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Full Length Article

Genome Wide Diversity in Bread Wheat Evaluated by SSR Markers

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Abstract

To determine the genetic diversity and genome wide allelic variation, a natural population of 105 bread wheat genotypes was analyzed. The 302 polymorphic SSR markers, distributed among homeologous genomes A, B and D were manipulated. A total 2308 alleles of 302 markers were observed with average density of 7.6 alleles per marker. Among the observed polymorphic alleles, 685, 869 and 754 for 102, 100 and 100 polymorphic SSR loci were belong to A, B and D genome, respectively. The maximum (0.89) polymorphic information contents (PIC) value was observed for markers *Xwmc95*, *Xbarc95* and *Xwmc399*. These markers with total 15 alleles possessed the 100–320, 100–280 and 120–280 base pair genomic range at chromosomes 1A, 7B and 4D, respectively. The average PIC values ranged from 0.33 to 0.89 with a mean value of 0.68 in A-genome, while both B and D-genomes had PIC values ranged from 0.37 to 0.89 showing the averaged values 0.75 and 0.72, respectively. Gene diversity (GD) values ranged from 0.45 to 0.90 across the three A, B, and D genomes. The mean values of gene diversity were identified in B-genome (0.78) followed by D- (0.77) and A-genome (0.71), which indicated the maximum variation in B-genome and graded the three genomes as B>D>A-genome for genomic variation. The UPGMA cluster DARWIN tree and STRUCTURE analysis classified the 105 bread wheat genotypes into 4 groups or clusters. Genotypes G-31, G-32 and G-33 contained the combination of genetic makeup from the cluster 2 and cluster 3, while the genotypes G-71 and G-72 contained the genetic constitution from cluster 3 and cluster 4 which showed the origin from these clusters assuming the similar descendants. Genetic distances among clusters clearly showed the variations and genetic diversity among each of the clusters. The current experiment indicates the reliability of SSR markers that would be beneficial and robust resource for future genetic and genomic studies, as genetic map construction, comparative genomic analyses, genetic diversity and molecular marker-assisted breeding in wheat for selection of promising genotypes.

Keywords: Genome; Wheat; Yield; Polymorphism; Molecular markers

Introduction

Bread wheat (*Triticum aestivum* L.; 2n=6x=42, AABBDD allohexaploid) having three A, B and D homeologous genomes, has been evolved through a series of natural crossing and the effect of polyploidy (Gill and Gill 1994). In the evolutionary pathway of modern wheat, the allopolyploidization was occurred twice. In first step *T. urartu* (diploid) hybridized with *Aegilops speltoides* (wild grass) that resulted into tetraploid *T. turgidum* and in the second step, tetraploid AABB, (2n=4x=28) crossed with diploid goat grass *A. tauschii* having the genome DD (2n=2x=14) which produced (hexaploid=AABBDD) modern wheat (Förster *et al.* 2012).

According to (Dixon *et al.* 2009), wheat demand is increasing faster and it is expected that it will be reach up to 40% in 2030. So, there is need to increase the wheat production to confirm the food security. There are many constraints which are responsible for lower wheat production, including poor quality of seed, using broadcasting method for sowing, late sowing, poor soil management, unbalanced fertilizer application, improper weed eradication, diseases and shortage of water, heat and drought stress due to climatic changes (Ahmed *et al.* 2017b). Among cereals crops, wheat crop status is imperative because of nutritional values and more consumption. Massive growth in population and the liberated life style has directed to new challenges/problems for wheat breeders to

create new wheat genotypes with prominent yield and improved quality seed (Ahmed *et al.* 2019).

Use of molecular markers have been demonstrated as a prominent tool in the assessment of polymorphism and interpretation of genomic association for intra and inter varieties to obtain desirable genes for the improvement in yield (Budak *et al.* 2015). These molecular markers extensively used in applied plant breeding such as identification of qualitative and quantitative attributes loci (QTLs) and find out their position on chromosome, gene pyramiding, gene cloning for desired attributes, genetic diagnostics, marker-assisted selection (MAS), functional characterization of germplasm, phylogenetic relationship, genetic diversity analysis for numerous crop plants (Mwadzingeni *et al.* 2017). These markers are detectible gene sequences, specifically situated in the genome and inherited in the successive generations (Ahmed *et al.* 2017a).

Molecular markers have been applied enormously in the evaluation of genetic diversity, mapping of genes and identify the location of QTLs on chromosomes in plants (Zhang *et al.* 2011). In the past, molecular marker techniques also useful to recognize the genomic regions which are linked to the phenotypic expression of characters and resultantly leads to marker assisted breeding (MAB) or marker assisted selection (MAS) for the development of desired variety (Roy *et al.* 2011). In plants molecular markers as SSR (simple sequence repeat) and SNPs (single nucleotide polymorphisms) are. Genetic maps of major field crops have been established by generating the data from these markers (Lopes *et al.* 2015). In most of the crop plants, DNA markers are abundant and easily measurable. Furthermore, these DNA markers are not influenced due to external factors and can be applied for grouping of individuals (Mwadzingeni *et al.* 2017). Profiling of DNA to evaluate whether genetically and phenotypically similar genotypes is valuable in crop improvement. PCR based molecular marker techniques are effective techniques in the exposure of variation at the DNA level and genetic association (Kumar *et al*. 2016).

Classification of DNA markers divided into three groups is based on: i) Inheritance pattern. ii) Gene action behavior (dominant or co-dominant). ii) Hybridization or PCR based molecular markers. Molecular markers like, SSRs are useful for the determination of genetic diversity consuming their potential for robotics, co-dominance inheritance is additional advantage and they disperse in the three genomes on the 21 chromosomes (Ahmed *et al.* 2017a). Due to the SSR markers' abundance, chromosome specificity, co-dominant in nature, highly polymorphic, outstanding reproducibility and evenly genome wide distributed have been favored over other markers (Kumar *et al*. 2016). For wheat crop, SSR markers previously used to demonstrate the genetic diversity in wild and domesticated species of wheat and their improved germplasm. Presence of maximum genetic diversity beneficial for the selection and development of promising wheat varieties (Lopes *et al.* 2015).

Knowledge about diversity and association between desired traits is productive in yield enhancement and to obtain the evidence about the genetic basis of various biological developments (Henkrar *et al.* 2016). Gene sequencing plans for the kingdom of Plantae is hard therefore, DNA marker and their relationship with various characters has delivered a required landmark for explanation of genetic diversity. Cell organelles and DNA components like mitochondrial, retro-transposons, and chloroplast-based markers exhibited genetic variation through complex genome coverage (Bassi *et al.* 2016). Thus, DNA markers like SSR markers seemed to be the best techniques for accurate assessment of diversity in crop plants and assortment of germplasm (Henkrar *et al.* 2016). Therefore, keeping in mind the above information, the current experiment was conducted to distinguish bread wheat genotypes based on their genetic basis through SSR markers. The main objective of this study is to estimate the genetic diversity and genome wide allelic variation of studied germplasm for further selection in any breeding program.

