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Investigation of Genetic Diversity in Afghan Bread Wheat Genotypes Using SSR and AFLP Markers

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ABSTRACT

Genetic diversity assessment is the principle component for conservation and characterization of germplasm. Genetic diversity study of Afghan bread wheat genotypes is a first step to identify and to select high performance genotypes and distribute to wheat breeding programs. The main objective of this study is to investigate of genetic diversity in 35 Afghan bread wheat genotypes by using Simple Sequence Repeat (SSR) and Amplified Fragment Length Polymorphism (AFLP) markers. DNA extraction according to Cetyl Trimethyl Ammonium Bromide (CTAB) method was conducted and the total genomic DNA was isolated from each variety. Sixty-four SSR primer markers were used and eighteen EcoRI+(N)/MseI+(N) primer combinations with their primer sequences were used for selective polymerase chain reaction (PCR) amplification. Every SSR and AFLP fragment was scored as present (1) or absent (0) within all genotypes under study. Marker/ Value ratio of pairwise genetic distance between genotypes according to the SSRs data was from 0.508 to 0.691 with an average distance of 0.599. Relatively different grouping pattern in comparison to AFLP data observed through cluster analysis. Both types of molecular markers (AFLP and SSR) used in this research proved to be suitable for investigating genetic diversity in the genotypes of Afghan bread wheat, however, AFLP markers gave better view of genetically relationships among genotypes than the SSR markers. The grouping generated by AFLP data showed a special agreement with the origin regions of genotypes (Ariana-07 and Mazar-99 originating from the north of Afghanistan, Lalmi-03 and Kabul-02. Large number of DNA bands identified with AFLP markers might provide a better estimation of genetic similarity than those of SSR markers.

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Introduction

Agriculture sector is the backbone of Afghanistan economy for sustainability and food security. Afghanistan produces about 2.3 million hectares wheat (*Triticum aestivum* L.) as a staple cereal food and has about 40 wheat varieties in its seed chain (FAO, 2017). Collecting wheat germplasm from specific geographic region will show high genetic variation. A study of genetic diversity among adapted varieties or elite genotypes breeding materials has a significant impact on crop improvement used for germplasm management and genotype selection for different breeding purposes (Fufa et al., 2005).

Genetic diversity assessment is a principle component for conservation and characterization of germplasm (Wenguang et al., 1998). Genetic diversity is based on pedigree analysis, phenotypic data or molecular markers. In each gene pool genetic drift, selection pressure and the relatedness of ancestors without a known pedigree are important to investigation of genetic diversity based on pedigree analysis (Soleimani et al., 2002). Different morphological and physiological traits have been studied as selection items for wheat breeding programs (Casadesus et al., 2007; Naghavi et al., 2007), but these studies have some serious limitation including low heritability and polymorphism and late expression may be controlled by pleiotropic gene effects and epistasis (Van Beuningen and Busch, 1997). These limitations made these markers to be replaced by DNA based markers such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLP), and Simple Sequence Repeats (SSRs). Using molecular markers is a complementary method to analyze genetic variation in different crop plants and wild type species because they are not influenced by pleiotropic gene effects and epistasis. In addition, in term of cost, polymorphism, reproductively and genetic distance estimation are very different, and

breeder can choose each of them by considering advantages and disadvantages of them (Gupta et al., 1996; Prasad et al., 2000). SSR markers is frequently used in most plant genomes and can be highly informative and reproducible (Gupta et al., 1996). Although AFLP analysis is laborious and time consuming, it can detect many polymorphisms with each specific primer combination (Mardi et al., 2006).

In recent years, low yield per unit area, low quality of bread wheat, lack of research activities on wheat as a staple food and lack of certified seeds for farmers are the biggest challenges of wheat production in Afghanistan. Therefore, a study of genetic diversity of Afghan bread wheat genotypes is first step to identify and selecting high performance genotypes and to distribute to wheat breeding programs. The main objective of this study is to investigate of genetic diversity in 35 Afghan bread wheat genotypes by using SSR and AFLP markers.

