust resistance at the seedling and adult plant stages. The findings presented here will help identify SHs with good agronomic features, resistant to karnal bunt and stripe rust thus suitable as sources of new genetic diversity for wheat breeding focusing on yield enhancement targeted on high 1000 kernel weight.

Materials and Methods

Plant material: The Elite-1 subset collection comprised of 95 primary synthetic hexaploid wheats derived from the cross combinations of 34 durum wheats and 74 *Ae. tauschii* accessions. The synthetic production protocol has been reported earlier (Mujeeb-Kazi *et al*., 1996). From the elite-1 entries 37 synthetic hexaploids with high grain weight were selected.

Disease scoring

Karnal bunt: The Elite-1 sub-set of SHs was screened for Karnal bunt in the fields of National Agricultural Research Center (NARC), Islamabad. Spike inoculations were done on five tillers taken at random from each entry at the boot stage (Zadoks *et al*., 1974**)** by injecting 1 ml/tiller of the sporidial suspension with a hypodermic syringe. At maturity, each inoculated spike was graded for infection (Warham *et al*., 1985) and the overall percentage infection calculated for each entry (Mujeeb-Kazi *et al*., 2007).

Stripe rust: Seedling tests were conducted under glass house conditions with stripe rust race having the virulence/avirulence formula *Yr3, Yr5, Yr8, Yr10, Yr15, YrSp, YrCV/ Yr1, Yr2, Yr6, Yr7, Yr9, Yr17, Yr18, Yr24, Yr26, Yr28, Yr29, Yr31*. Infection types were recorded three weeks after inculation on a 0-9 scale (McNeal *et al*., 1971), when susceptible check "Morocco" showed showing maximum infection. Plants having infection types 0-3 were considered as resistant, 4-6 as intermediate resistant and those having infection types 7-9 were highly susceptible. The Elite-1 high kernel weight lines were also subjected to stripe rust screening according to Ma *et al*., (1995) in the fields of National Agricultural Research Center (NARC), Islamabad. Data for disease severity and infection types were recorded when the susceptible check (Morocco) had reached a 100% severity. The severity was recorded as percent of the rust infection on the plants according to the modified Cobb scale (Peterson *et al*., 1948).

Total genomic DNA isolation: 10 cm long pieces of fresh leaf material were collected, frozen in liquid nitrogen and ground to a fine powder. 500µl DNA extraction buffer was added and mixed well. 500µl phenol: chloroform: isoamylalcohol (25:24:1) was added and well shaken. The eppendorf tubes were centrifuged for 3 minutes and the supernatant was transferred to a new tube. 500µl cold chloroform was added and mixed gently. It was centrifuged for 1 minute and the supernatant was transferred to a fresh tube. 50µl 3M sodium acetate (pH = 4.8) and 500µl isopropanol were added, mixed gently and centrifuged for 5 minutes. The supernatant was poured off and the pellet washed with 70% ethanol. The pellet was dried and resuspended in 50µl TE or double distilled water. After treatment with RNase the DNA concentration was measured by fluorometer DyNA Quant^{TM} 200. The total genomic DNA was diluted in double distilled water to a concentration of 5.0 ng/µl for PCR analysis.

PCR conditions and gel electrophoresis: PCR was performed in a 25µl reaction volume containing 10mM Tris-HCl (pH 8.3), 50mM KCl, 3mM $MgCl_2$, 100µM each of dATP, dCTP, dGTP, dTTP, 30ng of primer, 0.001% gelatin, 30ng of genomic DNA and 2 unit of *Taq* polymerase. For RAPD analysis, 50 random decamer primers (OPF, OPG and OPH series) were amplified using a thermal cycler (Hybaid touch down thermal cycling system). Following cyclic conditions were observed: one cycle of 94°C for 5 min; 40 cycles of 94°C for 1 min; 36°C for 1 min; 72°C for 2 min; followed by one cycle of 72°C for 10 min. For SSR analysis, the 77 primers developed by Roder *et al*., (1998) were selected; their names and designations are included in Table 4. Amplified products were resolved by 1.5% agarose/TBE gels stained with ethidium bromide and visualized under UV light chamber.

Data analysis: The loci were scored as present (1) or absent (0). RAPD markers data were analyzed as described by Nei and Li's (1979). The similarity matrix obtained was then used to construct a dendogram. For SSR method each fragment generated by a primer pair was considered to be an allele of the same marker locus. Fragment size was estimated by interpolation from the migration distance of marker fragments. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. Variability for each locus was measured using the polymorphism index content (PIC) (Anderson *et al*., 1993).

$$PIC = 1 - \sum p_i^2$$

where *pi* is the frequency of the *ith* allele. The polymorphic information content (PIC) was calculated for each SSR primer combination.