Materials and Methods

Germplasm collection

The total 105 bread wheat genotypes were studied in this experiment. According to the maintaining sources, the germplasm divided into three groups (Supplementary 1 mentioned in Ahmed *et al.* 2019 published paper). In first group the genotypes G-1 to G-20 developed in the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad (PBG-UAF), Pakistan, while second group genotypes G-21 to G-55 were from exotic source and third group genotypes G-56 to G-105 were from indigenous source.

Plant growing condition and DNA extraction

In green-house, wheat seeds were sown in small plastic trays for healthy seedlings at Department of Plant Breeding and Genetics (PBG), University of Agriculture Faisalabad (U.A.F.), Pakistan. After three weeks, fresh leaves were collected for DNA isolation using modified Cetyl-Trimethyl Ammonium Bromide (CTAB) method (Saghai-Maroof *et al.* 1984) in 96 well-plates. The concentration and quality of isolated DNA was assessed by Nano-drop (ND1000, Thermo Scientific, U.S.A.). In Fig. 1 the isolated genomic DNA of 105 bread genotypes was indicated.

SSR markers-based genotyping

The total 302 genome wide polymorphic SSR markers were selected for study. Among them, based on the consensus map Ta-SSR-2004, 102, 100 and 100 markers found at the A, B, and D homeologous genomes, respectively (Somers *et al.* 2004). According to this map each genome had 15

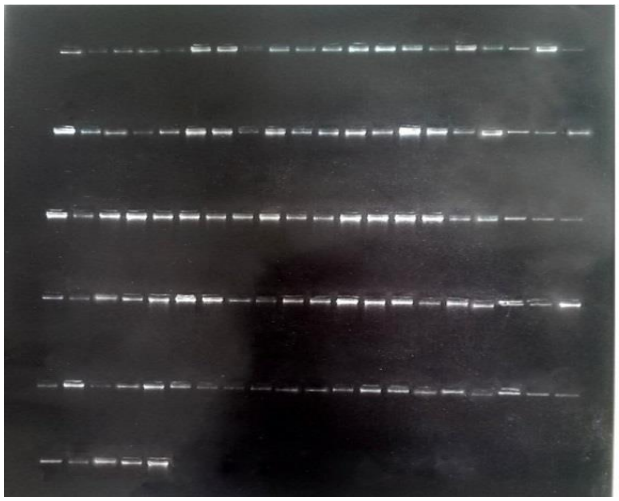

Fig. 1: Genomic DNA of 105 bread wheat genotypes isolated by CTAB method. Reading from left to right in 1st lane showing the genotypes from G-1 to G-20, 2nd lane from G-21 to G-40, 3rd lane from G-41 to G-60, 4th lane from G-61 to G-80, and 5th lane from G-81 to G-100, while 6th lane showing the last five genotypes from G-101 to G-105

polymorphic SSR markers located on each chromosome (1–7) except 3A, 3B and 3D chromosomes which had 13 polymorphic SSR markers on each chromosome, while 6B and 6D showed each 12 polymorphic SSR markers and 6A had 14 polymorphic SSR markers.

Using autoclaved Double Distilled Water 6.1 μL, Buffer (10X) 1 L, $MgCl_2$ (25 m*M*) 0.2 μL, dNTPs (2.5 m*M*) 0.3 μL, M13 fluorophores (10 μM) 0.08 μL, Taq DNA Polymerase (5 Units μL^{-1}) 0.2 μL, FORWARD Primer (10 μM) 0.02 μL and REVERSE Primer (10 μM) 8.0 μL with the Total Volume 8.0 μL for 1X (1 PCR reaction) and multiplied with 96 if used 96-well plate and further multiplied with 4 for 384-well plate. Here four types of M13 fluorophores were used, (1) FAM for Blue color (2) HEX Green color (3) NED for Yellow color and (4) PET for Red color peaks in capillary electrophoresis (John *et al.* 2012). After this, sealed the plate using the sealing mat (BIOEXPRESS T-3109-3). Centrifuged and put the plate in thermo-cycler using the following PCR steps. (1) Denature at 94°C for 5 min, (2) Denature at 94°C for 30 s and (3) Anneal at 60°C for 45 s (4) extension at 72°C for 60 s. In step 2 to 4 total 37 cycles were programmed. Finally extend at 72°C for 10 min.

After PCR, prepared the ABI plate, used one 384 well-plate to made DILUTION PLATE (GENEMATE T-6061-1) and another 384 well plate (single notch GENEMATE plate; GENEMATE T-3157-1) to made ABI plate along with four PCR amplified plates and one formamide plate (mixture of formamide dye with specific molecular weight marker or size standard) mix them into one plate. Finally, plates were ready for capillary electrophoresis in ABI (ABI Prism 3100 Genetic Analyzer, Applied Biosystems) (Daware *et al.* 2016). After running ABI, the data recorded and converted on Gene Mapper software which peaks (indicating the SSR base pair position just like on Gel electrophoresis) converted into numeric format like, 1 for presence and 0 for absence (Kujur *et al.* 2015).

Molecular data analysis

Polymorphic alleles were estimated in numeric by using Gene Marker on the basis of peaks which showed different allelic pattern of SSR markers. Four types of peaks were separated on the basis of different M13 fluorophores and then further aligned for genetic diversity and the genome wide allelic variation study. Total numbers of allele per markers and allelic frequency were measured through the statistical software GenAlEx version 6.5 (Smouse and Peakall 2012) and UPGMA (Un-weighted pair group method with arithmetic mean, or un-weighted neighbor joining tree) branching tree were created by statistical software DARWIN version 6 (Perrier *et al.* 2003) for the grouping of studied germplasm. POWER MARKER software version 3.23 (Liu and Muse 2005) applied for estimation of polymorphic information contents (PIC) values and gene diversity (GD). Bayesian clustering techniques was used to classify the group of genetically similar population *via* statistical software STRUCTURE v.2.3 (Pritchard *et al.* 2000). Web-based software package "Structure Harvester v0.6.93" was applied (Earl 2012) to obtain the maximum peak ''K'' which helpful for the visualization of STRUCTURE results to know the number of groups based on adhoc methods. Here "K" value selected from 1 to 10 in order to derive the reliable effects.

