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Full Length Article

Genome Wide Allelic Pattern and Genetic Diversity of Spring Wheat Genotypes through SSR Markers

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Abstract

Genetic diversity is basis for crop improvement. In wheat breeding programs, it is desirable to have large genetic diversity for the creation of new genotypes. Spring wheat genome was explored with 95 simple sequence repeat (SSR) markers dispersed all over the three A, B and D homeologous genomes in 105 spring wheat genotypes for determination of genetic diversity. Number of alleles ranging from 2 to 15 were generated across all the genomes with an average of 6.57 per SSR marker. The highest polymorphic information contents (PIC) value 0.89 for *gwm136* marker sited on 1A chromosome with 15 total number of alleles having the size range in base pairs 270–420 and 0.90 gene diversity. The maximum PIC values 0.86 and 0.81 were carried at *cfd020* and *wmc121* markers located on 5B and 7D chromosomes respectively while the lowest PIC values 0.36, 0.35 and 0.33 were detected at *cfa2155*, *wmc073* and *gdm153* markers located on 5A, 5B and 5D chromosomes among the A, B and D genomes respectively. Gene diversity and PIC values ranged from 0.41 and 0.33 to 0.90 and 0.89 respectively indicating the significant level of genetic diversity among spring wheat genotypes and these findings ranked as: genome A> genome B > genome D. The studied genotypes were distributed into four genetically different subdivisions based on STRUCTURE Bayesian approach and DARWIN model analysis. Three genotypes G31, G32 and G33 had the mixed genetic material from the group 2 and group 3 while G71 and G72 shared the genetic material from the group 3 and group 4 which indicates the origin of the genotypes from these groups having same ancestors. Present study showed the potentiality of SSR markers for the study of genetic diversity in spring wheat and this information would be a good tool for choosing the desirable genotypes in wheat breeding programs for creating promising spring wheat varieties.

Keywords: diversity, origin, genotypes, alleles, cluster.

Introduction

Spring wheat (*Triticum aestivum* L.) belongs to the most diverse family Poaceae of the plant kingdom. It produces edible grains and has been a chief source of staple food for human and feed for animals. According to Gupta *et al*., (2008) it feeds 40% of the world's population in daily routine and subsidizes 20% consumption in terms of protein and total calories. It has one of the largest and most complex genomes among other cereal crops (Arif *et al*., 2010). It is allohexaploid with three A, B and D homeologous genomes. The haploid genome size of bread wheat is approximately 17GB in contrast with the human genome five times more (Abebe and Leon, 2012; Faheem *et al.*, 2015). The plodding growth in population demands with the passage of time a considerable increase in its production. The greatest way to enhance wheat productivity is through genetic manipulation so achieving the maximum yield (Ahmed *et al.*, 2017). Therefore, it is crucial to study the mode of inheritance and estimate the genetic diversity at allelic levels to initiate dynamic spring wheat breeding programs.

Molecular markers such as simple sequence repeats (SSRs) are directly responsible for the estimation of genetic diversity having their potential for automation, codominance inheritance are surplus benefits and they cover all the twenty one wheat chromosomes on A, B and D genomes (Plaschke *et al*., 1995). Microsatellite markers or SSRs are one of the most influential molecular marker classification due to highly polymorphic, chromosome specific, highly reproducible and co-dominant nature in eukaryotic genomes (Zhang *et al*., 2010). Due to high accuracy of SSR markers, they have been successfully used for genotype identification of Sorghum (Li *et al.*, 2005); rice (Rahman *et al*., 2009) and pigeon pea (Khalekar *et al*., 2014).

In case of wheat, SSR markers have been utilized to illustrate genetic diversity for the wild relatives of wheat (Hammer *et al*., 2011) and for improved wheat germplasm (Tulin *et al*., 2016). Since, varieties with similar morphological characteristics are increasing promptly every year, this is very essential to validate wheat varieties using molecular markers to distinguish similar varieties/lines and establish diversity among them for the improvement of

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future wheat breeding program, because large genetic diversity should exist for the development of promising genotypes. Hence, keeping in view the above facts, the present study was undertaken to discriminate genotypes based on their genetic basis using SSR markers. The basic aim of this research is to determine the genome wide allelic pattern and genetic diversity of the investigated genotypes.