Genetic relationships among cultivars were calculated by pooling the RAPD and SSR data with the simple matching coefficient (Nei, 1979) using NTSYS-pc version 2.0 software. The resulting similarity matrix was subjected to cluster analysis by the unweighted pair-group method with the arithmetic averages (UPGMA) using NTSYS-pc.

Results and Discussion

Morphological features and resistance to karnal bunt and stripe rust: Results regarding some quantitative morphological features are presented in Table 1. The qualitative morphological features with resistance to karnal bunt and stripe rust are depicted in Table 2.

The thousand grain weight ranged from 53.1 to 67.6 g with an average of 58.37g (Table 1). The co-efficient of variation was 4.46%. Seven SHs have thousand grain weight greater than 60g. All of these entries were resistant to moderately resistant to stripe rust except SH-13. Days to flowering ranged from 85-112 days with an average of 98 days, while days to physiological maturity ranged from 127-148 days with an average of 136 days. It was important to observe that SH-6 and SH-13 had grain weight more than 60 gram, were resistant to karnal bunt and took the least time to mature. These promising features make important genotypes good candidates for deployment in wheat improvement programs. Keeping focus on thousand grain weight, transfer of this trait to the descendents is very important for yield improvement. Candidate synthetics with desired traits upon crossing with elite bread wheat cultivars normally have a 1000-grain weight around 40g result in F_1 seed that via top-crossing or limited back-crossing give filial advanced materials categorized F8 derivatives have been generated. From a wide array of breeding output data that has been generated more than 1100 synthetic derivatives were studied for their thousand grain weight (Unpublished data) and segregation diversity was observed. We were unable to find any transgressive segregation at the

upper spectrum. One of the advanced line derived from SH-6 (62.2g) had thousand grain weight 57.6g where the bread wheat parent had 37.5 g thousand grain weight. Similarly advanced lines derived from SH-17 ranged from 36.7 to 50g where the synthetic parent had 58.9 g and the bread wheat parent had 35.3 g of TGW. Earlier findings are also available where the significant importance of this trait in synthetic hexaploids is evident. Pestsova *et al*., (2001) used substitution lines of Chinese Spring where the D-genome chromosomes were replaced by the homologous chromosomes from synthetic hexaploids and different yield contributed traits were studied. It was observed that introduction of 3D and 7D chromosomes from synthetic hexaploids had a positive effect for thousand grain weight and number of grains per spike. Until now the genetics of yield related trait is not well understand at the molecular level and to date no gene is cloned for yield related characteristics in wheat due to the poor understanding of the genetic/molecular mechanisms of these traits. In this context these synthetic hexaploids are important genetic resources to understand the genetics of higher grain weight and to identify QTLs underlying this trait. It is important that many yield contributing characters had negative association among them like thousand grain weights had negative correlation with number of grains per spike (Xiang-Zheng *et al*., 2008). Therefore identifying the QTLs contributing for higher grain weight and being independent of other yield related attributes is very important and this has been studied that synthetic hexaploids carrying alleles for higher grain weight increased grain weight from 2.3-4.8 g without decreasing grain number and spike number. However this needs further validation and requires fine mapping of these QTLs with advanced marker techniques (like SNPs and microarrays).

Table 1. Quantitative morphological descriptors of high 1000-grain weight synthetic hexaploids (*Triticum turgidum* x *Aegilops tauschii*; 2n = 6x = 42, AABBDD).