Material and Methods

Plant Materials

Previously we gathered more than 250 cultivars and advanced lines (such as landraces, elite genotypes, generation of the advanced backcross populations or recombinant inbreed lines) from Afghanistan's Ministry of Agriculture, Irrigation and Livestock, ICARDA and CIMMYT region offices in the country and other international related organization. This study was conducted in agriculture research farms in Kabul University and Badam Bagh, during three wheat harvesting seasons 2014-2015, 2015-2016 and 2016-2017. The genetic materials include thirty-five different local wheat genotypes adapted and introduced by Afghanistan's Ministry of Agriculture, Irrigation and Livestock. These common genotypes had good agronomic characteristics and performed well under Kabul agro-ecological conditions in recent years. More descriptions of these genotypes is presented in Table 1.

DNA Extraction

To obtain material for DNA extraction, according to Cetyl Trimethyl Ammonium Bromide (CTAB) method (Saghai-Maroof et al., 1984), 5 seeds of each examined genotypes were germinated and allowed to develop for 3 weeks under glasshouse conditions. Five cm leaf segments were picked up from each genotypes and used to create a pooled leaf sample. The leaf tissue was used to extract DNA. The final DNA pellet was suspended in 50 µL TE buffer (10 mM TRIS-HCl, pH 8.0; 1 mM EDTA).

Table 1 The Descriptions of Afghan Common Wheat Genotypes Used in This Research (2014-2017).

Genotype Name	Source	Growth Type	Pedigree
Solh-02	CIMMYT	Winter	OK82282//BOW//NKT/F4/
Gul-96	ICARDA	Winter	ID8009994.W./VEE 2WM-OWM-OSE-1YCOYC
Ghazna-97	CIMMYT	Winter	AGRI/NAG
Bakhtawar-92	CIMMYT	Winter	JUP/BJY/URES CM7458-4Y-1M-3Y-08-OSY
Ghori-96	CIMMYT	Spring	PRL"S"/PEW CM59377-3AP-1AP-3AP-2AP- 1AP-OAP
HD-2285	CIMMYT	Spring	HD1912-1592/hd1962E4870-K65XHD2160/ HD2186
Inqlab-91	Pakistan	Spring	PB19545-9A-0A-OPAK
Balkh-66	India	Spring	HD-2232
Nangarhar-64	CIMMYT	Spring	WL-711
Chonte	Afghanistan	Winter	SERI.1B*2/3/KAUZ*2/BOBWHITE//KAUZ/4/PBW 343*2/KUKUNA[3692]
PBW-154	India	Spring	HD2177/HD2160
Takhar-96	CIMMYT	Spring	VEE#7/OPATA
Snb-01	CIMMYT	Spring	Snb's'/5/Maya74's'/On//II60.147/3/Bb/GII/4/Chat's'
HUW-234	India	Spring	HUW12/Sparrow/HUW12
Dayma-96	CIMMYT	Spring	HD2206/HORK//BUC/BUL
MH-97	Pakistan	Spring	Attila CM8583-504-OM-OY-OSY-OAP
Rana-96	ICARDA	Facultative	CA8055/6/PATOR/CAL/3/76//BB/CN015/CAL//CNOSN64/4/CNO//NAD/CH 2AP-2AP-2AP-1AP-OAP
Irena/Weaver	CIMMYT	Facultative	IRENA/Weaver/CMBW90M294.1-1M-020Y-010M-010Y- 6M-015Y-0Y
Lalmi-03	ICARDA	Facultative	FLORKWA-3 IC84-0074-02AP-3002-1APOL-OAP
Sheshambagh-08	CIMMYT	Facultative	SW89.5181/KAUZ
Ariana- 94	Afghanistan	Winter	BOBWHITE/NACOZARI-76//VEERY/3/BLUEJAY/COCORAQUE-75[1922]; CHINA-13//GLENNSON-M-81[3589]
Amu-99	ICARDA	Facultative	Bloyka-ICW84-0008-013AP-300L-OAP
Kabul-02	Afghanistan	Facultative	HD-3280
Darulaman-07	CIMMYT	Facultative	Weaver/4/Nac/Th.ac//3*PVN/3/mirlo/bucCID/SID:133428/104
Roshan-96	ICARDA	Facultative	BLOUNDAN/3/Bb/7C*2//Y50E/KAL*3
Mazar-99	ICARDA	Facultative	PASTURE CM85295-0101TOPY-2M-OYOM- 3Y-OM
Herat-99	ICARDA	Facultative	MYNA/VUL//PRL CM97958-OM-7Y-030M-030M-84-OM
Croc-01	CIMMYT	Facultative	CROC_1/AE.SQ (205) KAUZ/3/PASTOR
Drokhshan-08	CIMMYT	Facultative	CNDO/R143/ENTE/MEXI_2/3/
Parvan-02	CIMMYT	Facultative	CHTO/ARDEA//SRN_2 CD74825-C-5M-1Y-040M-2YRC-2M-0YRC
Lalmi-02	CIMMYT	Facultative	BOBWHITE/MN IC88-063-1AP-OL-1AP-2AP-OTS-OAP
Pamir-94	CIMMYT	Winter	YMH/TOB/3/LIRA SWM16
Koshan-09	Afghanistan	Spring	BABAX/Lr42//BABAX*2/VIVITSI[3686]
Lalmi-01	ICARDA	Facultative	FOW-1 SWM11147-1AP-2AP-1AP-1AP-OAP
Ariana-07	CIMMYT	Facultative	Pastor/3/kauz*2/Opata//Kauz/CID/SID:133513/256