Materials and Methods

Experiment Material

A representative collection of 105 genotypes were procured from the department of Plant Breeding and Genetics University of Agriculture Faisalabad (PBG-UAF). The name, pedigree (if available) and origin of these 105 accessions are listed in Table 1. The 50 accessions are indigenous and widely cultivated varieties, which were produced from different research institutes of Pakistan, 35 exotic accessions and 20 accessions developed in PBG-UAF Pakistan used in wheat breeding program.

Plant Growing Conditions and DNA Extraction

Seeds were sown in small plastic pots in green house, where normal agronomical practices were followed for raising healthy plants during the season 2016. After three weeks, leaves were collected for DNA extraction. Genomic DNA extracted from approximately ~ 0.2 g fresh leaf tissue using the modified cetyl-trimethyl ammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984). The quality and concentration of extracted DNA was estimated by Nano-drop (ND1000, Thermo Scientific, USA) and also followed by gel electrophoresis using known concentrated DNA.

Moleculer Marker and PCR Profile

A total of 95 SSR markers, which were distributed evenly over the 21 wheat chromosomes, were selected and synthesized according to the information available in Grain Genes database (Table 2). Genome-wide 95 SSRs comprised of 57, 19 and 19 markers were specific to A, B, and D homeologous genomes of spring wheat for determination of genetic diversity. Total reaction volume was 20 µL for PCR amplifications, having the 2 µL genomic DNA used as template, 2 µL 10X buffer containing 1.5 mg $MgCl_2$, 0.1 µL *Taq* polymerase of 5 Units μL^{-1}, 0.4 µL 10 mM dNTPs, and 2 µL (forward and reveres) of 2.5 µM primer. PCR profile contained total 35 cycles at 94ºC for 45s, specific primer pairing at optimum annealing temperature for 45s, and 72ºC for 60s, and the last step extension at 72ºC for 10 min. Amplified PCR products were examined by using 2% agarose gel electrophoresis (AGE) and visualized under Ultra violet light.

Molecular Data Analysis

Visualized polymorphic bands were calculated in numeric format like, 1 for presence and 0 for absence and data further aligned for the genome wide allelic pattern and genetic diversity analysis. Total number of alleles per locus and allele frequency were determined using the statistical software GenAlEx version 6.5 (Peakall and Smouse, 2012) and UPGMA (Unweighted pair group method with arithmetic mean, or unweighted neighbor joining tree) tree construction branching clusters were developed using statistical software DARWIN version 6 (Perrier *et al*., 2003) for the classification of population into sub population. POWER MARKER software version 3.23 (Liu and Muse, 2005) used for calculation of polymorphic information content (PIC) values, gene diversity and Bayesian clustering method was applied to identify clusters of genetically similar individuals using the statistical software STRUCTURE v.2.3 (Pritchard *et al*., 2000). A burn-in length of 10^4 cycles (to minimize the effect of starting configuration), a simulation run of 10^6 cycles, and the admixture model option were applied in the STRUCTURE program. Web-based software package "Structure Harvester v0.6.93" was used (Earl and vonHoldt, 2012) to derived the peak or optimal number of clusters ''K.'' this is permits the visualization of the STRUCTURE results to understand the number of clusters based on ad-hoc techniques. We chose cluster values (K) ranging from 1 to 10 and six independent runs for each value in order to obtain consistent results.