S. No.	Pedigree	DF	DPM	PH	TGW
SH-1	DOY1/Ae. tauschii (188)	89*	130*	110	59.6*
SH-2	ALTAR 84/Ae. tauschii (192)	102*	136	95*	59.7*
SH-3	ALTAR 84/Ae. tauschii (198)	99	140*	120*	57.0*
SH-4	CPI/GEDIZ/3/GOO//JO69/CRA/4/Ae. tauschii (208)	100	132*	110	59.4*
SH-5	D67.2/P66.270//Ae. tauschii (213)	100	144*	110	55.6*
SH-6	ROK/KML//Ae. tauschii (214)	85*	127*	115*	62.2*
SH-7	D67.2/P66.270//Ae. tauschii (217)	110*	148*	105*	55.5*
SH-8	YUK/Ae. tauschii (217)	93*	130*	115*	59.0*
SH-9	ALTAR 84/Ae. tauschii (219)	100	134*	95*	55.9*
SH-10	DVERD_2/Ae. tauschii (221)	96	134*	105*	57.7*
SH-11	D67.2/P66.270//Ae. tauschii (221)	110*	144*	105*	59.6*
SH-12	D67.2/P66.270//Ae. tauschii (222)	110*	148*	105*	53.1*
SH-13	ACO89/Ae. tauschii (309)	85*	127*	125*	60.5*
SH-14	68112/WARD//Ae. tauschii (369)	96	134*	115*	55.6*
SH-15	DOY1/Ae. tauschii (511)	96	130*	110	58.1
SH-16	DOY1/Ae. tauschii (515)	96	144*	110	60.1*
SH-17	68.111/RGB-U//WARD/3/FGO/4/RABI/5/Ae. tauschii (629)	96	134*	125*	58.9*
SH-18	68.111/RGB-U//WARD/3/FGO/4/RABI/5/Ae. tauschii (878)	93	134*	125*	58.2
SH-19	68.111/RGB-U//WARD/3/FGO/4/RABI/5/Ae. tauschii (878)	93	130*	125*	55.4*
SH-20	CROC_1/Ae. tauschii (879)	96	144*	100*	57.4*
SH-21	68.111/RGB-U//WARD/3/FGO/4/RABI/5/Ae. tauschii (882)	96	130*	125*	55.9*
SH-22	CROC_1/Ae. tauschii (518)	85*	127*	110	57.8*
SH-23	SNIPE/YAV79//DACK/TEAL/3/Ae. tauschii (629)	106*	144*	110	67.6*
SH-24	D67.2/P66.270//Ae. tauschii (633)	117*	150*	105*	58.3
SH-25	SNIPE/YAV79//DACK/TEAL/3/Ae. tauschii (700)	99	134*	115*	57.9*
SH-26	SNIPE/YAV79//DACK/TEAL/3/Ae. tauschii (877)	100	136	105*	60.8*
SH-27	GAN/Ae. tauschii (897)	96	130*	120*	56.8*
SH-28	YAV_2/TEZ//Ae. tauschii (895)	96	130*	105*	57.6*
SH-29	DOY1/Ae. tauschii (333)	108*	140*	90*	55.7*
SH-30	DOY1/Ae. tauschii (428)	99	134*	140*	59.0*
SH-31	DOY1/Ae. tauschii (458)	96	130*	135*	60.4*
SH-32	GREEN/Ae. tauschii (458)	89*	127*	95*	56.9*
SH-33	CETA/Ae. tauschii (174)	102*	138*	100*	56.8*
SH-34	ALTAR84/Ae. tauschii (502)	84*	137	140*	63.2*
SH-35	CROC_1/Ae. tauschii (517)	96	134*	90*	57.1*
SH-36	ALTAR 84/Ae. tauschii (220)	99	134*	100*	60.0*
SH-37	D67.2/P66.270//Ae. tauschii (659)	112*	144*	120*	59.5*
Average		97.97	135.76	111.6	58.37
CV (%)		7.96	4.86	11.37	4.46
CD (0.05)		5.06	2.28	3.7	0.42

DF=Days to flowering; **DPM**=Days to physiological maturity; **PH**= Plant Height; **TGW**= Thousand grain weight;
*Significant difference from mean at $CD_{0.05}$

Table 2. Qualitative morphological descriptors, response to karnal bunt and stripe rust of high 1000-grain weight synthetic hexaploids (*Triticum turgidum* ×*Aegilops tauschii*; 2n = 6x = 42, AABBDD).

Name	PUB	PIG	AWN	KB	Yr(S)	Yr(A)
SH-1	+	+	LB	0	1	0
SH-2	-	+	LB	0	0	5MRR
SH-3	-	-	LB	0	78	5R
SH-4	+	-	W	0	0	0
SH-5	-	+	DB	0	12	10S
SH-6	-	+	DB	0	56	20MS
SH-7	-	+	DB	0	7	10MS
SH-8	+	+	W	0	0	40S
SH-9	-	+	W	0	4	40S
SH-10	+	-	B	0	78	50S
SH-11	-	+	W	0	1	30MR
SH-12	-	+	LB	0	34	MSS
SH-13	+	+	DB	0	89	60S
SH-14	+	-	LB	0	67	40MSS
SH-15	-	+	LB	0	12	40MRMS
SH-16	+	+	LB	0	0	20S
SH-17	+	-	LB	0	0	20MRR
SH-18	+	+	B	0	12	40MS
SH-19	+	+	B	0	12	20MRMS
SH-20	+	+	LB	0	23	60MSS
SH-21	+	+	LB	0	45	20MS
SH-22	-	+	LB	0	45	40MS
SH-23	+	+	B	0	0	0
SH-24	+	+	LB	0	8	40MS
SH-25	+	-	LB	0	8	30MS
SH-26	+	-	LB	0	8	0
SH-27	-	+	LB	0	34	60S
SH-28	+	-	LB	0	12	80S
SH-29	+	-	DB	0	78	80S
SH-30	+	+	LB	0	34	10R
SH-31	+	-	LB	0	89	0
SH-32	-	-	LB	0	0	0
SH-33	-	+	LB	0	0	20MRMS
SH-34	-	-	B	0	78	5R
SH-35	+	-	LB	0	1	10MR
SH-36	+	+	DB	0	12	50S
SH-37	-	+	LB	0	0	0

