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Full Length Article

Characterization of Synthetic Wheat Germplasm using Morphological and Molecular Markers

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Abstract

The present study was conducted to characterize the germplasm of synthetic hexaploid wheat using forty genotypes during two wheat season (2014–16). The experiment was conducted in randomized complete block design (RCBD) with two replications. At maturity, the genotypes were characterized for both morphological traits *i.e*., plant height, peduncle length, extrusion length, spike length, awn length, number of spikelets per spike, thousand grain weight, yield per spike and number of grains per spike, and molecular markers using Inter Simple Sequence Repeats (ISSR) markers. Analysis of variance revealed significant differences among genotypes for all the studied traits. Year factor also showed significant effect for all the traits except spikelets per spike and number of grains per spike. Year × genotype interaction showed significant effect for all the traits except plant height. Among ISSR markers, UBC-810 and 807 showed maximum polymorphic bands. UBC-810 appeared as the most diverse marker because it accounted maximum polymorphism information content (*PIC*) value 0.48. Minimum *PIC* value (0.287) was revealed by the primer UBC-814. Dendrogram grouped the genotypes into four clusters on the basis Jaccard's distance. Genetic similarity among genotypes was ranged from 17 to 76%. © 2019 Friends Science Publishers

Keywords: ISSR; Molecular markers; Synthetic wheat; Polymorphism

Introduction

Wheat is the major cereal grain crop which provides ~28% world's total edible dry matter contents and fulfills 60% energy requirements in many developing countries across the globe (Cakmak, 2008; Wang *et al*., 2011). Wheat is an allohexaploid which belongs to family poaceae. Its genome (AABBDD) is derived from natural crossing between cultivated tetraploid species *Triticum turgidum* L. (AABB) and *Aegilops tauschii* (Matsuoka, 2011). The process of formation of allohexaploid wheat can be artificially produced by crossing tetraploid and diploid species developing synthetic hexaploid wheat (Kihara and Lilienfeld, 1949; Mujeeb-Kazi *et al*., 1996). These synthetic wheats work as the genetic bridge between modern wheat cultivars and wild wheat (Calderini and Ortiz-Monasterio, 2003). These synthetics have been successfully used to introgress the genetic material into modern wheat from their progenitor species *Ae. tauschi* and *T. turgidum* (Trethowan and Mujeeb-Kazi, 2008; Talbot, 2011).

Exploration of wheat genome faces difficulties due to large genome size (17,000 Mb) and high rate of repetitive sequences (Brenchley *et al*., 2012). Management of such large genomic resources is very complex process. These genetic resources play key role in sustainable agriculture development. Therefore, collection and evaluation of these genetic resources should be done on priority basis (Arif *et al*., 2010).

As synthetic derivatives play important role under stress therefore, assessment of genetic diversity could be useful in identification of parents for breeding programs (Tascioglu *et al*., 2016). Success of any breeding programs depends upon the identification of parents and the distance between the parents in order to obtain transgressive segregants (Khodadadi *et al*., 2011). Various methods are being used for the identification of genetic diversity using morphological, molecular, biochemical and pedigree data of germplasm lines (Mohammadi and Prasanna, 2003). Value of heterosis determines the success level which depends upon genetic distance between parents.

Morphological markers are used to determine the performance of accession under field conditions. Therefore selection of the parents can be made on the basis of their phenotypic performance. Phenotype of an individual is

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resultant of interaction between genotype and environment. Environmental conditions may vary from location to location which results in expression of phenotypes. Therefore, selection only on the basis of morphological characters cannot be relied (Najaphy *et al.,* 2012).