Results

Genome Wide Allelic Pattern

Among the selected 95 SSR microsatellite markers 57, 19 and 19 were found to be located on A, B and D homeologous genomes respectively. The total number of alleles per marker ranged from 2 to 15, polymorphic information content (PIC) ranged from 0.330 to 0.891 and gene diversity from 0.419 to 0.903 across all the genomes (Table 2). The highest PIC value in *gwm136* marker for A genome 0.891 at chromosome 1A with 15 total number of alleles having the size range in base pairs 270–420 with 0.903 gene diversity. The maximum PIC value was 0.860 of B genome was detected at *cfd020* marker found on 5B chromosome with the 14 total number of alleles having the size range in base pairs 290–420 with 0.876 gene diversity. The D genome was conceded at *wmc121* marker positioned on 7D chromosome for maximum PIC 0.812 value with 9 total number of alleles having the size range in base pairs 280–350 with 0.837 gene diversity, while the lowest PIC values 0.366, 0.353 and 0.330 were detected at *cfa2155*, *wmc073* and *gdm153* markers located on 5A, 5B and 5D chromosomes among the A, B and D genomes respectively.

Table 1: Genotypes code, name, pedigree and origin of 105 spring wheat genotypes

Code	Name	Pedigree	Code	Name	Pedigree
G1	9493	LU26S/PB96	G54	Gutha	GAMENYA//GABO*3/KHAPSTEIN(M-146)/3/(66-W-02)FALCON*3/CHILE-1-B
G2	9496	5039/Rawal87	G55	Sunstar	CONDOR,AUS/4/2*WW-15/3/STEINWEDEL/YAROSLAV-EMMER//LA-PREVISION
G3	9508	5039/PB96	G56	Watan01	LU26/HD 2179
G4	9515	4770/PB70	G57	AARI-2011	SH-88/90A-204//MH97
G5	9610	Pasban90/4943	G58	Aas-2011	PRL/PASTOR//2236(V6550/SUTLEH-86)
G6	9618	Pb96/Pasban90	G59	Abadgar-93	PSN/BOW
G7	9675	Pasban90/WLRG4 1-8 (1993-94)	G60	Anmol-91	KVZ/TRM//PTM/ANA
G8	9707	8060/Rawal87	G61	AS-2002	URES/BOW`S
G9	9736	Inq91/30th SAWSN 30 (1998-99)	G62	Auqab-2000	CROW'S'/NAC//BOW'S'
G10	9796	BOWN 14 (1999-2000)/Kohistan97	G63	Bahawal-97	PFAU'S'/SERI
G11	9797	BOWN 11 (1999-2000)/Kohistan97	G64	Bahawalpur-2000	AU/UP301//GLL/Sx/3/PEW S/4/MAI S/MAY A S//PEWS
G12	9869	WON 13 (2001-02)/Iqbal2000	G65	Bahtawar-94	Mentana/Mayo-48//4-11
G13	9870	WON 13 (2001-02)/Iqbal2000	G66	Bakar-2002	P20102/PIMA/SKA/3/TTR'S'/BOW'S'
G14	9877	9244/PBW222	G67	Bakhtawar-93	AU/UP301//GLL/SX/3/PEW/4/MAI/MAYA//PEW
G15	9883	9244/Iqbal2000	G68	Bars-2009	PFAU/SERI//BOW
G16	9930	22 SAWSN 14 90 (2005-06)/9258	G69	Bathoor-2008	URES/JUN//KAUZ
G17	9970	DN49/Sahar2006	G70	Chakwal-50	ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA
G18	9889	9244/Parwaz94	G71	Chakwal-86	FORLANI/ACC//ANA or Fln/ACS//ANA
G19	9970	DN49/Sahar2006	G72	Chakwal-97	BUC'S'/FCT'S'