PUB= Pubescence; **PIG**= Anthocyanin pigmentation; **AWN**= Awn color (LB: light brown; B: Brown; DB: dark brown); **KB**= Resistance to Karnal bunt; **YR (S)**= Reaction to Stripe rust at seedling stage; **YR (A)**: Reaction to stripe rust at adult plant stage

In all these Elite-1 selected synthetic hexaploid lines high level of resistance against karnal bunt was observed during artificial KB stress screening (Table 2). Resistance to KB in synthetic hexaploids is the cumulative function of *Ae. tauschii* and durum cultivars as most of the durum parents are known to be field resistant to karnal bunt (Mujeeb-Kazi *et al*., 2006). In earlier studies where stringent greenhouse tests were done, the field durum resistant evaluation did show susceptibility. Synthetics however maintained their immunity in such controlled tests suggesting that resistance was contributed by the D-genome donor *Ae. tauschii* accessions.

In case of stripe rust resistance, 21 (62.1%) out of 37 SHs were found resistant at the seedling stage, out of which 10 were completely to near immune. Out of these 21 SHs, 6 (SH-1, 4, 23, 30, 32, and 37) were also found to be resistant at adult plant stage leading to the conclusion that these may have major stripe rust resistance genes. SH-3, SH-31 and SH-34 were found susceptible at seedling stage and resistant at adult plant stage suggesting the presence of adult plant resistance genes indicative of durable resistance promise. Four SHs (SH-10, 13, 14 and 29) were susceptible at both seedling and adult plant stage. Seven SHs (SH-6, 9, 12, 20, 21, 22 and 27) showed moderate response to stripe rust at seedling and adult plant stage which may be attributed to the presence of quantitative resistance genes. KB and stripe rust are the major biotic threats to wheat in this ecological region.

The synthetics hence form the following two categories based upon resistance screening for stripe rust:

a) Entries possessing seedling and adult plant resistance, and
b) Entries with seedling susceptibility and adult plant resistance suggesting durable resistance gene influence.

Synthetic hexaploids from both categories can thus be exploited for bread wheat improvement

Molecular diversity based on RAPDs and SSRs

RAPDs: The genetic diversity among the SHs was assessed using RAPDs revealed the following results. In case of RAPDs, only 20 primers generated polymorphisms and the capability of these 20 primers to generate RAPD markers ranged from 8 to 16 genotypes of the SHs. Range of scorable bands was from 250 bp to 2500 bp. Total 72 different bands were generated by these RAPD primers with an average of 3.6 bands per primer. In the present study RAPD primers amplified between 1 and 6 different bands per genotype. It is well documented in wheat and in other plant species, that not all of the amplified RAPD bands are scorable and useful as markers. This problem may be, in part, due to the relatively low resolving power of the agarose gels commonly used for RAPD analysis (Mahmood *et al.,* 2011; Plaschke *et al*., 1995). The mean similarity indices for the 37 SHs ranged from the 71% to 96%, indicating medium to high polymorphism at the DNA level among the selected synthetic hexaploid lines. For instance the similarity among the synthetics having grain weight more than 60g is given (Table 3). This showed that even the promising genotypes for higher grain weight are diverse at the genetic level and their deployment in a breeding program will not reduce the genetic base of the output product. RAPD marker technique is not considered very efficient for diversity analysis and there are better marker systems available which can dissect the genotypes at molecular level even where RAPDs fail to assess any diversity. However, RAPDs are still good to get first hand information about genetic diversity independently of the morphological features without a confounding affect of the environmental. Therefore the information generated from the RAPD markers in this study can help to exploit these synthetics to improve grain weight along with the resistance to karnal bunt and stripe rust when breeding programs are confronted by narrow genetic base for wheat improvement. Exploiting the unique genetic diversity of the synthetics has a greater comparative advantage over conventional diversity as the alien D genome accessional novel input so far is minimal in wheat varieties.

Table 3. Similarity matrix among synthetic hexaploids having thousand grain weight >60g, based on RAPD and SSR markers.