Table 2 SSR Markers Name, Chromosomal Location and Number of Alleles Scored.

Row	Marker Name	Chromosomal Location	Number of Alleles	Row	Marker Name	Chromosomal Location	Number of Alleles
1	GWM164	1A-L	3	33	GWM251	4B-L	8
2	GWM497	1A-L, 2A-L, 3D-L	14	34	GWM107	4B-L	6
3	GWM259	1B-L	10	35	GWM149	4B-L	6
4	GWM153	1B-L	11	36	GWM608	4D-L	4
5	GWM337	1D-S	11	37	GWM156	5A-L	3
6	GWM357	1A-L	1	38	GWM304	5A-S	7
7	GWM274	1B-L, 7B-L	6	39	GWM335	5B-L	5
8	GWM359	2A-L	3	40	GWM443	5B-S	2
9	GWM558	2A-S	4	41	GWM554	5B-L	1
10	GWM372	2A-L	3	42	GWM371	5B-L	10
11	GWM55	2B-L	15	43	GWM540	5B-S	2
12	GWM148	2B-L	6	44	GWM639	5D-L	9
13	GWM120	2B-L	13	45	GWM271	5D-L	3
14	GWM249	2D-L	8	46	GWM190	5D-S	0
15	GWM210	2D-L	5	47	GWM427	6A-L	8
16	GWM484	2D-L	3	48	GWM459	6A-L	6
17	GWM539	2D-S	1	49	GWM169	6A-L	7
18	GWM102	2D-L	6	50	GWM334	6A-L	5
19	GWM261	2D-L	10	51	GWM626	6B-S	4
20	GWM32	3A-S	6	52	GWM70	6B-L	0
21	GWM369	3A-S	4	53	GWM613	6B-L	13
22	GWM247	3B-L	9	54	GWM132	6B-L	7
23	GWM493	3B-L	3	55	GWM469	6D-L	15
24	GWM340	3B-L	6	56	GWM325	6D-L	5
25	GWM114	3B-L	16	57	GWM233	7A-L	1
26	GWM3	3D-L	2	58	GWM130	7A-L	2
27	GWM314	3D-L	10	59	GWM60	7A-L	5
28	GWM383	3D-L	10	60	GWM46	7B-S	5
29	GWM165	4A-S, 4B-L, 4D-L	1	61	GWM43	7B-S	10
30	GWM397	4A-L	10	62	GWM111	7D-L	2
31	GWM160	4A-L	10	63	GWM44	7D-L	9
32	GWM538	4B-L	1	64	GWM437	7D-L	12
Mean							6.29

Table 3 AFLP Markers Name, Primer Sequences and Polymorphic Fragments Scored.