Results

Genome wide allelic variation

The total 302 genome wide polymorphic SSR markers selected for study, out of them 102, 100 and 100 found at the A, B, and D homeologous genomes, respectively. From each genome 15 polymorphic SSR markers located on each chromosomes except 3A, 3B and 3D chromosomes had each 13 polymorphic SSR markers, while 6B and 6D showed each 12 polymorphic SSR markers and 6A had 14 polymorphic SSR markers as displayed in Table 1. The total number of alleles in all genome was 2308 for 302 polymorphic SSR markers. Out of these, the total 685, 869 and 754 alleles were recorded for 102, 100 and 100 polymorphic SSR loci in A, B and D genome respectively. The mean value of polymorphic information content was 0.72, and the gene diversity (GD) value was 0.76 among the genome wide 302 polymorphic SSR markers. The total number of alleles (TNA) per marker ranged from 2 to 16 in A genome with the average value of 6.72 while both B and D genome showed 3 to 15 total numbers of alleles having the mean values of 8.69 and 7.54, respectively.

Table 1: List of polymorphic 302 SSR markers used to evaluate 105 bread wheat genotypes

S. No	MN	CL	TNA	PSM	SRBP	GD	PIC	S. No	MN	CL	TNA	PSM	SRBP	GD	PIC
1	*Xgdm33*	1A	15	92.38	120-280	0.90	0.88	152	Xbarc68	4B	5	94.29	220-260	0.76	0.72
2	*Xgwm136*	1A	9	100.00	210-290	0.80	0.76	153	Xgwm495	4B	5	94.29	220-260	0.72	0.67
3	*Xgwm11*	1A	5	94.29	220-260	0.76	0.72	154	Xgwm113	4B	15	100.00	100-320	0.90	0.89
4	*Xcfa2226*	1A	5	94.29	220-260	0.72	0.67	155	Xbarc25	4B	8	100.00	80-150	0.79	0.76
5	*Xwmc33*	1A	11	100.00	100-200	0.86	0.84	156	Xbarc20	4B	13	100.00	170-290	0.86	0.84
6	*Xwmc336*	1A	11	97.14	100-200	0.77	0.73	157	Xbarc163	4B	7	100.00	50-120	0.76	0.72
7	*Xwmc95*	1A	15	100.00	100-320	0.90	0.89	158	Xwmc692	4B	7	85.71	160-220	0.83	0.81
8	*Xwmc24*	1A	7	93.33	120-190	0.78	0.74	159	Xbarc109	4B	8	100.00	110-190	0.84	0.82
9	*Xbarc83*	1A	4	93.33	120-150	0.64	0.56	160	Xwmc617	4B	8	100.00	80-150	0.79	0.76
10	*Xgwm164*	1A	5	94.29	140-180	0.73	0.68	161	Xcfd5	5B	13	100.00	170-290	0.86	0.84
11	*Xbarc28*	1A	5	94.29	140-180	0.69	0.63	162	Xwmc773	5B	14	100.00	100-280	0.88	0.87
12	*Xgwm135*	1A	14	100.00	100-280	0.88	0.87	163	Xwmc630	5B	8	98.10	120-190	0.81	0.79
13	*Xbarc17*	1A	8	98.10	120-190	0.81	0.79	164	Xwmc47	5B	3	96.19	240-260	0.51	0.45
14	*Xbarc145*	1A	3	96.19	240-260	0.51	0.45	165	Xgwm443	5B	8	100.00	80-150	0.79	0.76
15	*Xwmc59*	1A	4	96.19	230-260	0.64	0.59	166	Xcfa2121	5B	13	100.00	170-290	0.86	0.84
16	*Xbarc212*	2A	6	97.14	120-170	0.77	0.73	167	Xwmc740	5B	8	100.00	80-150	0.79	0.76
17	*Xwmc382*	2A	8	100.00	120-190	0.86	0.84	168	Xgwm66	5B	13	100.00	170-290	0.86	0.84
18	*Xcfd36*	2A	8	100.00	100-170	0.79	0.76	169	Xgwm68	5B	7	100.00	50-120	0.76	0.72
19	*Xgwm359*	2A	5	95.24	200-240	0.74	0.69	170	Xbarc89	5B	7	85.71	160-220	0.83	0.81
20	*Xwmc149*	2A	7	90.48	110-170	0.78	0.75	171	Xgwm371	5B	8	100.00	110-190	0.84	0.82
21	*Xwmc453*	2A	6	100.00	270-320	0.70	0.64	172	Xgwm499	5B	8	83.81	140-230	0.81	0.78
22	*Xgwm339*	2A	8	98.10	120-190	0.73	0.70	173	Xwmc537	5B	5	100.00	260-300	0.69	0.63
23	*Xgwm448*	2A	2	100.00	200-210	0.50	0.37	174	Xcfd7	5B	7	98.10	120-190	0.77	0.73
24	*Xgwm95*	2A	10	100.00	90-190	0.77	0.73	175	Xwmc289	5B	5	100.00	190-230	0.78	0.74
25	*Xwmc702*	2A	7	100.00	80-140	0.75	0.71	176	Xgwm613	6B	3	98.10	210-230	0.46	0.38
26	*Xgwm328*	2A	5	94.29	150-190	0.74	0.69	177	Xwmc486	6B	3	98.10	170-190	0.60	0.53