	SH-6	SH-13	SH-16	SH-23	SH-26	SH-31	SH36
SH-6	1.000						
	1.000						
SH-13	0.861	1.000					
	0.778	1.000					
SH-16	0.917	0.833	1.000				
	0.804	0.795	1.000				
SH-23	0.861	0.778	0.889	1.000			
	0.772	0.725	0.780	1.000			
SH-26	0.806	0.722	0.833	0.778	1.000		
	0.740	0.693	0.774	0.759	1.000		
SH-31	0.819	0.764	0.847	0.792	0.875	1.000	
	0.740	0.748	0.736	0.721	0.736	1.000	
SH-36	0.889	0.806	0.833	0.778	0.722	0.736	1.000
	0.748	0.719	0.740	0.733	0.753	0.838	1.000

Upper values are based on RAPD markers and lower ones represent similarity based on SSR markers.

SSRs: The basic information revealed from SSR analysis is described in Table 4. Of the 77 SSR markers employed, 25 were specific for the A-genome, 28 for B and 24 for the D-genome. These 77 microsatellites amplified 452 alleles of which 431 (95.35%) were polymorphic. The number of alleles revealed by each SSR marker ranged from 2 to 16 with an average of 5.95 alleles per SSR locus. The number of polymorphic alleles by each SSR marker locus ranged from 2 to 16 with an average of 5.67. PIC value ranged from 0.05 for the marker Xgwm403-1B to 0.9 for the marker Xgwm-219-6B and Xgwm234-5B with an average of 0.58. When the three genomes were compared maximum PIC was observed for the B genome (0.61) followed by the D genome (0.55). PIC value observed in this study is higher than that reported by Bohn *et al.*, (1999), Manifesto *et al*., (2001) and Kuleung *et al*., (2006). SSRs revealed lower genetic similarity as compared to RAPDs in synthetic hexaploids having grain weight more than 60g. This indicated higher polymorphism depicted by SSRs as compared to RAPDs adds strength to the fact that SSRs are a better marker system for revealing genetic diversity. In addition, the SSR markers did reveal genetic similarity among the SH accessions regardless of their grain weight which indicated that in wheat these SSR markers are valuable and reliable for examining relationships of wheat genotypes. The results have provided an insight into the prevalent genetic diversity of wheat that should facilitate efficient utilization and management of SH germplasm. It is our intention to devise a molecular marker–based strategy for selecting parents to create elite lines based on their parental genetic diversity and add efficiency plus cost effectiveness to the recombination breeding aspects by targeted use of SH parents identified by marker tools.

Consensus genetic diversity based on RAPDs and SSRs: Bivariate datasets generated from RAPD and SSR markers were pooled and subjected to cluster analysis. The resulting dendrogram is presented as Fig. 1. This dendrogram depicted high sub-clustering which proves high genetic variability among SHs (Fig. 1). The dendrogram revealed three main clusters, grouping the genotypes on similarity basis at the molecular level. It is important to observe that cluster analysis discriminated these SHs regardless of their parentage because many SHs with similar durum parents fell in different clusters. Hence our objectives to identify diversity within the 37 SH entries for high thousand grain weight with KB and Yr resistance present were well met. Diagnosing variation allowed us to select "some" SHs with diversity in a reasonable number for use in breeding in a targeted manner. This objective was fulfilled with RAPDs and SSRs.

National yield priorities of all introduced elite materials from CIMMYT and ICARDA approach 9 tons/ha (i.e. 90 mounds/acre). Country mean yield levels across its environments of irrigated and rainfed cultivation areas are between 26-30 maunds/acre. Thus an enormous yield gap prevails and numerous factors influence the low final output value. Program to enhance yield *per se* are few and use of large spikes, multiple ovaries, pentaploid breeding and wheat/alien chromosome translocations are suggested. Exploiting high 1000-grain weight is another attractive option and from our strategy it comes from unique genetic synthetic stocks that warrant greater emphasis. This study demonstrates that high grain weight exists and a separate study (Unpublished data) has shown that it is inherited. RAPD and SSRs have narrowed down some combinations for breeding that also possess KB and Yr resistance allowing effective pyramiding of these traits and making breeding efficient.

Table 4. Basic information about SSR markers used in diversity of synthetic hexaploids having higher grain weight.