G20	9764	WLRG 3 1-8 (1993-94)/5039	G73	Shafaq-2006	LU 26/HD 21790/ 2*INQALAB 91
G21	BWL-812	C 591/RN//JN/3/CHR/HD 1941	G74	Fakhar. Sarhad	NORD-DESPREZ(ND)/VG-9144//KALYANSONA/BLUEBIRD/3/YACO/4/VEERY-5
G22	PBW-175	HD2160/4/JN/GAGE//JN/KALYANSONA/3/V-18/C-273; HD-2160/WG-1025;	G75	Fareed-2006	PT'S'/3/TOB/LFN//BB/4/BB/HD-832-5//ON/5/G-V/ALD'S'//HPO
G23	Anza	LERMA-ROJO-64//NORIN-10/BREVOR/3/3*ANDES-ENANO	G76	FD-2008	PBW65/2*Pastor
G24	PBW 222	NP 890 /HD 2160	G77	FD-83	FURY//KAL/BB
G25	HD 2307	HD-2160/116-1-3	G78	FD-85	MAYA/MON//KVZ/TRM
G26	DPW-621-50	KAUZ//ALTAR-84/(AOS)AWNED-ONAS/3/MILAN/KAUZ/4/HUITES	G79	GA 2002	DWL5023/SNB//SNB
G27	PBW 343	NORD-DESPREZ/VG-1944//KALYANSONA//BLUEBIRD/3/YACO (SIB)/4/VEERY-5	G80	Glaxy-2013	Punjab96/87094/MH-97
G28	HD 2967	ALD/COC//URES/HD2160M/HD2278	G81	Gomal-2008	Atilla
G29	BWL-1793	ND/VG9144 //KAL/BB/3/YCO"S'/4/VEE#5 "S'	G82	Hashim-2008	JUP/ALD'S'//KLT'S'/3/VEE'S'/6/BEZ//TOB/8156/4/ON/3/6*TH/KF//6*LEE/KF/5
G30	BWL-9022	N/A	G83	Inq-91	WL 711/CROW "S"
G31	BWL-0924	N/A	G84	Iqbal-2000	BURGUS/SORT 12-13//KAL/BB/3/PAK 81
G32	C-78711	N/A	G85	Kaghan-93	TTR/JUN
G33	C-252782	N/A	G86	Khyber-87	KVZ/TRM//PTM/ANA
G34	BWL-1771	N/A	G87	Kohistan-97	V-1562//CHRC'S'/HORK/3/KUFRA-I/4/CARP'S'/BJY'S'
G35	C-252874	N/A	G88	Kohinoor-83	ORE F1 158/FDL//MFN/2*TIBA63/3/COC
G36	C-252803	N/A	G89	Kohsar-95	PSN/BOW
G37	C-118737	N/A	G90	Lasani-2008	LUAN/KOH-97
G38	C-128196	N/A	G91	Ufaq-2002	V.84133/V83150
G39	C-335716	N/A	G92	Marvi-2000	CMH-77A917/PKV 1600//RL6010/6*SKA
G40	C-212185	N/A	G93	Maxi-Pak 65	PJ/GB55 or PJ62/GB55
G41	C-32586	N/A	G94	Mehran-89	KVZ/BUHO//KAL/BB
G42	C-532653	N/A	G95	MH-97	NORD-DESPREZ(ND)/VG-9144//KALYANSONA/BLUEBIRD/3/YACO/4/VEERY-5
G43	C-437081	N/A	G96	Millat-2011	CHENAB2000/INQ-91
G44	C-410028	N/A	G97	Mirij-2008	SPARROW/INIA//V.7394/WL711/13/BAUS
G45	BWL-0814	N/A	G98	Moomal-2002	BUC or BUCS/4/TZPP/IRN46
G46	Sonara-64	N/A	G99	Mugall-99	OPATA/BOW'S'
G47	PBN-51	BUCKBUCK/FLICKER/BUCKBUCK(SIB)/(SIB)FLICKER/(SIB)VEERY	G100	NARC-2009	INQALAB 91*2/TUKURU
G48	C-586642		G101	Nifa-barat 2010	FRET2
G49	Sakha	INIA-66(SIB)/NAPO-63	G102	Nowshera-96	BUC/FLK//MYNA/VUL
G50	PBW 621	KAUZ/ALTAR-84/(AOS)AWNED-ONAS/3/MILAN/KAUZ/4/HUITES	G103	Pak-81	KVZ/BUHO//KAL/BB
G51	C-296299	N/A	G104	parwaz-94	V.5648/PARULA or V.5648/PRL
G52	Bareukee	N/A	G105	Pasban-90	KVZ/3/TOB/CTFN/BB/4/BLO/5/VEE#5/6/BOW/3/YD//BB/CHA
G53	Redfiled	N/A			

G1-G20 (origin) PBU-UAF= Department Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
G21-G55 (origin) Exotic = Foreign (other than Pakistan) spring wheat genotypes
G56-G105 (origin) Indigenous = Pakistani spring wheat genotypes