27	*Xwmc819*	2A	8	100.00	80-150	0.79	0.76	178	Xgwm132	6B	15	100.00	100-320	0.90	0.89
28	*Xgwm47*	2A	13	100.00	170-290	0.86	0.84	179	Xwmc79	6B	14	100.00	100-280	0.88	0.87
29	*Xcfd168*	2A	7	100.00	50-120	0.76	0.72	180	Xgdm113	6B	8	98.10	120-190	0.81	0.79
30	*Xwmc181*	2A	7	85.71	160-220	0.83	0.81	181	Xwmc494	6B	3	96.19	240-260	0.51	0.45
31	*Xgwm369*	3A	8	100.00	110-190	0.84	0.82	182	Xgwm361	6B	15	92.38	120-280	0.90	0.88
32	*Xwmc532*	3A	2	100.00	160-170	0.50	0.37	183	Xbarc146	6B	9	100.00	210-290	0.80	0.76
33	*Xwmc11*	3A	4	100.00	240-270	0.54	0.49	184	Xbarc198	6B	5	94.29	220-260	0.76	0.72
34	*Xwmc215*	3A	4	100.00	160-190	0.55	0.44	185	Xbarc127	6B	5	94.29	220-260	0.72	0.67
35	*Xgwm155*	3A	8	100.00	200-270	0.76	0.73	186	Xgwm133	6B	15	100.00	100-320	0.90	0.89
36	*Xgwm2*	3A	6	100.00	100-150	0.76	0.72	187	Xbarc24	6B	14	100.00	100-280	0.88	0.87
37	*Xgwm32*	3A	7	100.00	80-150	0.82	0.79	188	Xgwm569	7B	8	98.10	120-190	0.81	0.79
38	*Xwmc651*	3A	3	100.00	270-290	0.62	0.54	189	Xwmc606	7B	3	96.19	240-260	0.51	0.45
39	*Xwmc627*	3A	2	100.00	250-260	0.49	0.37	190	Xgwm537	7B	8	100.00	80-150	0.79	0.76
40	*Xwmc527*	3A	7	99.05	170-230	0.81	0.77	191	Xgwm68	7B	13	100.00	170-290	0.86	0.84
41	*Xgwm391*	3A	7	99.05	130-190	0.82	0.79	192	Xbarc85	7B	7	100.00	50-120	0.76	0.72
42	*Xwmc264*	3A	4	100.00	200-230	0.68	0.61	193	Xwmc426	7B	7	85.71	160-220	0.83	0.81
43	*Xgwm162*	3A	5	100.00	90-130	0.62	0.58	194	Xgwm46	7B	8	100.00	110-190	0.84	0.82
44	*Xwmc516*	4A	6	98.10	200-250	0.65	0.58	195	Xwmc475	7B	8	100.00	80-150	0.79	0.76
45	*Xbarc206*	4A	7	100.00	130-190	0.81	0.78	196	Xbarc267	7B	13	100.00	170-290	0.86	0.84
46	*Xwmc15*	4A	6	99.05	290-340	0.62	0.54	197	Xbarc95	7B	15	100.00	100-320	0.90	0.89
47	*Xwmc491*	4A	11	100.00	230-350	0.82	0.80	198	Xwmc396	7B	14	100.00	100-280	0.88	0.87
48	*Xgwm601*	4A	7	91.43	240-300	0.84	0.81	199	Xwmc653	7B	8	98.10	120-190	0.81	0.79
49	*Xwmc617*	4A	9	99.05	180-280	0.71	0.66	200	Xgwm274	7B	3	96.19	240-260	0.51	0.45
50	*Xgwm397*	4A	9	99.05	140-220	0.86	0.84	201	Xgwm302	7B	8	100.00	80-150	0.79	0.76
51	*Xbarc170*	4A	13	98.10	240-420	0.77	0.74	202	Xwmc723	7B	13	100.00	170-290	0.86	0.84
52	*Xwmc468*	4A	7	100.00	150-220	0.78	0.75	203	Xgwm147	1D	7	100.00	50-120	0.76	0.72
53	*Xgwm565*	4A	11	100.00	70-180	0.82	0.80	204	Xbarc149	1D	7	85.71	160-220	0.83	0.81
54	*Xcfd257*	4A	4	87.62	240-270	0.73	0.67	205	Xgwm33	1D	8	100.00	110-190	0.84	0.82
55	*Xwmc283*	4A	12	99.05	100-260	0.82	0.79	206	Xcfd21	1D	15	92.38	120-280	0.90	0.87
56	*Xwmc232*	4A	5	95.24	140-190	0.61	0.53	207	Xgwm106	1D	9	100.00	210-290	0.80	0.76
57	*Xbarc78*	4A	6	97.14	140-190	0.73	0.68	208	Xbarc119	1D	5	94.29	220-260	0.76	0.72
58	*Xwmc722*	4A	5	100.00	150-190	0.68	0.62	209	Xbarc99	1D	5	94.29	220-260	0.72	0.67
59	*Xbarc69*	5A	7	100.00	100-190	0.64	0.57	210	Xbarc169	1D	8	100.00	80-150	0.79	0.76
60	*Xwmc173*	5A	3	97.14	210-230	0.50	0.44	211	Xbarc66	1D	13	100.00	170-290	0.86	0.84
61	*Xcfa2076*	5A	5	99.05	130-170	0.71	0.65	212	Xgwm642	1D	14	100.00	100-280	0.88	0.87
62	*Xbarc10*	5A	5	99.05	150-190	0.77	0.72	213	Xcfd63	1D	8	98.10	120-190	0.81	0.79
63	*Xgwm443*	5A	8	83.81	140-230	0.81	0.78	214	Xgdm126	1D	3	96.19	240-260	0.51	0.45
64	*Xwmc713*	5A	5	100.00	260-300	0.69	0.63	215	Xgdm11	1D	8	100.00	80-150	0.79	0.76
65	*Xgwm154*	5A	7	98.10	120-190	0.77	0.73	216	Xwmc405	1D	13	100.00	170-290	0.86	0.84
66	*Xcfa2190*	5A	5	100.00	190-230	0.78	0.74	217	Xbarc62	1D	7	100.00	50-120	0.76	0.72
67	*Xgwm129*	5A	3	98.10	210-230	0.46	0.37	218	Xgwm210	2D	7	85.71	160-220	0.83	0.81