Molecular characterization of genotypes provides insight in their genetic background. Use of molecular markers for identification of parents is more reliable and cost effective strategy (Sajjad *et al.,* 2018). A number of DNA markers have been utilized in exploring the genetic diversity existed in crop plants and their wild relatives. Polymerase chain reaction (PCR) based molecular markers include inter simple sequence repeats (ISSRs) have been widely used in various areas of plant research (Karaca and Izbirak, 2008). ISSR markers are easy to handle, highly informative and repeatable, hence repeated sequences are abundant throughout the genome. Najaphy *et al.* (2012) showed that ISSR markers provide adequate polymorphism and reproducible fingerprinting profile for genetic characterization of wheat. Therefore, the study was planned to characterize the synthetic wheat germplasm using morphological and molecular markers. The parents identified in this study could be used for genetic improvement in wheat breeding programs.

Materials and Methods

Plant Material and Cite Information

Forty hexaploid synthetic derivative wheat lines (*T. aestivum* L.) were evaluated at the experimental area of the Department of Plant Breeding and Genetics, Bahauddin Zakariya University, Multan, situated at 30.1917° N (latitude) and 71.4654° E (longitude). List of genotypes used in this study is presented in Table 1. The experimental field soil was clay-loamy in texture.

Phenotypic Evaluation

Genotypes were sown in field by hand drill method using Randomized Complete Block Design (RCBD) with 2 replications. Experiment was conducted for two consecutive years 2014–2015 and 2015–2016. Sowing was done on 15th Nov during both years. Genotypes were raised in rows of 4.5 m in length. Plant to plant distance was maintained 22 cm by thinning. Field was irrigated 4 times to get healthy crop. All the recommended agronomic practices were followed throughout the cropping season. Following plant traits were measured at maturity from five plants (Pask *et al.*, 2012); *i.e.*, plant height (PH), peduncle length (PL), extrusion length (EL), awn length (AL), spike length (SL), no. of spikelets per spike (SL/S), 1000 grain weight (TGW), no. of grains per spike (NG/S) and yield per spike (Y/S).

DNA Extraction and ISSR Assay

One spike per genotype was harvested separately, to obtain pure seed samples for DNA extraction. Spikes were threshed manually and five uniform healthy seeds were taken. These uniform seeds were sown in polythene bags filled with peat moss and placed at 22°C in an incubator. After 15 days to sowing, approximately 1 cm^2 young leaf tissue (0.5 g) was harvested for DNA isolation and further molecular studies. Genomic DNA was extracted by using pestle and mortar by following the method (Khan *et al.*, 2004).

PCR assay was performed in a 20 μL volume. PCR reaction mixture contained of I μL of genomic DNA and 12.8 μL of sterile ultra-pure deionized water (d_2H_2O), 2.0 μL of 10X PCR buffer, 2.5 μL of $MgCl_2$, 0.5 μL of dNTPs (10 m*M*), 1.0 μL of primer and 0.2 μL of Taq Polymerase. The cycling conditions were as initial 5 min at 95°C (denaturation step), followed by 30 cycles of 1 min at 95ºC (denaturation step), 52ºC for 1 min (annealing step, temperature optimized for each primer), 72ºC for 2 min (elongation step), and 72ºC for 10 min (final extension), then kept at 4°C. PCR product was visualized by 2% agarose gel electrophoresis. For ISSR markers, 18 markers were tested as single primers for the amplification of genomic DNA. Out of these, 11 primers produced polymorphic band patterns.

Data Analysis

To check difference for studied traits analysis of variance was performed as suggested by Steel *et al.* (1997). The diversified genotypes were selected on the basis of Principal Component Analysis (PCA) (Ogunbayo *et al.,* 2005) using XLSTAT software. The statistically significant principal components were selected using eigen values criteria as established by Kaiser (1960).

For molecular data analysis, DNA bands generated by ISSR primers were scored visually on the basis of their presence (1) and absence (0). Binary data sheet was generated on the basis of primer scoring. To group the genotypes on the basis of similarity, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis was performed using NTSYS-PC Version 2.0 software. Polymorphism Information Content (PIC) value was determined as calculated by Ashraf *et al.* (2016). Confusion probability (cj) and Discriminating power (dj) were identified as determined by (Anderson *et al.,* 1993).