Row	MN*	Primer Sequence**		PFS
1	E31/M47	E31 5'GACTGCGTACCAATTCAAA	M47 5'GATGAGTCCTGAGTAACAA	10
2	E31/M50	E31 5'GACTGCGTACCAATTCAAA	M50 5'GATGAGTCCTGAGTAACAT	16
3	E31/M52	E31 5'GACTGCGTACCAATTCAAA	M52 5'GATGAGTCCTGAGTAACCC	18
4	E31/M59	E31 5'GACTGCGTACCAATTCAAA	M59 5'GATGAGTCCTGAGTAACTA	17
5	E32/M47	E32 5'GACTGCGTACCAATTCAAC	M47 5'GATGAGTCCTGAGTAACAA	19
6	E32/M50	E32 5'GACTGCGTACCAATTCAAC	M50 5'GATGAGTCCTGAGTAACAT	15
7	E32/M52	E32 5'GACTGCGTACCAATTCAAC	M52 5'GATGAGTCCTGAGTAACCC	25
8	E32/M59	E32 5'GACTGCGTACCAATTCAAC	M59 5'GATGAGTCCTGAGTAACTA	17
9	E38/M47	E38 5'GACTGCGTACCAATTCACT	M47 5'GATGAGTCCTGAGTAACAA	10
10	E38/M50	E38 5'GACTGCGTACCAATTCACT	M50 5'GATGAGTCCTGAGTAACAT	8
11	E38/M52	E38 5'GACTGCGTACCAATTCACT	M52 5'GATGAGTCCTGAGTAACCC	22
12	E38/M62	E38 5'GACTGCGTACCAATTCACT	M62 5'GATGAGTCCTGAGTAACTT	14
13	E41/M47	E41 5'GACTGCGTACCAATTCAGG	M47 5'GATGAGTCCTGAGTAACAA	36
14	E41/M52	E41 5'GACTGCGTACCAATTCAGG	M52 5'GATGAGTCCTGAGTAACCC	21
15	E41/M62	E41 5'GACTGCGTACCAATTCAGG	M62 5'GATGAGTCCTGAGTAACTT	23
16	E46/M47	E46 5'GACTGCGTACCAATTCATT	M47 5'GATGAGTCCTGAGTAACAA	12
17	E46/M52	E46 5'GACTGCGTACCAATTCATT	M52 5'GATGAGTCCTGAGTAACCC	18
18	E46/M62	E46 5'GACTGCGTACCAATTCATT	M62 5'GATGAGTCCTGAGTAACTT	7
Mean				17.11

MN: Marker Name, PFS: Polymorphic Fragments Score, *This AFLP primers were abbreviated in accordance with the standard nomenclature of AFLPs (https://wheat.pw.usda.gov). ** E: EcoRI adaptor, M: MseI adaptor.

Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphisms (AFLPs) analyses

Total genomic DNAs were isolated from each variety. Sixty-four SSR primer markers were used following Roder et al. (1998), see Table 2 for details. Also AFLP analysis was conducted by using enzyme combination EcoRI and MseI in accordance with method of Vos et al. (1995). Eighteen EcoRI+(N)/MseI+(N) primer combinations with their primer sequences were used for selective polymerase chain reaction (PCR) amplification (Table 3), (Eivazi et al, 2008).