Table 2: 95 SSR markers used for 105 wheat genotypes

MN	CL	GD	PIC	TNA	SRBP	MN	CL	GD	PIC	TNA	SRBP
barc0017	1A	0.897	0.883	15	250-320	wmc492	5A	0.875	0.857	12	60-160
barc0083	1A	0.797	0.765	9	252-290	barc0003	6A	0.733	0.681	4	188-230
barc0158	1A	0.759	0.717	5	232-243	gwm334	6A	0.502	0.375	2	110-220
cfa2219	1A	0.722	0.672	5	250-300	wmc201	6A	0.719	0.665	5	230-260
gwm135	1A	0.859	0.839	11	120-160	wmc553	6A	0.802	0.773	9	340-370
cfa2226	1A	0.766	0.729	11	180-220	wmc243	6A	0.473	0.398	4	180-230
gwm136	1A	0.903	0.891	15	270-420	cfa2257	7A	0.667	0.590	3	140-280
gwm164	1A	0.779	0.739	7	130-200	gwm060	7A	0.778	0.738	5	180-220
gwm357	1A	0.637	0.559	4	130-210	gwm233	7A	0.604	0.527	3	130-220
wmc183	1A	0.727	0.679	5	150-210	barc0008	1B	0.540	0.492	4	240-270
wmc278	1A	0.694	0.630	5	150-170	gwm018	1B	0.548	0.441	4	180-240
wmc312	1A	0.883	0.869	14	220-260	wmc044	1B	0.764	0.732	8	200-250
wmc716	1A	0.814	0.790	8	130-190	wmc367	1B	0.755	0.716	6	110-150
barc0083	1A	0.514	0.448	3	250-320	wmc216	1B	0.823	0.794	7	80-140
cfa2219	1A	0.637	0.588	4	250-310	gwm429	2B	0.618	0.543	6	300-330
gwm357	1A	0.768	0.727	6	130-210	wmc027	2B	0.821	0.796	11	230-390
wmc278	1A	0.862	0.841	8	160-220	wmc418	3B	0.728	0.673	4	240-260
barc0119	1A	0.792	0.760	8	102-154	barc0163	4B	0.712	0.652	5	140-170
gwm011	1A	0.738	0.689	5	203-240	gwm513	4B	0.767	0.723	5	160-250
cfa2129	1A	0.783	0.746	7	120-170	gdm146	5B	0.775	0.746	8	140-170
cfd059	1A	0.701	0.644	6	278-325	wmc073	5B	0.489	0.353	3	190-220
gdm033	1A	0.730	0.701	8	126-197	wmc160	5B	0.837	0.811	8	120-180
barc0148	1A	0.502	0.375	2	200-240	cfd020	5B	0.876	0.860	14	290-420
cfd015	1A	0.768	0.731	10	160-240	wmc397	6B	0.495	0.423	3	160-220
wmc336	1A	0.747	0.708	7	90-120	wmc737	6B	0.807	0.776	10	280-330
wmc011	1A	0.736	0.690	5	160-190	gwm146	7B	0.756	0.710	5	140-180
gwm666	1A	0.791	0.757	8	90-130	gwm297	7B	0.553	0.463	3	160-290
cfd030	1A	0.864	0.844	13	170-300	wmc076	7B	0.778	0.738	6	220-270
barc0028	1A	0.756	0.717	7	60-120	cfa2147	1D	0.625	0.544	3	290-320
wmc059	1A	0.833	0.805	7	150-210	cfd092	1D	0.492	0.370	2	250-270
wmc469	1A	0.844	0.819	8	110-190	gdm111	1D	0.806	0.774	7	190-210
wmc009	1A	0.501	0.374	2	160-210	wmc175	2D	0.836	0.810	7	250-300
gwm047	2A	0.819	0.788	7	150-210	wmc601	2D	0.709	0.659	9	180-300
barc0124	2A	0.676	0.607	4	220-250	barc0042	3D	0.817	0.792	12	100-250
wmc261	2A	0.623	0.580	5	100-120	gdm072	3D	0.609	0.527	5	137-210
cfd168	2A	0.647	0.580	6	210-260	gwm624	4D	0.807	0.776	8	134-250
wmc453	2A	0.813	0.783	7	140-190	wmc285	4D	0.687	0.626	5	270-300
cfa2076	3A	0.861	0.840	9	140-250	wmc331	4D	0.773	0.732	7	130-260
gwm004	3A	0.770	0.735	13	240-450	cfd040	5D	0.745	0.693	4	160-190
gwm494	3A	0.784	0.748	7	170-210	gdm153	5D	0.419	0.330	2	120-140
barc0197	3A	0.821	0.795	11	60-180	wmc233	5D	0.649	0.579	4	250-270
cfa2256	4A	0.731	0.682	6	150-180	barc0054	6D	0.795	0.758	7	60-110
gwm160	4A	0.681	0.622	5	180-260	barc0096	6D	0.700	0.640	4	180-230
wmc089	4A	0.637	0.565	7	100-140	cfd076	6D	0.780	0.745	8	150-220
barc0184	4A	0.496	0.442	3	220-280	wmc121	7D	0.837	0.812	9	280-350
barc0186	5A	0.779	0.738	5	190-230	wmc463	7D	0.733	0.683	6	100-160
cfa2155	5A	0.448	0.366	3	220-290	wmc671	7D	0.835	0.809	8	50-140
wmc110	5A	0.604	0.530	3	173-230						