Results

Analysis of variance showed significant differences among wheat synthetics for all the morphological characters *viz.*, plant height (PH), peduncle length (PL), extrusion length (EL), awn length (AL), spike length (SL), spikelets per spike (SL/S), thousand grain weight (TGW), number of grain per spike (NG/S) and yield per spike (Y/S). Year factor showed significant effect on all the traits except SL/S and NG/S (Table 2). Y×G interactions showed

Table 1: List of wheat synthetic genotypes used in this study

Code	Genotype	Parentage
G1	BW/SH-5	TURACO/5/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA (205)/3/3×BUC/6/FCT/6/DOY1/AE.SQUARROSA (458)
G2	BW/SH-126	OPATA//CETA/AE.SQUARROSA (1027)
G3	BW/SH-038	MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD/4/KAMBARA
G4	BW/SH-161	CHAPIO/INQALAB 91/4/PICUS/3/KAUZ×2/BOW//KAUZ
G5	BW/SH-037	MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/PASTOR/ 4/SARSABZ
G6	203	OPATA//DOY1/AE.SQUARROSA(458)
G7	181	OPATA//68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878)
G8	171	OPATA//68.112/WARD//AE.SQUARROSA(369)
G9	BW/SH-162	CHAPIO/INQALAB 91/4/PICUS/3/KAUZ×2/BOW//KAUZ
G10	BW/SH-033	CHIR3/CBRD//OPATA
G11	BW/SH-16	GAN/AE.SQUARROSA(897)//OPATA/3/D67.2/P66.270//AE.SQUARROSA(223)
G12	BW/SH-108	MAYOOR/TK SN1081/AE.SQUARROSA (222)/ 3/PASTOR/4/CROC_1/AE.SQUARROSA(444)
G13	BW/SH-164	CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ
G14	BW/SH-147	KAMBARA/INQALAB
G15	BW/SH-86	OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878)
G16	BW/SH -030	GANAE.SQUARROSA(236) //CETA/AE.SQUARROSA (895)/ 3/MAIZ/4/INQALAB91/5/ BKH-94
G17	BW/SH -039	PBW-343/6/YAV_3/SCO//JO69/CRA/3/YAV79/4/ AE.SQUARROSA(498)/5/OPATA
G18	BW/SH -048	OPATA/PASTOR
G19	BW/SH -26	OPATA//68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878)
G20	BW/SH -138	BKH-93/4/MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT
G21	BW/SH -28	OPATA//68.112/WARD//AE.SQUARROSA(369)
G22	BW/SH -121	OPATA//ALTAR 84/AE.SQUARROSA(J BANGOR)
G23	BW/SH -114	OPATA//DOY1/AE.SQUARROSA(458)
G24	BW/SH -74	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/ 5/OAPTA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/ AE.SQUARROSA(783)
G25	BW/SH -71	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208) /5/OAPTA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783)
G26	204	PASTOR/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD
G27	BW/SH -154	CHAPIO/INQALAB 91/4/PICUS/3/KAUZ×2/BOW//KAUZ
G28	BW/SH -151	CHAPIO/INQALAB 91/4/PICUS/3/KAUZ×2/BOW//KAUZ)
G29	BW/SH -53	ALTAR/84/AE.SQUARROSA(224)//2×YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA (222)/4/KUKUN/5/GAN/AE.SQUARROSA(248)
G30	BW/SH -6	OPATA//CETA/AE.SQUARROSA(895)
G31	BW/SH -92	CNDO/R143//ENTE/MEXI_2/3/AE.SQUARROSA(TAUS)/ 4/WEAVER/5/2×KAUZ/6/ DOY1/AE.SQUARROSA) (458)
G32	BW/SH -129	OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878)
G33	BW/SH -107	OPATA//ALTAR 84/AE.SQUARROSA
G34	182	CHAPIO/INQALAB 91/4/PICUS/3/KAUZ×2/BOW//KAUZ)
G35	186	CHAPIO/INQALAB 91/4/PICUS/3/KAUZ×2/BOW//KAUZ
G36	BW/SH -87	INQALAB 91/TSAPKI//SCA/AE.SQUARROSA(518)
G37	BW/SH -153	CHAPIO/INQALAB 91/4/PICUS/3/KAUZ×2/BOW//KAUZ
G38	BW/SH -140	PASTOR/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD
G39	BW/SH -105	DOYI/AE.SQUARROSA(1018) x CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OPATA
G40	BW/SH -56	ALTAR 84/AE.SQUARROSA (224)//2×YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA (222)/4/KUKUN/5/GAN/AE.SQUARROSA(248)