Table 1: Continued

Table 1: Continued

68	*Xbarc117*	5A	3	98.10	170-190	0.60	0.53	219	Xcfd36	2D	8	100.00	110-190	0.84	0.82
69	*Xbarc180*	5A	12	99.05	60-190	0.88	0.86	220	Xwmc818	2D	8	83.81	140-230	0.81	0.78
70	*Xbarc56*	5A	8	97.14	90-190	0.77	0.75	221	Xgwm455	2D	5	100.00	260-300	0.69	0.63
71	*Xbarc186*	5A	3	100.00	170-190	0.49	0.39	222	Xwmc503	2D	7	98.10	120-190	0.77	0.73
72	*Xgwm156*	5A	8	95.24	100-180	0.84	0.81	223	Xgwm261	2D	5	100.00	190-230	0.78	0.74
73	*Xwmc795*	5A	16	100.00	240-400	0.88	0.86	224	Xwmc470	2D	3	98.10	210-230	0.45	0.37
74	*Xgwm334*	6A	4	100.00	160-190	0.74	0.69	225	Xbarc59	2D	3	98.10	170-190	0.60	0.53
75	*Xbarc206*	6A	2	98.10	120-130	0.45	0.33	226	Xwmc453	2D	14	100.00	100-280	0.88	0.87
76	*Xbarc23*	6A	4	99.05	240-270	0.65	0.58	227	Xwmc81	2D	8	98.10	120-190	0.81	0.79
77	*Xbarc3*	6A	4	99.05	160-190	0.73	0.68	228	Xgwm102	2D	3	96.19	240-260	0.51	0.45
78	*Xbarc195*	6A	2	98.10	110-120	0.50	0.37	229	Xgwm515	2D	8	100.00	80-150	0.79	0.76
79	*Xbarc48*	6A	5	100.00	230-270	0.72	0.66	230	Xbarc145	2D	13	100.00	170-290	0.86	0.84
80	*Xbarc146*	6A	9	97.14	240-370	0.80	0.77	231	Xcfd2	2D	7	100.00	50-120	0.76	0.72
81	*Xbarc165*	6A	4	98.10	160-190	0.47	0.40	232	Xcfd10	2D	7	85.71	160-220	0.83	0.81
82	*Xwmc672*	6A	3	93.33	160-180	0.50	0.42	233	Xgwm114	3D	8	100.00	110-190	0.84	0.82
83	*Xwmc201*	6A	10	97.14	240-330	0.81	0.78	234	Xbarc68	3D	8	83.81	140-230	0.81	0.78
84	*Xgwm570*	6A	7	98.10	60-120	0.79	0.76	235	Xcfd141	3D	5	100.00	260-300	0.69	0.63
85	*Xgwm617*	6A	4	98.10	160-190	0.70	0.64	236	Xgwm183	3D	7	98.10	120-190	0.77	0.73
86	*Xgwm169*	6A	8	96.19	150-220	0.78	0.75	237	Xbarc128	3D	5	100.00	190-230	0.78	0.74
87	*Xwmc417*	6A	3	97.14	130-150	0.67	0.59	238	Xwmc43	3D	3	98.10	210-230	0.45	0.37
88	*Xgwm666*	7A	5	93.33	180-220	0.78	0.74	239	Xcfd34	3D	3	98.10	170-190	0.60	0.53
89	*Xgwm233*	7A	3	100.00	120-140	0.60	0.53	240	Xwmc529	3D	8	83.81	140-230	0.81	0.78
90	*Xgwm350*	7A	5	94.29	140-180	0.76	0.71	241	Xgwm456	3D	5	100.00	260-300	0.69	0.63
91	*Xgwm471*	7A	3	96.19	160-180	0.55	0.46	242	Xwmc492	3D	7	98.10	120-190	0.77	0.73
92	*Xgwm60*	7A	6	100.00	220-270	0.78	0.74	243	Xcfd201	3D	5	100.00	190-230	0.78	0.74
93	*Xwmc283*	7A	9	100.00	240-320	0.84	0.81	244	Xwmc630	3D	3	98.10	210-230	0.45	0.37
94	*Xbarc154*	7A	6	100.00	100-160	0.73	0.68	245	Xcfd127	3D	3	98.10	170-190	0.60	0.53
95	*Xwmc826*	7A	8	98.10	50-140	0.84	0.81	246	Xwmc285	4D	14	100.00	100-280	0.88	0.87
96	*Xbarc174*	7A	14	100.00	100-280	0.88	0.87	247	Xwmc818	4D	8	98.10	120-190	0.81	0.79
97	*Xbarc23*	7A	8	98.10	120-190	0.81	0.79	248	Xwmc52	4D	3	96.19	240-260	0.51	0.45
98	*Xwmc17*	7A	3	96.19	240-260	0.51	0.45	249	Xwmc457	4D	8	100.00	80-150	0.79	0.76
99	*Xwmc65*	7A	8	100.00	80-150	0.79	0.76	250	Xgwm165	4D	13	100.00	170-290	0.86	0.84
100	*Xbarc121*	7A	13	100.00	170-290	0.86	0.84	251	Xwmc206	4D	7	100.00	50-120	0.76	0.72
101	*Xcfd20*	7A	7	100.00	50-120	0.76	0.72	252	Xwmc331	4D	7	85.71	160-220	0.83	0.81
102	*Xcfa2019*	7A	7	85.71	160-220	0.83	0.81	253	Xcfd84	4D	8	100.00	110-190	0.84	0.82
103	*Xgwm608*	1B	8	100.00	110-190	0.84	0.82	254	Xgwm194	4D	8	83.81	140-230	0.81	0.78
104	*Xgwm550*	1B	15	92.38	120-280	0.90	0.88	255	Xwmc825	4D	5	100.00	260-300	0.69	0.63
105	*Xwmc798*	1B	9	100.00	210-290	0.80	0.76	256	Xgwm609	4D	7	98.10	120-190	0.77	0.73
106	*Xwmc406*	1B	5	94.29	220-260	0.76	0.72	257	Xwmc720	4D	5	100.00	190-230	0.78	0.74
107	*Xgwm33*	1B	5	94.29	220-230	0.72	0.67	258	Xwmc48	4D	3	98.10	210-230	0.45	0.37
108	*Xgwm18*	1B	15	100.00	100-320	0.90	0.89	259	Xwmc489	4D	3	98.10	170-190	0.60	0.53
109	*Xwmc813*	1B	8	100.00	80-150	0.79	0.76	260	Xwmc399	4D	15	100.00	120-280	0.90	0.89
110	*Xbarc181*	1B	13	100.00	170-290	0.86	0.84	261	Xbarc130	5D	8	100.00	80-150	0.79	0.76
111	*Xgwm374*	1B	7	100.00	50-120	0.76	0.72	262	Xgwm190	5D	13	100.00	170-290	0.86	0.84
112	*Xwmc416*	1B	7	85.71	160-220	0.83	0.81	263	Xcfd189	5D	7	100.00	50-120	0.76	0.72
113	*Xwmc134*	1B	8	100.00	110-190	0.84	0.82	264	Xwmc150	5D	7	85.71	160-220	0.83	0.81
114	*Xwmc631*	1B	15	100.00	100-320	0.90	0.89	265	Xwmc608	5D	8	100.00	110-190	0.84	0.82
115	*Xwmc673*	1B	14	100.00	100-280	0.88	0.87	266	Xgwm358	5D	15	92.38	120-280	0.90	0.88
116	*Xcfa2147*	1B	8	98.10	120-190	0.81	0.79	267	Xcfd266	5D	9	100.00	170-290	0.80	0.76
117	*Xwmc44*	1B	3	96.19	240-260	0.51	0.45	268	Xcfd17	5D	5	94.29	220-260	0.76	0.72
118	*Xwmc661*	2B	8	83.81	140-230	0.81	0.78	269	Xgdm136	5D	5	94.29	220-260	0.72	0.67
119	*Xwmc35*	2B	5	100.00	260-300	0.69	0.63	270	Xgwm174	5D	13	100.00	170-290	0.86	0.84
120	*Xwmc25*	2B	7	98.10	120-190	0.77	0.73	271	Xcfd7	5D	7	100.00	50-120	0.76	0.72
121	*Xwmc213*	2B	5	100.00	190-230	0.78	0.74	272	Xcfd12	5D	7	85.71	160-220	0.83	0.81
122	*Xgwm257*	2B	3	98.10	210-230	0.45	0.37	273	Xwmc95	5D	8	100.00	110-190	0.84	0.82
123	*Xgwm429*	2B	3	98.10	170-190	0.60	0.53	274	Xwmc97	5D	8	100.00	80-150	0.79	0.76
124	*Xgwm148*	2B	15	100.00	100-320	0.90	0.89	275	Xwmc357	5D	13	100.00	170-290	0.86	0.84
125	*Xgwm374*	2B	8	100.00	80-150	0.79	0.76	276	Xcfd49	6D	8	100.00	110-190	0.84	0.82
126	*Xbarc167*	2B	13	100.00	170-290	0.86	0.84	277	Xcfd135	6D	14	100.00	100-280	0.88	0.87
127	*Xwmc498*	2B	7	100.00	50-120	0.76	0.72	278	Xcfd75	6D	8	98.10	120-190	0.81	0.79
128	*Xgwm388*	2B	7	85.71	160-220	0.83	0.81	279	Xgwm469	6D	3	96.19	240-260	0.51	0.45
129	*Xgwm120*	2B	8	100.00	110-190	0.84	0.82	280	Xcfd9	6D	8	100.00	80-150	0.79	0.76
130	*Xgwm47*	2B	15	92.38	120-280	0.90	0.88	281	Xcfd132	6D	13	100.00	170-290	0.86	0.84
131	*Xwmc332*	2B	9	100.00	210-290	0.80	0.76	282	Xcfd19	6D	7	100.00	50-120	0.76	0.72
132	*Xwmc434*	2B	5	94.29	220-260	0.76	0.72	283	Xgwm325	6D	7	85.71	160-220	0.83	0.81
133	*Xwmc430*	3B	5	94.29	220-260	0.72	0.67	284	Xcfd37	6D	8	100.00	110-190	0.84	0.82
134	*Xbarc92*	3B	8	98.10	120-190	0.81	0.79	285	Xcfd287	6D	8	100.00	110-190	0.84	0.82
135	*Xwmc597*	3B	3	96.19	240-260	0.51	0.45	286	Xbarc175	6D	14	100.00	100-280	0.88	0.87
136	*Xwmc808*	3B	15	92.38	120-280	0.90	0.88	287	Xbarc96	6D	8	98.10	120-190	0.81	0.79