MN= marker name, CL=Chromosome Location, GD= Gene diversity, PIC= Polymorphic Information Contents, TNA=total number of alleles. SRBP=Size range in base pairs

The average number of alleles per locus 6.8, the mean number of different alleles with a frequency >= 5% is 4.516 with standard error value 0.162. Number of effective alleles averaged 4.166 having standard error value 0.175. Private alleles means number of alleles unique to a single population, there was no existence of private alleles. The mean of heterozygosity (He) allelic pattern 0.718 with standard error 0.012 and unbiased expected heterozygosity (uHe) mean was 0.721 with the standard error value 0.012 and Shannon's Information Index mean value 1.488 having the standard value 0.044 suggesting that there is abundant genetic diversity at SSR loci within these genotypes (Table 3).

Genetic Diversity

Bayesian method executed in statistical software package STRUCTURE used to estimate the genetic structure of 105 spring wheat genotypes and the results suggest that top number of K=4 indicating the studied genotypes divided into four subgroups (Fig. 1A). Each color in Fig. 2 exhibits the separate group and whole population divided into 4 sub populations. Molecular DARWIN cluster analysis (Fig. 1B) and STRUCTURE Bayesian analysis showed that wheat populations from Department of Plant Breeding and Genetics UAF had genetic diversity and not existed in the same group which clearly indicate that these genotypes

Table 3: Allelic pattern for 95 SSR in 105 spring wheat genotypes

Population	Mean value	Standard Error
Na= No. of Alleles	6.579	0.323
Na Freq.>= 5%= No. of Alleles with a Frequency >=5%=	4.516	0.162
Ne= No. of Effective Alleles = 1 / (Sum pi^2)	4.166	0.175
I= Shannon's Information Index = -1* Sum (pi * Ln (pi))	1.488	0.044
No. of private Alleles = Unique to a Single Population	0.000	0.000
He= Heterozygosity = 1 - Sum pi^2	0.718	0.012
uHe= Unbiased Expected Heterozygosity = (2N / (2N-1)) * He	0.721	0.012

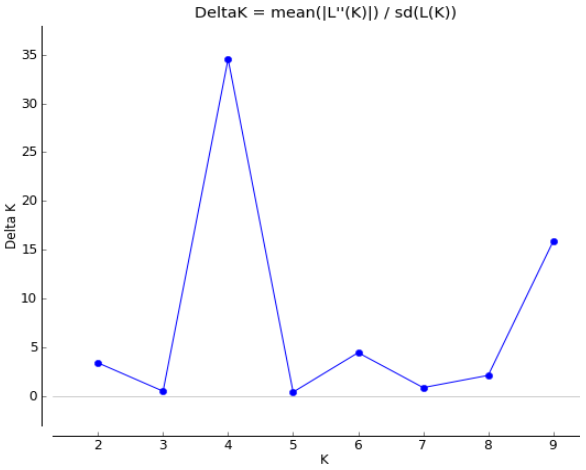

Fig. 1A: The result obtained of 105 genotypes using 95 SSR marker from Structure Harvester analysis