Table 2: Mean squares values of synthetic wheat genotypes for studied traits over two years

SOV	DF	PH	PL	EL	SL	AL	SL/S	TGW	Y/S	NG/S
Year	1	615.7**	13.5**	238.8**	44.7**	5.7**	3.60	536.1**	0.93**	5.26
Rep	1	6.21	0.003	0.24	0.04	0.00	1.50	20.7*	0.41**	133.9**
Gen	39	331.5**	59.2**	52.3**	5.9**	2.1**	8.1**	53.2**	0.14**	40.4**
Y*R	1	0.26	0.008	1.03*	0.05	0.02	0.92	1.40	0.0115	1.68
Y*G	39	1.35	13.1**	1.4**	0.8**	1.2**	3.7**	11.2**	0.04**	8.9**
R*G	39	3.87**	0.25	0.3**	0.08	0.09	1.55	2.17	0.02*	6.4**
Error	39	1.27	0.38	0.12	0.08	0.05	1.34	2.36	0.007	2.44
Total	159									

*=Significant at <0.05, **=highly significant at <0.01, PH: plant height, PL: peduncle length, EL: extrusion length, AL: awn length, SL: spike length, SL/S: no. of spikelets per spike, TGW: 1000 grain weight, Y/S: yield per spike and NG/S: no. of grains per spike

significant effect for all the traits except PH. Y×R showed non-significant effect for all the traits except EL which showed significant differences.

Mean performance of genotypes showed that for plant height, genotypes (G10) appeared as the shortest genotypes with 60.40 cm and 63.50 cm for both years, respectively (Table 3). Tallest genotype (G7) attained 99 cm and 103.98 cm height for both years, respectively. For yield related traits, value of TGW was ranged from 28.46 g to 46.91 g and from 32.29 g to 50.35 g for both years, respectively. In

Table 3: The range and mean values synthetic wheat of all the studied traits over two years

Traits	Range 1st year	Mean 1st year	Range 2nd year	Mean 2nd year
PH (cm)	60.40-99.05	79.73	63.50-103.98	83.74
PL (cm)	29.55-47.55	38.55	29.45-47.55	38.5
EL (cm)	8.7-23.00	15.85	11.90-25.05	18.5
SL (cm)	7.45-12.85	10.15	7.45-12.85	10.15
AL (cm)	4.80-8.15	6.5	4.10-8.25	6.17
SL/S	13-19	16	14-23	18.15
TGW (g)	28.46-46.91	37.7	32.29-50.35	41.32
Y/S (g)	1.02-1.67	1.35	1.05-2.08	1.6
NG/S	27-44	35.5	31-46	38.5

PH: plant height, PL: peduncle length, EL: extrusion length, AL: awn length, SL: spike length, SL/S: no. of spikelets per spike, TGW: 1000 grain weight, Y/S: yield per spike and NG/S: no. of grains per spike