Every SSR and AFLP fragment was scored as present (1) or absent (0) within all genotypes under study and to estimate the genetic similarities (GS) between pairs, binary matrix was used by applying Nei and Li coefficient (Nei and Li, 1979). Therefore, the coefficient of dissimilarity (GD) between pairs calculated by GD = 1 - GS. A cluster analysis was carried out using the unweighted pair grouping method of arithmetic averages. The analyses

were conducted with NTSYS-PC software (Rohlf, 2000). The support values for the level of confidence at the nodes of the AFLP, SSR and AFLP+SSR dendrograms were analyzed by 1000 bootstrap resampling using PHYLIP 3.57c computer software (Eivazi et al., 2008; Felsenstein, 1995).

Results

SSRs Analyses

Sixty-four wheat SSR loci produced a total of 491 alleles across all the genotypes related to grain yield and other agronomical traits under research. The number of alleles per locus ranged from 1 to 16, with an average of 6.29 alleles per locus (See Table 2). Marker/ Value ratio of pairwise genetic distance between genotypes is measured. According to the SSRs data this ratio was from 0.508 to 0.691 with an average distance of 0.599 (Table 4). Relatively different grouping pattern in comparison to AFLP data observed through cluster analysis (Fig. 1). In the results of clusters, Koshan-09 and Lalmi-01 were placed in the same cluster and also Kabul-02 and Lalmi-03 were assigned in same cluster. Chonte was separated from Kabul-02 and grouped with Darulaman-07, Roshan-96 and Herat-99. In both data, Ariana-07 was distinct from the other clusters (Fig. 1 and Fig. 3), by considering of genetically content of them.

AFLPs Analyses

An analysis of amplified fragment length polymorphisms in 35 Afghan wheat genotypes based on eighteen primer combination constituted a total of 320 polymorphic amplified DNA fragments. Estimates of genetic diversity based on AFLP data varied from 0.425 to 0.819 with an average of 0.622 (Table 4). Grouping based on AFLP data revealed relative association with origin of genotypes region (Fig. 2). In the AFLP grouping, two genotypes, Ariana-07 and Mazar-99 originating from the north of Afghanistan were closely grouped together. Genotypes Lalmi-03, provided from ICARDA materials and Kabul-02, which have good tolerance to drought, were also clustered together, with high bootstrap value.

AFLPs & SSRs Analyses

Amplified fragment length polymorphisms and SSR combined data analysis revealed a different grouping pattern compared with the individual methods. Based on this grouping, genotype Ariana-07 was differentiated from the others, which, has similar results of AFLP clustering analysis and Gul-98, Bakhtawar-92, Ghori-96, HD-2285, Inqlab-91, Balkh-66, Nangarhar-64, PBW-154, HUW-234, Dayma-96, Rana-96, Amu-99, Darulaman-07, Roshan-96, Herat-99 and Croc-01 showed very close relationships and grouped in one cluster (Fig. 3).

Discussion

Both types of molecular markers (AFLP and SSR) used in this research showed to be suitable for investigating genetic diversity in the genotypes of Afghan bread wheat. However, according to the Table 4, AFLP markers provided better view of genetically relationships among genotypes than the SSR markers. The grouping generated by AFLP data showed a special agreement with the origin regions of genotypes (Ariana-07 and Mazar-99 originating from the North of Afghanistan, Lalmi-03 obtained from ICARDA and Kabul-02. A large number of DNA bands identified with AFLP markers might provide a better estimation of genetic similarity than those of SSR markers in wheat and maize, respectively, (Almanza-Pinzon et al., 2003, Barbosa et al., 2003)

Table 4 Marker/Value ratio of pairwise genetic distance matrices based on SSR and AFLP Markers among 35 Afghan Bread Wheat Genotypes.