Table 1: Continued

137	*Xwmc51*	3B	9	100.00	210-290	0.80	0.76	288	Xwmc646	7D	3	96.19	240-260	0.51	0.45
138	*Xbarc173*	3B	5	94.29	220-260	0.76	0.72	289	Xwmc506	7D	8	100.00	80-150	0.79	0.76
139	*Xwmc615*	3B	5	94.29	220-260	0.72	0.67	290	Xbarc184	7D	13	100.00	170-290	0.86	0.84
140	*Xwmc653*	3B	8	100.00	80-150	0.79	0.76	291	Xwmc450	7D	7	100.00	50-120	0.76	0.72
141	*Xwmc418*	3B	13	100.00	170-290	0.86	0.84	292	Xgwm635	7D	7	85.71	160-220	0.83	0.81
142	*Xgwm131*	3B	7	100.00	50-120	0.76	0.72	293	Xbarc70	7D	8	100.00	110-190	0.84	0.82
143	*Xcfd283*	3B	7	85.71	160-220	0.83	0.81	294	Xcfd41	7D	8	83.81	140-230	0.81	0.78
144	*Xbarc229*	3B	8	100.00	110-190	0.84	0.82	295	Xcfd26	7D	5	100.00	260-300	0.69	0.63
145	*Xgwm108*	3B	8	100.00	80-150	0.79	0.76	296	Xcfd31	7D	7	98.10	120-190	0.77	0.73
146	*Xwmc632*	4B	13	100.00	170-290	0.86	0.84	297	Xcfd21	7D	5	100.00	190-230	0.78	0.74
147	*Xgwm547*	4B	14	100.00	100-280	0.88	0.87	298	Xwmc438	7D	3	98.10	210-230	0.45	0.37
148	*Xwmc125*	4B	8	98.10	120-190	0.81	0.79	299	Xcfd14	7D	3	98.10	170-190	0.60	0.53
149	*Xbarc10*	4B	3	96.19	240-260	0.51	0.45	300	Xcfd193	7D	8	83.81	140-230	0.81	0.78
150	*Xgwm6*	4B	15	92.38	120-280	0.90	0.88	301	Xwmc150	7D	5	100.00	260-300	0.69	0.63
151	*Xbarc60*	4B	9	100.00	210-290	0.80	0.76	302	Xcfd25	7D	5	100.00	190-230	0.78	0.74

MN= marker name, CL=Chromosome Location, TNA=total number of alleles, PSM= Polymorphism, SRB=Size range in base pairs, GD= Gene diversity and PIC= Polymorphic Information Content

Table 2: Mean Allelic variations across 302 polymorphic SSRs in studied wheat germplasm

Population	Mean	Standard Error
No. of Average Alleles (Na)	7.642	0.204
No. of Alleles with a Frequency >=5% (Na Freq.>= 5%)	5.212	0.098
No. of Effective Alleles (Ne) = 1 / (Sum pi^2)	4.905	0.116
Shannon's Information Index (I) = -1* Sum (pi * Ln (pi))	1.650	0.026
No. of private Alleles = Unique to a Single Population	0.000	0.000
Heterozygosity (He) = 1 - Sum pi^2	0.754	0.007
Unbiased Expected Heterozygosity (uHe)= (2N / (2N-1)) * He	0.758	0.007

Polymorphic information contents (PIC) values range from 0.33 to 0.89 in A genome with the mean value of 0.68, while in both B and D genome it ranged from 0.37 to 0.89 with average of 0.75 and 0.72, respectively. Gene diversity (GD) values ranged from 0.45 to 0.90 across the three A, B, and D homeologous genomes with averaged values of 0.73, 0.79 and 0.76, respectively (Table 1). The mean value of allele per locus was 7.64 having the standard error (SE) value as 0.204. The mean number of different alleles with a ≥ 5% frequency was 5.212 with SE value 0.098. The average number of effective alleles was 4.905 with SE value 0.116. Shannon's Information Index (I) value was 1.650 with the SE values of 0.026 as shown in Table 2. The mean of heterozygosity (He) value was 0.754 in allelic variation with SE value 0.007 and unbiased expected heterozygosity (uHe) average value was 0.758 with the SE value 0.007 (Table 2).

Among 102 polymorphic SSR markers in A genome 44 markers showed 100% polymorphism in 105 studied wheat genotypes, while 11 markers showed 99% followed by 14 markers showed 98%, 8 markers had 97% and the remaining markers showed 96 to 84% polymorphism (Table 1). In A-genome the maximum PIC values was 0.89 for marker *Xwmc95* with 15 alleles followed by 0.88 in *Xgdm33* with 15 alleles and 0.87 in *Xbarc174* with 14 alleles. These markers (*Xwmc95*, *Xgdm33* and *Xbarc174*) located at the chromosomes 1A, 1A and 7A with the size range of 100–320, 120–280 and 100–280 base pairs and the gene diversity values of 0.90, 0.89, and 0.88, respectively. In A-genome the lowest PIC values 0.33 and 0.37 detected at *Xbarc206* and *Xgwm129* were found on chromosome 6A and 5A with the value of gene diversity 0.45 and 0.46 having the size range in base pairs 120–130 and 210–230 showing the 2 and 3 total numbers of alleles respectively.