S. No.	Marker	TA	SB	PIC	S. No.	Marker	TA	SB	PIC
1.	Xgwm33-1A	8	69	0.62	42.	Xgwm234-5B	16	117	0.9
2.	Xgwm99-1A	7	62	0.66	43.	Xgwm371-5B	3	53	0.39
3.	Xgwm136-1A	6	32	0.72	44.	Xgwm193-6B	6	52	0.42
4.	Xgwm164-1A	4	45	0.49	45.	Xgwm219-6B	16	159	0.9
5.	Xgwm497-1A	4	29	0.67	46.	Xgwm508-6B	5	48	0.72
6.	Xgwm71.1-2A	5	98	0.7	47.	Xgwm613-6B	3	4	0.41
7.	Xgwm359-2A	2	2	0.21	48.	Xgwm626-6B	4	68	0.55
8.	Xgwm497-2A	4	58	0.57	49.	Xgwm43-7B	5	133	0.82
9.	Xgwm558-2A	5	67	0.66	50.	Xgwm46-7B	5	5	0.65
10.	Xgwm2-3A	7	46	0.27	51.	Xgwm68-7B	5	114	0.75
11.	Xgwm391-3A	6	31	0.72	52.	Xgwm146-7B	4	68	0.72
12.	Xgwm4-4A	10	11	0.79	53.	Xgwm340-7B	6	37	0.73
13.	Xgwm160-4A	6	44	0.41	54.	Xgwm106-1D	4	48	0.55
14.	Xgwm610-4A	4	69	0.64	55.	Xgwm232-1D	5	51	0.62
15.	Xgwm126-5A	5	8	0.72	56.	Xgwm458-1D	4	37	0.53
16.	Xgwm617-5A	7	89	0.75	57.	Xgwm642-1D	11	134	0.87
17.	Xgwm135-6A	4	15	0.47	58.	Xgwm102-2D	6	30	0.73
18.	Xgwm169-6A	4	44	0.21	59.	Xgwm261-2D	6	72	0.67
19.	Xgwm459-6A	2	2	0.5	60.	Xgwm515-2D	4	34	0.71
20.	Xgwm494-6A	2	54	0.42	61.	Xgwm645-3D	2	12	0.05
21.	Xgwm570-6A	4	47	0.39	62.	Xgwm3-3D	3	17	0.57
22.	Xgwm130-7A	4	15	0.48	63.	Xgwm183-3D	7	43	0.51
23.	Xgwm332-7A	9	90	0.74	64.	Xgwm383-3D	8	64	0.73
24.	Xgwm350-7A	3	14	0.37	65.	Xgwm583-3D	4	43	0.28
25.	Xgwm635-7A	4	14	0.62	66.	Xgwm608-4D	8	59	0.69
26.	Xgwm140-1B	8	38	0.38	67.	Xgwm182-5D	5	33	0.57
27.	Xgwm264-1B	8	125	0.81	68.	Xgwm190-5D	5	55	0.75
28.	Xgwm403-1B	2	9	0.05	69.	Xgwm292-5D	6	10	0.62
29.	Xgwm550-1B	12	53	0.78	70.	Xgwm565-5D	10	125	0.86
30.	Xgwm47-2B	8	51	0.76	71.	Xgwm55-6D	4	61	0.72
32.	Xgwm210-2B	5	87	0.67	72.	Xgwm325-6D	4	30	0.4
33.	Xgwm257-2B	12	71	0.71	73.	Xgwm469-6D	13	98	0.84
34.	Xgwm112-3B	5	62	0.56	74.	Xgwm44-7D	8	106	0.75
35.	Xgwm264-3B	11	77	0.87	75.	Xgwm428-7D	12	36	0.22
36.	Xgwm284-3B	4	36	0.71	76.	Xgwm437-7D	5	42	0.46
37.	Xgwm493-3B	4	44	0.65	77.	Xgwm635-7D	2	4	0.06
38.	Xgwm533.1-3B	3	14	0.1		**A-genome**	**5.04**	**42.4**	**0.55**
39.	Xgwm6-4B	6	37	0.76		**B-genome**	**6.67**	**60.26**	**0.61**
40.	Xgwm149-4B	4	7	0.14		**D-genome**	**6.08**	**51.83**	**0.57**
41.	Xgwm191-5B	10	58	0.54		**Average**	**5.95**	**51.66**	**0.58**

TA= Total alleles; **SB**=scorable bands; **PIC**= Polymorphic information content