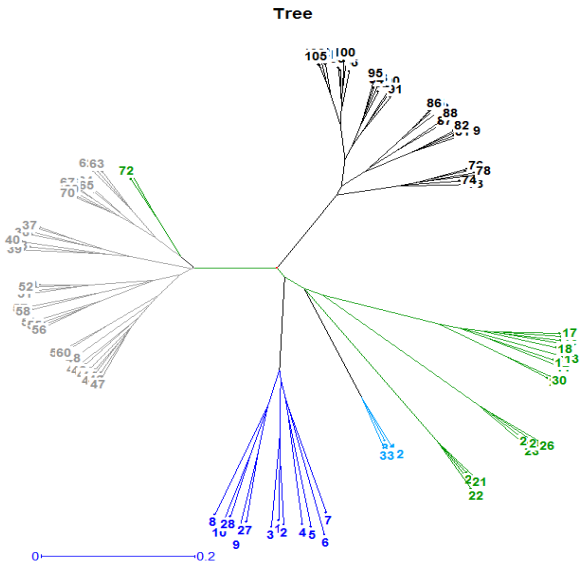

Fig. 1B: The UPGMA cluster tree analysis show the grouping of the 105 wheat genotypes in four clusters and showing the genetic distances

originated from different ancestors. Further assessment of each cluster or group revealed that genotypes G1–G10 and G27–G28 were found to fall into the same group; whereas the G11–G26 and G29–G30 genotypes

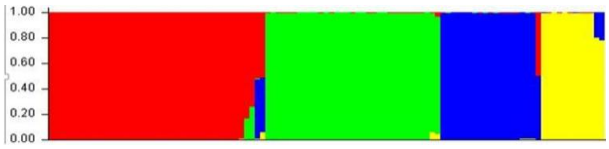

Fig. 2: Population structure of 105 spring wheat genotypes based on Bayesian approach analyzed with 95 SSR markers observing four clusters, K=4. The different colors representing the different groups which containing the used material corresponding to Table 1

were completely appeared in the second group. The third group formed by a mixture of the different genetic material contained G34–G70 genotypes. The fourth group consisted of G73–G105 genotypes. As a substitute way of predicting the diversity of the genotypes, branching tree cluster based on the Nei's genetic distance from 95 SSR markers were made using DARWIN analysis and got the same results, which were observed from the STRUCTURE Bayesian analysis.

Discussion

SSR markers have been extensively used to detect variability in wheat genotypes and to evaluate their genetic diversity. The highest number of alleles per marker was detected in genome A with the value of 15, compared to 14 and 12 for genomes B and D respectively (Table 2). Senturk-Akfirat *et al.* (2011) used 65 simple sequence repeat (SSR) markers which covered the three A, B and D genomes of spring wheat to estimate the genetic variation with 1 to 4 SSR markers for 21 chromosome. These results are comparable with the results of Liu *et al.*, 2007. These values are higher than those found by Dreisigacker *et al.* (2004) they found that the number of alleles were 5 (A genome), 6 (B genome) and 5 (D genome). A higher average number of alleles were found by Dvojkovi, 2009 (D = 9.65, A = 8.86, B = 8.93). Marker detecting the lowest total number of alleles showed lowest gene diversity than those detected maximum total number of alleles revealed the higher gene diversity. In our study based on the number of alleles per marker A genome showed the highest diversity and the D genome had the lowest diversity. Similar findings were described by Salem *et al.* (2008), where they reported that the total number of alleles per marker ranged from 2–15. Jain *et al.* (2004) observed in spring wheat the