In B-genome, among 100 polymorphic SSR markers the 57 markers showed 100% polymorphism in 105 studied wheat genotypes followed by 13 markers showed 98% and the remaining markers showed 96 to 84% polymorphisms as displayed in Table 1. The highest PIC value 0.89 of B genome was detected at *Xbarc95*, *Xgwm133*, *Xgwm132*, *Xgwm113*, *Xgwm148*, *Xwmc631* and *Xgwm18* markers, found on 7B, 6B, 6B, 4B, 2B, 1B, 1B chromosome with the 15 total number of alleles and the size range base pairs 100–280 having the 0.90 gene diversity in these markers. The lowest PIC values 0.37 and 0.38 identified at *Xgwm257* and *Xgwm613* were located on chromosome 2B and 6B having the values of gene diversity 0.45 and 0.46 respectively showing the size range in base pairs 210–230 with the 3 total numbers of alleles in above mention markers in B-genome.

The total 100 polymorphic SSR markers in D-genome, out of them 55 markers showed 100% polymorphism in 105 studied wheat genotypes, followed by 20 markers showed 98% and the remaining markers showed 96 to 88% polymorphisms were mentioned in Table 1. The D genome was conceded at *Xwmc399*, *Xgwm358* and *Xcfd21* marker found on 4D, 5D and 1D chromosomes having maximum PIC values 0.89, 0.88 and 0.87 respectively with 15 total number of alleles and having the size range of base pairs 120–280 with the value of 0.90 gene diversity in all mentioned markers. The lowest PIC values in D genome was 0.37 which identified in these markers namely, *Xwmc470*, *Xwmc43*, *Xwmc48* and *Xwmc438* were situated

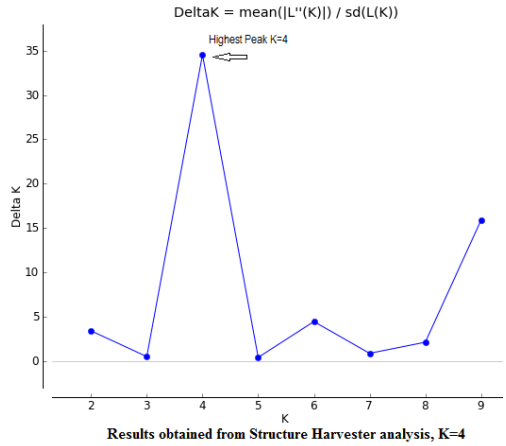

Fig. 2: This result achieved of 105 bread wheat genotypes using 302 polymorphic SSR markers from Structure Harvester analysis. It's based on the second order derivation on the variance of the maximum likelihood estimation of your model given a specific K. Delta K shows only the uppermost clustering level and number of subpopulations in main population

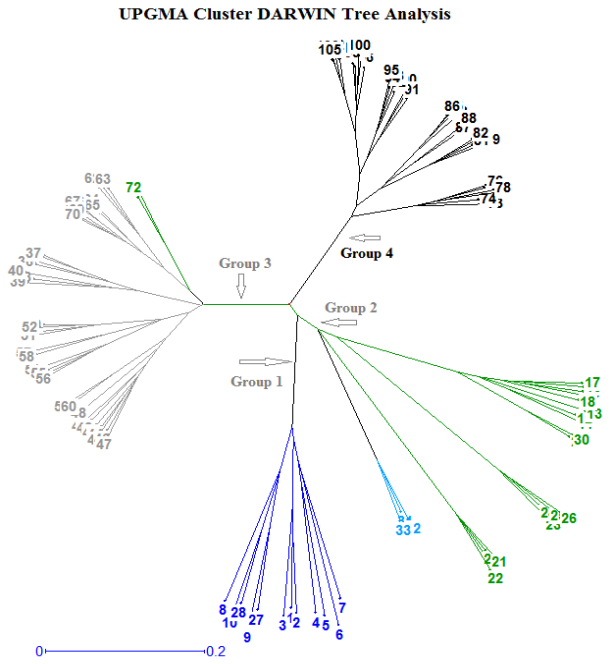

Fig. 3: UPGAMA DARWIN tree displaying the distribution of the 105 bread wheat genotypes in four groups, and presenting the genetic similarities and dissimilarities within and between the groups

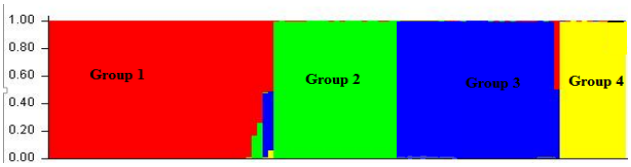

Fig. 4: Population structure of 105 bread wheat genotypes based on Bayesian method analyzed with 302 polymorphic SSRs detecting 4 groups. The dissimilar colors in this figure demonstrating the different group

on chromosome 2D, 3D, 4D and 7D respectively having the values of gene diversity 0.45 showing the size range in base pairs 210–230 with the 3 total numbers of alleles in these markers.

Genetic diversity

Bayesian technique implemented in statistical software STRUCTURE to access the genetic structure of studied germplasm and the outcomes showed that highest (peak) number of K=4 demonstrating the germplasm distributed into 4 sub-population (Fig. 2). Different types colored in Fig. 2 exhibits the distinct group and overall germplasm allocated into four sub-groups. Molecular UPGMA cluster DARWIN tree analyses and STRUCTURE Bayesian results exhibited that genotypes from department of PBG-UAF containing genetic diversity and were not present in the similar cluster which undoubtedly show that these genotypes derived from diverse forefathers. Additionally, evaluation of each group exposed that genotypes G-1 to G-10 and G-27 to G-28 located in the similar cluster, while the G-11 to G-26 and G-29 to G-30 genotypes were entirely seemed in the second cluster. The third cluster composed of a combination of the diverse genotypes had G-34 to G-70. The fourth cluster contained the G-73 to G-105. Similar results obtained from the STRUCTURE Bayesian and DARWIN tree analyses using 302 polymorphic SSR markers in 105 bread wheat genotypes (Fig. 3 and 4).