Table 4: Eigen values and squared cosines of the variables for PCA first year

Traits	PC 1	PC 2	PC 3	PC 4
Eigen value	2.2085	1.8858	1.6272	1.2061
Variability (%)	24.5391	20.9537	18.0805	13.4009
Cumulative %	24.5391	45.4928	63.5734	76.9743
Traits	PC 1	PC 2	PC 3	PC 4
PH	0.1005	0.4938	0.0068	0.0013
PL	0.0256	0.6120	0.1084	0.0503
EL	0.1168	0.5480	0.0007	0.0279
SL	0.1425	0.0000	0.5377	0.1231
AL	0.3504	0.0685	0.0113	0.0252
SL/S	0.0271	0.0027	0.0835	0.7750
TGW	0.1319	0.0588	0.6192	0.1386
Y/S	0.7274	0.0837	0.1031	0.0298
NG/S	0.5864	0.0184	0.1565	0.0350

Values in bold correspond for each variable to the factor for which the squared cosine is the largest
PH: plant height, PL: peduncle length, EL: extrusion length, AL: awn length, SL: spike length, SL/S: no. of spikelets per spike, TGW: 1000 grain weight, Y/S: yield per spike and NG/S: no. of grains per spike

Table 5: Eigen value and squared cosines of the variables for PCA second year

Traits	PC 1	PC 2	PC 3	PC 4
Eigen value	2.3553	1.8957	1.3605	1.1035
Variability (%)	26.1700	21.0634	15.1168	12.2607
Cumulative %	26.1700	47.2334	62.3502	74.6110
Traits	PC 1	PC 2	PC 3	PC 4
PH	0.1373	**0.2126**	0.0285	0.1688
PL	0.0022	**0.7131**	0.0315	0.0488
EL	0.0020	**0.5268**	0.0397	0.2455
SL	0.0952	0.1224	0.0503	**0.4813**
AL	0.0000	0.1062	**0.6410**	0.0113
SL/S	0.0226	0.0002	**0.4041**	0.0608
TGW	**0.5850**	0.0849	0.0944	0.0628
Y/S	**0.9582**	0.0005	0.0029	0.0215
NG/S	**0.5528**	0.1289	0.0682	0.0026

Values in bold correspond for each variable to the factor for which the squared cosine is the largest
PH: plant height, PL: peduncle length, EL: extrusion length, AL: awn length, SL: spike length, SL/S: no. of spikelets per spike, TGW: 1000 grain weight, Y/S: yield per spike and NG/S: no. of grains per spike

2015, the genotype G21 showed maximum Y/S (1.67 g) whereas, the genotype G39 showed minimum Y/S (1.02 g). Similarly, during 2016, the genotype G3 attained minimum Y/S (1.05 g) whereas; the genotype G16 accounted maximum Y/S (2.08 g). Range of all other traits is represented in Table 3.

Principle Component Analysis (PCA)

Principal component analysis performed on morphological data showed that during both years first 4 PCs accounted more than 70% variation. Among these 4 PCs, first 2 PCs being the most diverse PCs were given due importance. It was observed that during both years 1st PC was more related to yield related traits (Y/S, NG/S and TGW) whereas, 2nd PC was more related to plant developmental traits (PH, PL and EL) (Table 4 and 5). Genetic diversity among genotypes for the traits related to first 2 PCs and association among various traits was analyzed by biplot analysis. The genotypes G5, G9, G15, G16, G33, G34, G35 and G37 appeared as the most diverse genotypes during both years which showed involvement of genetic factors in

presence of diversity. The angles between two vectors demonstrated correlation between these variables. The angle less than 90 degree indicated positive correlation and the angle more than 90 degree showed negative correlation among the variables. It was observed that SL/S showed negative correlation with yield related traits. The genotypes close to the vectors of a trait demonstrated its high contribution in that trait. The genotypes which showed highest contribution in yield related traits include G32, G21, G22 and G23 whereas, genotypes which performed high in plant developmental traits include G15, G35 and G38 (Fig. 1 and 2). From these results, a selection for the.

Molecular Characterization

All the accessions were characterized using ISSR markers (Fig. 3 and 4). Eleven primers showed polymorphism, which were used to generate genetic similarity matrix and dendrogram (Fig. 5). The primers UBC-818 and UBC-825 depicted major polymorphic alleles (7) followed by primer UBC-845 which scored (6) polymorphic alleles. Only 2 polymorphic alleles were accounted by UBC-814 (Table 6). The genetic resemblance among studied genotypes was ranged from 17 to 76%.