number alleles per locus ranged from 3 to 22 with the mean of 7.8. PIC value also exhibited a significant variability and positively correlated with the total number of alleles and gene diversity for SSR microsatellites in this study. Moleculer analysis of our experiment indicated that the genetic diversity based on PIC values ranked as genome A> genome B > genome D. The allele size range in base pairs also highly correlated with the total number of alleles which is close to the result revealed by Zheng *et al*. (2009). These findings (Table 3) reliable with earlier effort done by Herrera *et al.* (2008), who evaluated the genetic diversity, which significantly correlated with the total number of alleles and allele size range in base pairs. The DARWIN tree (UPGMA cluster) analysis and STRUCTURE sub populations directed to the grouping of the 105 wheat genotypes in four subgroups. In wheat breeding program these approaches also used by Khodadadi *et al*. (2011) and got the informative results and have been used in other plant species by Sakiroglu *et al*. (2010). In our study distance between clusters specify the differences among 105 spring wheat genotypes and all clusters genetically different to each other. The more genetic distance among clusters shows that genetically they are diverse. Basically this is a sign of genetic dissimilarity among the groups (branch) and genetic similarity within each group (branch) as shown in Fig. 1B. Many scientists determined the genetic diversity such as Zhang *et al.* (2010) and Khodadadi *et al.* (2011) and Tulin *et al*. (2016) used the STRUCTURE and DARWIN analysis and obtained four subpopulations within selected elite spring wheat genotypes. Also, Chen *et al*. (2012) obtained the three subgroups specifically separated conferring to their geographical origin of 90 Chinese bread wheat genotypes using 269 simple sequence repeat markers. Production of new wheat varieties should attain the significant level of genetic diversity and distant hybridization should be adopted in wheat germplasm. Maximum genetic diversity indicate the variation in our studied wheat genotypes doubtfully that the breeding material was introduced from other sources or may be mechanical mixing. Some high yielding PBG-UAF wheat genotypes included in first group, in this cluster G27 and G28 genotypes fell and showed no genetic dissimilarity within this cluster (Fig. 1A and 2). The second cluster constituted 18 genotypes which belong to the PBG-UAF origin and some are exotic origin, which indicates that these have the same genetic constitution due to common ancestors. The third group formed by a mixture of the different genetic material which imply different ancestry of these genotypes. The third and fourth groups both having the (35+32=67) genotypes some extent results are valid according to the already known origin. The three genotypes G31, G32 and G33 had the mixed genetic material from the group two and group three comprising genotypes while the G71 and G72 shared the genetic materials from the group 3 and group 4, this indicates that the origin of the genotypes from these groups have the same ancestors. According to given origin information from maintainer of studied genotypes and pedigree record, we had three types of populations but on genetic basis the population divide into four clusters so the results are not unexpected. Genetic diversity determination would be aid to preserve the diverse genotypes for the improvement and boost up the future wheat breeding program (Zhang *et al*., 2011). Those genotypes which have diverse genetic basis, can be designated for desirable combinations with the target to develop the genetic base and their performance for complex and valuable indices. Present study on genome wide allelic pattern and genetic diversity of spring wheat would be beneficial for designing the future studies on wheat genetic resources and improve the wheat breeding program for developing promising wheat varieties.

Conclusion

Genetic diversity is the base of any genetic improvement breeding program. Therefore, it is necessary to investigate genetic diversity in wheat germplasm in order to broaden the genetic base in future wheat breeding. Gene diversity and PIC value ranged from 0.41 and 0.33 to 0.90 and 0.89 respectively indicating the significant level of genetic diversity among 105 wheat genotypes. The maximum PIC value 0.89 and lowest PIC value 0.33 were found among the genomes A and D respectively. Genetic diversity among homeologous genome ranked as: genomes A> genome B> genome D, based on PIC values. The studied genotypes divided into four genetically different subgroups based on STRUCTURE Bayesian and DARWIN analysis. The documentation of genetic diversity would be a good tool for choosing the desirable genotypes in wheat breeding programs. The present investigations also showed clear-cut identity of studied genotypes, which would be of great utility for the protection of Plant Breeder's Rights.

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