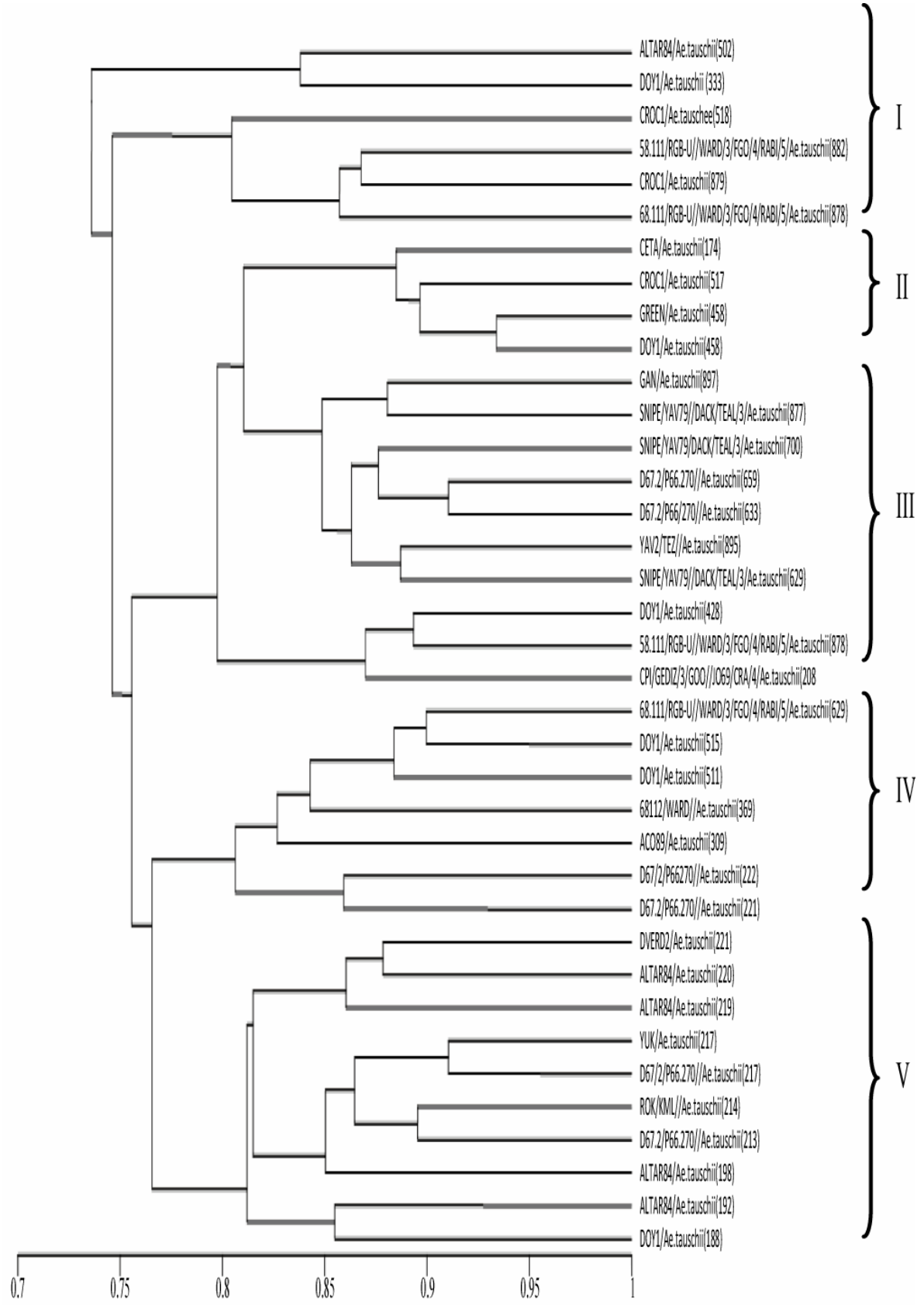

Fig. 1. Consensus dendrogram based on SSR and RAPDs pooled data of synthetic hexaploids; Genotypes with bold pedigree had grain weight >60g.

Acknowledgements

We acknowledge the contributions of Dr. Atiq-ur-Rahman Rattu and Muhammad Fayyaz (Crop Disease Research Program (CDRP), National Agriculture Research Center (NARC), Islamabad, Pakistan).

References

Anderson, J.A., J.E. Churchill, S.D. Autrique, S. Tanksley and M.E. Sorrells. 1993. Optimizing parental selection for genetic linkage maps. *Genome*, 36: 181-188.

Bibi, A., S. Ajmal, A. Rasheed, A. Gul-Kazi and A. Mujeeb-Kazi. 2012. High molecular weight (HMW) glutenin subunit compositions of the Elite-II synthetic hexaploid wheat sub-set (*Triticum turgidum* × *Aegilops tauschii*; 2n=6x=42; AABBDD). *Plant Genetic Resources*, 10: 1-4.

Bohn, M., H.F. Utz and A.E. Melchinger. 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. *Crop Sci.,* 39: 228-237.

Brocklehurst, P.A. 1977. Factors controlling grain weight in wheat. *Nature*, 266: 348-349.

Calderini, D.F. and I. Ortiz-Monasterio. 2003. Crop physiology and metabolism: grain position affects grain macronutrient and micronutrient concentrations in wheat. *Crop Sci.,* 43: 141-151.

Campbell, K.J., C.J. Bergman, D.G. Gualberto, J.A. Anderson, M.J. Giroux, G. Hareland, R.G. Fulcher, M.E. Sorrells and P.L. Finney. 1999. Quantitative trait loci associated with kernel traits in a soft × hard wheat cross. *Crop Sci.,* 39: 1184-1195.