Dendrogram grouped the genotypes into two major clusters (A and B). Major cluster B was further subdivided into three sub groups B1, B2 and B3. The genotypes grouped into same group are more similar as compared to the genotypes located in the other groups. Genotypes G19, G20, G22, G23 and G32 were located in the same group A. Subgroup B1 contained only 1 genotype G6 whereas, the genotypes G3, G4, G5, G8, G10, G12, G24, G25, G9, G33, G15, G18, G13, G14, G16, G17, G26, G28 and G27 were located in sub cluster B2. Genotypes G1 and G11 were located in sub cluster B3. These results showed the genetic association among genotypes on the basis of molecular markers.

Markers Discriminating Indices of ISSR

PIC value of the primers was ranged from 0.287 (UBC-814) to 0.478 (UBC-810). The *Cj* values of primers varied from 0.55 (UBC-845) to 0.705 (UBC-814). The *Dj* values of primers varied from 0.647 (UBC-814) to 0.745 (UBC-810) as depicted in Table 7.

Discussion

Wheat contributes significantly in world's food production and world food security mainly depends upon its sustainable production. To meet the challenge of ever increasing world's population and fluctuating climatic conditions genetic resources for improved adaptation and yield must be identified (Reynolds *et al.*, 2015). Extensive breeding efforts have resulted in decreased genetic diversity in bread wheat.

Table 6: No. of polymorphic and monomorphic bands obtained with ISSR markers

Primer	Polymorphic bands	Monomorphic bands	% of Polymorphism
UBC-807	5	0	100
UBC-810	5	0	100
UBC-812	4	1	75
UBC-813	3	1	66.7
UBC-814	2	0	100
UBC-815	4	0	100
UBC-818	7	3	70
UBC-825	7	2	77.7
UBC-835	5	2	71.4
UBC-845	6	1	85.7
UBC-846	5	2	71.4

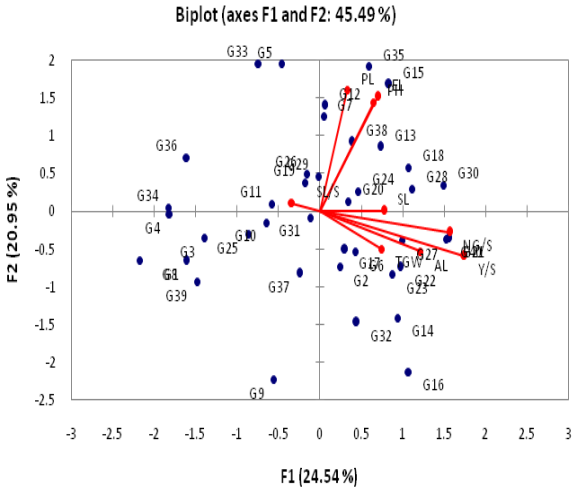

Fig. 1: Biplot for Principle component analysis first year

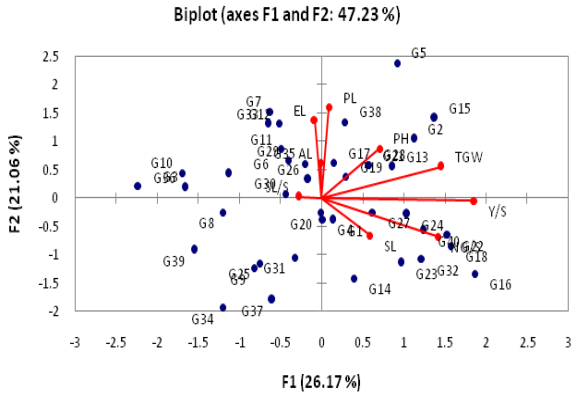

Fig. 2: Biplot plot for Principle component analysis second year

Success of any breeding program depends upon the availability of genetic diversity. Therefore, utilization of synthetic wheat to introduce new allelic combination was started in previous centaury (Mujeeb-Kazi *et al.*, 1996).