Parameter	SSR	AFLP	SSR + AFLP
Maximum	0.691	0.819	0.709
Minimum	0.508	0.425	0.498
Mean	0.599	0.622	0.603

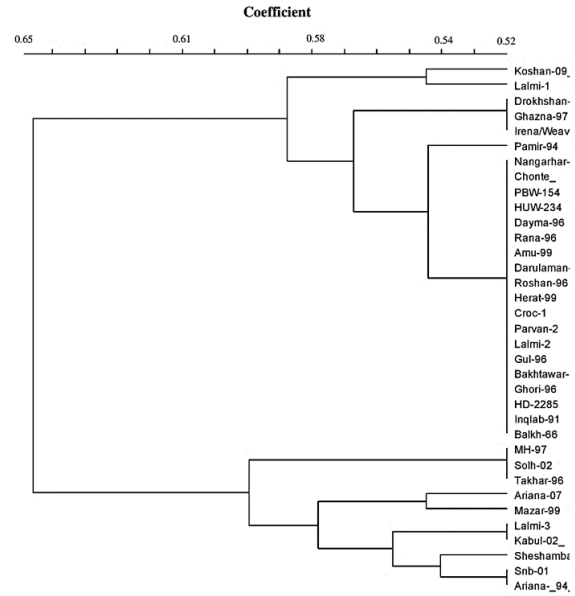

Figure 1 Arithmetic averages dendrogram of unweighted pair grouping method on 35 Afghan wheat genomes based on genetic distances computed from simple sequence repeats markers (SSRs), coefficients are bootstrap values (%) obtained from 1000 replicate analyses

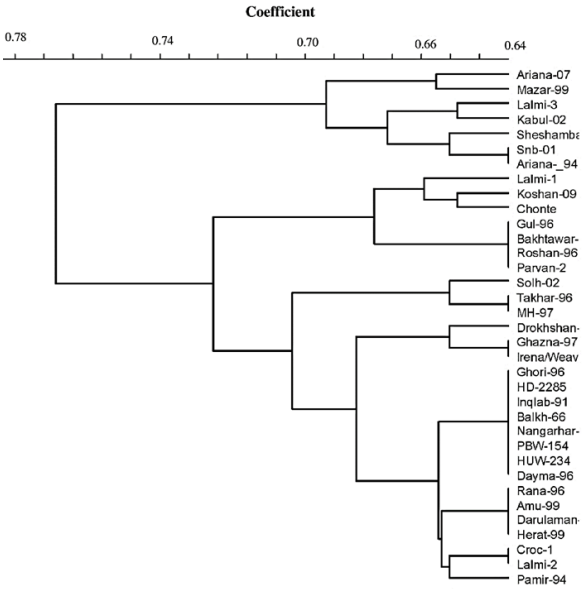

Figure 2 Arithmetic averages dendrogram of unweighted pair grouping method on 35 Afghan wheat genomes based on genetic distances computed from amplified fragment length polymorphisms (AFLPs), coefficients are bootstrap values (%) obtained from 1000 replicate analyses

AFLP marker analysis by 18 clusters in genetic distance 0.64 (Fig. 2) is useful for identifying polymorphic molecular markers on the genotypes, Therefore, these markers were useful for evaluating genetic diversity among and within species (Shoaib et al., 2006, Altıntaş et al., 2007). As well by considering the highest mean of marker by value, 0.622 (Table 4) in genetic distance by AFLP technique, the primers developed for population are relevant to related taxa (Sasanuma et al., 2002).

Conclusion

In the study showed that collecting wheat germplasm from specific geographic region showed high genetic variation. Considering the importance of morphological assessment, the characterization of wheat gene pool by using DNA fingerprinting techniques such as marker assisted selection via AFLP and SSR molecular markers is an initial step in wheat breeding. This provides a tool to assess genetic diversity for finding high yield varieties. In summary, we conclude that the AFLP loci tested here in the genotypes, generally have more dominant inheritance versus SSR regions. The frequencies of polymorphic bands in diverse germplasm are in the range that enables map-based diversity studies (Hazen et al., 2002). Furthermore, the magnitude and pattern of genetic variation observed in this study will be useful for wheat breeders to apply the genotypes as parents in the breeding programs.

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