Dreccer, F.M., G.M. Borgognone, F.C. Ogbonnaya, R.M. Trethowan and B. Winter. 2007. CIMMYT-selected derived synthetic bread wheats for rainfed environments: yield evaluation in Mexico and Australia. *Field Crops Res.,* 100: 218-228.

Kuleung, C., P.S. Baenzieger and I. Dweikat. 2006. Evaluation of diversity of Triticale with wheat and rye SSR markers. *Crop Sci.,* 46: 1692-1700

Ma, H., R.P. Singh and A. Mujeeb-Kazi. 1995. Resistance to stripe rust in *Triticum turgidum, T. tauschii* and their synthetic hexaploids. *Euphytica,* 82: 117-124.

Mehmood, T., A. Siddiqua, A. Rasheed and N. Nazar. 2011. Evaluation of genetic diversity in different Pakistani wheat land races. *Pak. J. Bot.,* 43: 1233-1239.

Manifesto, M.M., A.R. Schlatter, H.E. Hopp, E.Y. Suarez and J. Dubcovsky. 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop Sci.,* 41: 682-690.

McNeal, F.H., C.F. Konzak, E.P. Smith, W.S. Tate and T.S. Russel. 1971. A uniform system for recording and processing cereal research data. *USDA-ARS*., 34: 31-121.

Mujeeb-Kazi, A., V. Rosas and S. Roldan. 1996. Conservation of genetic variation of *Triticum tauschii* (Coss.) Schmalh (*Aegilops squarossa* auct. Non L.) in synthetic hexaploid wheats (*T. turgidum* L. s. lat. x *T. tauschii*; 2n=6x=42; AABBDD) and its potential utilization for wheat improvement. *Genet Resour. Crop Evol.,* 43: 129-134.

Mujeeb-Kazi, A., G. Fuentes-Davilla, A. Gul and J.I. Mirza. 2006. Karnal bunt resistance in synthetic hexaploid wheats (SH) derived from durum wheat x *Aegilops tauschii* combinations and in some SH x bread wheat derivatives. *Cereal Res. Commun.,* 34: 1199-1205.

Mujeeb-Kazi, A., A. Gul, M. Farooq, S. Rizwan and J.I. Mirza. 2007. Genetic diversity of *Aegilops variabilis* (2n=4x=28; UUSS) for wheat improvement: Morpho-cytogenetic characterization of some derived amphiploids and their practical significance. *Pak. J. Bot.,* 39: 57-66.

Nei, N. and W. Li. 1972. Mathematical world for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.,* 76: 5269-5273.

Pestsova, E.G., A. Borner and M.S. Roder. 2001. Development of a Set of *Triticum aestivum-Aegilops tauschii* Introgression Lines. *Hereditas*, 135: 139-143.

Peterson, R.F., A.B. Campbell and A.E. Hannah. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Research*, 26: 496-500.

Plaschke, J., M.W. Ganal and M.S. Roder. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theor. Appl. Genet.,* 91: 1001-1007.

Rajaram, S. 2005. Role of conventional plant breeding and biotechnology in future wheat production. *Turk. J. Agric. For.,* 29: 105-111.

Rasheed, A., T. Mahmood, A. Gul-Kazi, A. Ghafoor and A. Mujeeb-Kazi. 2012. Allelic variation and composition of HMW-GS in advanced derivaties from D- genome synthetic hexaploid × Bread wheat cross combinations. *J. Crop Sci. and Biotech.,* 15: 1-7.

Roder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy and M.W. Ganal. 1998. A microsatellite map of wheat. *Genetics*, 149: 1-17.

Roder, M.S., X.Q. Huang and A. Borner. 2008. Fine mapping of the region on wheat chromosome 7D controlling grain weight. *Funct. Integr. Genomics,* 8: 79-86.

Trethowan, R.M. and A. Mujeeb-Kazi. 2008. The use of novel germplasm resources to improve the environmental stress tolerance of hexaploid wheat. *Crop Sci.,* 48: 1255-1265.

Warham, E.J., A. Mujeeb-Kazi and V. Rosas. 1986. Karnal bunt (*Tilletia indica*) resistance screening of *Aegilops* species and their practical utilization for *Triticum aestivum* improvement. *Can. J. Plant Pathol.,* 8: 65-70.

Xiang-Zheng, L., W. Jin, Z. Rong-Hua, R. Zheng-Long and J. Ji-Zeng. 2008. Mining favorable alleles of QTLs conferring thousand grain weight from synthetic wheat. *Acta Agron. Sin*., 34: 1877-1884.

Zadoks, J.C., T.T. Chang and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Res.,* 14: 415-421.

(Received for publication 18 May 2011)