The present study was performed on synthetic wheat derivative genotypes to assess the genetic diversity and utilization of this genetic diversity in breeding programs.

Table 7: Markers discriminating indices of ISSR markers and their sequences

Primer	Sequences (5' - 3')	*PIC*	*Cj*	*Dj*
UBC-807	AGAGAGAGAGAGAGAGT	0.387	0.602	0.698
UBC-810	GAGAGAGAGAGAGAGAT	0.478	0.509	0.745
UBC-812	GAGAGAGAGAGAGAGAA	0.473	0.514	0.742
UBC-813	CTCTCTCTCTCTCTCTT	0.309	0.682	0.658
UBC-814	CTCTCTCTCTCTCTCTA	0.287	0.705	0.647
UBC-815	CTC CTCTCTCTCTCTG	0.436	0.552	0.723
UBC-818	CACACACACACACACAG	0.387	0.602	0.698
UBC-825	ACACACACACACACACT	0.393	0.596	0.701
UBC-835	AGAGAGAGAGAGAGAGYC	0.433	0.555	0.722
UBC-845	CTCTCTCTCTCTCTCTRG	0.438	0.55	0.725
UBC-846	CACACACACACACACART	0.436	0.552	0.723

UBC= University of British Columbia

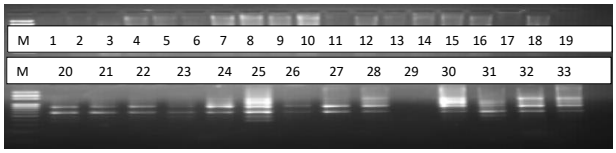

Fig. 3: ISSR amplification of 33 synthetic hexaploid wheat genotypes generated with primer UBC-825

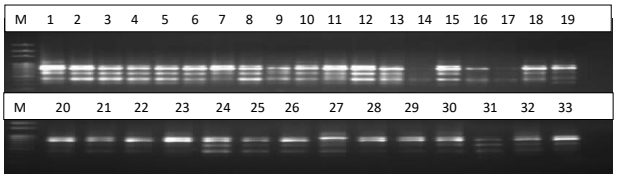

Fig. 4: ISSR amplification of 33 synthetic hexaploid wheat genotypes generated with primer UBC-846

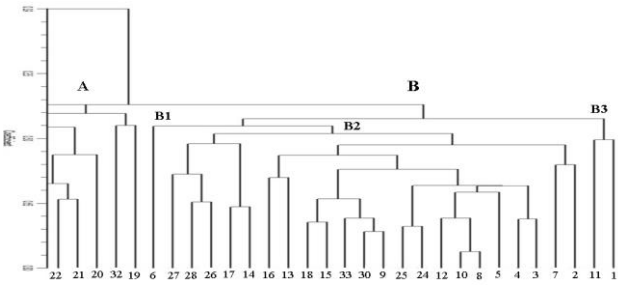

Fig. 5: Dendrogram generated on the basis of molecular characterization

Synthetic wheat germplasm has been explored for genetic diversity in various studies (Mujeeb-Kazi, 2003, Mujeeb-Kazi *et al.,* 2008; Hanif *et al.,* 2014; Masood *et al.,* 2016; Tariq *et al.*, 2018). In the current study, characterization of the germplasm was done both at morphological and molecular level (Ahmad *et al.,* 2014).