Discussion

Molecular markers like simple sequence repeats (SSRs) have been extensively used to detect variability in wheat genotypes and to evaluate their genetic diversity. The PIC values of SSR markers could be used to access the amount of genetic variability in plant sciences. When the PIC value is greater than 0.5 the marker is suggested to be of maximum diversity, if the PIC values is less than 0.25 the marker is suggested to be of minimum diversity (Ramadugu *et al.* 2015) and (Sönmezoğlu and Terzi 2018). In this experiment most of the markers having PIC values greater than 0.5 which indicate the presence of high allelic diversity in studied germplasm. SSR markers have also been widely applied to perceive gene variability in wheat germplasm and to estimate their genetic diversity (Raza *et al.* 2019). The mean values and SE values presented in Table 2 proposing that there is great genetic diversity at SSR loci among studied germplasm. In current study no presence of rare alleles (number of alleles unique to a single population) similar study was conducted by (Sajjad *et al.* 2018). The maximum mean values of gene diversity were identified in B-genome (0.78) followed by D-genome (0.77)

and A-genome (0.71) which suggesting that the B-genome showed more variation and the existence of genetic diversity in studied germplasm (Kumar *et al.* 2016). In bread wheat, genome wide 65 SSRs specifically 1–4 markers for each chromosome were applied previously by wheat breeders (Wang *et al.* 2013; Ahmed *et al.* 2017a) to determine the genetic diversity. Those markers perceiving the minimum total number of alleles displayed minimum gene diversity as compared to those which have high total number of alleles depicted the maximum gene diversity (Salehi *et al.* 2018; Sönmezoğlu and Terzi 2018). In current experiment, based on the mean values of total number of alleles per markers in B-genome exhibited the maximum genetic diversity as compared to the D-genome followed by A-genome which showed the minimum genetic diversity. Similar findings were described by wheat scientists (Ahmed *et al.* 2017a) where they described that the total number of alleles per markers ranged from 2–15. Our results are not similar with the findings of Tascioglu *et al.* (2016), they reported the lower values as compared to current experiment. They observed the average value of total numbers of allele 5 in A-genome and D-genome while 6 in B-genome. Dvojković (2010) described the higher total number of alleles as 8.86, 8.893 and 9.65 for A, B and D-genome respectively as compared to this study.

The total numbers of allele per locus ranged from 3 to 22 with the mean value of 7.8 was previously reported by plant scientists is similar to this experiment (Jain *et al.* 2004). In current study PIC values revealed a significant positive association with the gene diversity (GD) and total numbers of allele for SSR markers. All the results (GD, TNA and PIC values) suggested the existence of genetic diversity and ranked it as B-genome > D-genome > A-genome among the three genomes. The allelic SRBP (size range in base pairs) strongly associated with the TNA which is closed to the previously reported findings of wheat breeders (Akfirat and Uncuoglu 2013). The results reported by wheat scientists about strong association of TNA and allelic SRBP also supported the results of current study (Herrera *et al.* 2008; Kumar *et al.* 2016). Current study on genetic diversity and genome wide allelic variation in bread wheat genotypes may be favorable for planning the future strategies on wheat genetic resources and better the wheat breeding scheme for development of novel wheat genotypes.

The UPGMA cluster DARWIN tree and STRUCTURE analysis concentrating to the distribution of 105 bread wheat genotypes into 4 subgroups or clusters (Fig. 3 and 4). These techniques have been applied in wheat breeding scheme by many scientists and were obtained the explanatory outcomes (Ahmed *et al.* 2017a; Salehi *et al.* 2018). In current experiment, distances among cluster or group clearly show the variations between 105 bread wheat genotypes and all subgroups showed genetically diverse to one another. The presence of maximum genetic distance between clusters indicates that they were genetically dissimilar from each other. Fundamentally, this is the indication of genetic divergence between the clusters or groups and resultantly the presence of more genetic diversity in studied germplasm. There is a minor genetic distance among the genotypes within each cluster or group which shows the genetic similarity among 105 genotypes, closer genotypes showed more genetic similarity as mentioned in the Figure 3. Several wheat breeders evaluated the genetic diversity (Tascioglu *et al.* 2016; Ahmed *et al.* 2017a) using the similar techniques which were applied in current study and they got the similar results. Using 296 SSRs in 90 bread wheat genotypes by (Chen *et al.* 2012) and they observed the 3 clusters which convening the geographical origin and genetic diversity among germplasm. Development of novel bread wheat genotypes should be attaining the significance level of genetic diversity. Presence of more variation in 105 bread wheat genotypes which indicate the maximum genetic diversity, fearlessly, that the studied germplasm introduced from different sources or assumable mechanical mixing.

According to the provided pedigree record there are three groups of 105 bread wheat genotypes as shown in Supplementary Table 1. In first group, genotypes G-1 to G-20 which was developed in PBG-UAF, while in second group the genotypes G21 to G-55 were from exotic source, and in third group, genotypes G-56 to G-105 was from indigenous sources. But according to molecular analysis these genotypes divided into four clusters or groups. Wheat genotypes developed in PBG-UAF comprised the cluster 1, genotypes G-27 and G-28 are also included in this cluster which exhibited the genetic similarity with each other. Total 18 genotypes constituted in cluster 2, among them, some genotypes related to the PBG-UAF sources and some genotypes were from exotic sources. It exhibited that these genotypes originated from the ancestors of similar genetic makeup. Total 67 wheat genotypes were appeared in cluster 3 and 4. Out of them, 35 genotypes included in cluster 3 and 32 genotypes included in cluster 4. These genotypes produced by a mixture of the diverse genetic constitutions which suggesting the diverse pedigrees of these genotypes. Genotypes G-31, G-32 and G-33 contained the combination of genetic makeup from the cluster 2 and cluster 3 covering genotypes. The genotypes G-71 and G-72 contained the genetic constitution from cluster 3 and cluster 4 which showed that their origin from these clusters and assuming the similar descendants. Particularly, results were useable conferring to the previously known pedigree record and origin of wheat genotypes. Genetic diversity evaluation could be helpful to identify the different genotypes for the advancement and improve the future wheat breeding scheme (Yadav and Chand 2018; Ahmed *et al.* 2019; Lazzaro *et al.* 2019). The genotypes with different genetic makeup can be selected for desirable combinations to develop complex and significant attributes to obtaining maximum yield. Discrimination of wheat genotypes based on their genetic basis would be useful for effective and early selection of desired genotypes in wheat breeding scheme for

developing promising wheat genotypes.

Conclusion

A natural population of 105 bread wheat genotypes was genotyped with 302 polymorphic SSR loci, and a total 2308 alleles with average density of 7.64 alleles per marker were observed to determine the genetic diversity and genome wide allelic variation. The maximum (0.89) polymorphic information contents (PIC) value was observed for markers *Xwmc95*, *Xbarc95* and *Xwmc399*. These markers with maximum alleles (15) possessed the 100–320, 100–280 and 120–280 base pair genomic range at chromosomes 1A, 7B and 4D, respectively. These three and other similar SSR markers can use to evaluate the diversity and to classify any of the natural population of wheat. The polymorphic information contents (PIC) and Gene diversity (GD) values indicated the maximum genetic variation in B-genome followed by D- and A-genomes. It can be concluded that any of the agronomic traits linked to the B-genome can get the advantage of maximum genetic variation in a selection process. The UPGMA cluster DARWIN tree and STRUCTURE analysis classified the 105 bread wheat genotypes into four clusters. The clusters information can help to reduce the redundancy among genetically similar accession and to select the genotypes of diverse genetic back ground in any wheat breeding program.

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