Presence of significant variation among genotypes, year and G X E factor indicated that the germplasm is of diverse nature (Birsin, 2005; Ahmad *et al.,* 2015). Variation among genotypes was also confirmed by the differences in range of means (Table 3) and dispersion of genotypes on two dimensional ordinations in biplot analysis (Fig. 1 and 2). Assessment of genetic diversity and contribution of individual traits in overall diversity was determined by PCA (Panthee *et al.,* 2006). During both years analysis PC1 accounted maximum variation followed by PC2. Mohammadi and Prasanna (2003) observed that when there is high correlation among various traits then 1st few PCs account maximum diversity similarly when there is less diversity then extent of correlation decreases. Interestingly, 1st PC accounted maximum variation for yield related traits and 2nd PC for plant developmental traits. High loading values for yield and developmental traits also indicated that these traits are positively associated with each other. Therefore, selection for yield related traits such as thousand grain weight, number of grains per spike and yield per spike can be made from 1st PC. Similar results have also been observed in wheat (Ahmad *et al*., 2015) and in oat (Iannucci *et al*., 2011).

For improved classification of genotypes, ISSR markers were employed in this study (Ashraf *et al*., 2016). Among molecular markers, ISSR markers have proved to be very efficient systems in identifying large number of polymorphic bands which targets multiple simple sequences repeats loci across the genome (Dalamu *et al.,* 2012).

Molecular characterization in the current study revealed high level of genetic diversity in synthetic wheat genotypes. Tariq *et al.* (2018) also observed higher level of genetic diversity in synthetic wheat hybrids as compared to Pakistani origin wheat landraces. Sajjad *et al.* (2018) investigated decreasing trends of genetic diversity in Pakistani wheat genotypes. Therefore, exploration of synthetic wheat genotypes for genetic diversity analysis and utilization of this genetic diversity would be advantageous for wheat breeding and cultivar improvement program in Pakistan (Masood *et al.,* 2016). Dendrogram based on ISSR markers grouped the genotypes into 4 clusters on the basis of genetic similarity matrix. In the current study two methods were used for clustering of genotypes i) PCA biplot (morphological dataset) ii) dendrogram (molecular dataset). Both datasets showed differences in clustering of genotypes that might be due to the presence of polygenic inheritance for most of the traits which greatly affected by environmental factors. Other reason may be that most of the regions of the genome are not expressed and molecular markers may be located throughout the genome in coding or non-coding regions (Dalamu *et al.,* 2012; Ashraf *et al.,* 2016). Sofalian *et al.* (2008) also reported high level of genetic diversity in 39 wheat genotypes using ISSR markers. Ahmad *et al.* (2008) described that morphological characterization of genotypes should be accompanied with molecular characterization to determine the true association among genotypes (Teng *et al*., 2002).

Effectiveness of molecular markers depends upon their efficiency, especially when primers are being utilized for genetic diversity analysis. For dominant markers PIC value ranges from 0 to 0.5 and higher PIC values exhibit higher genetic diversity. Dj and Cj values are also very important in determining the efficiency of primers. Primers

with high PIC and Dj values coupled with low Cj value are considered more effective than others. The primer which is considered excellent for genetic diversity gives maximum *PIC* value among the genotypes (Riek *et al*., 2001). In the present study PIC values were ranged from 0.29 to 0.48. Maximum PIC value was exhibited by primer UBC-810 which accounted maximum genetic diversity. The minimum genetic diversity was exposed by the primer UBC-814 due to low PIC value (0.287). Therefore, this primer is not more efficient for genetic evaluation. The PIC values observed in this study were at par with those observed in other study using ISSR markers (Singh *et al*., 2009). UBC-810 appeared as the most efficient primer due to high PIC value coupled with high value of Dj and low value of Cj. From above results it can be concluded that UBC-810 is very powerful in revealing allelic diversity and have highest discriminatory power for genotypes (Sharma *et al*., 2009).

Conclusion

From the results it can be concluded that the genotypes G32, G21 and G22 could be used as parents in yield enhancement breeding programs. More interestingly, cluster analysis on the basis of molecular characterization also grouped these genotypes into same cluster which revealed similarity in genetic background of these genotypes. Among ISSR markers UBC-810 appeared as the powerful marker in exploring genetic diversity in wheat synthetics.

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