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ASSESSMENT OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF SOME SOFT AND HARD WHEAT VARIETIES BASED ON SSR MARKER

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ABSTRACT:
Wheat (*Triticum spp*.) is one of the most important cereal crops in Iraq and the world. It includes many species and varieties. The two major cultivated species of wheat are, durum wheat (*Tritium durum* Desf.) which is tetraploid (2n= 28) and the common wheat (*Triticum aestivum* L.) which is hexaploid (2n = 42). Ten wheat varieties from both species were examined using ten Simple sequence repeat (SSR) markers (WMC17, WMC20, WMC21, WMC24, WMC25, WMC48, WMC50, WMC283, Xgwm11 and Xgwm626). Various genetic parameters were calculated using Power Marker V3.25 software. A total of 156 alleles were detected in both species. The gene diversity in wheat varieties from both species collectively varied from 0.85 to 1.00, which indicates considerable genetic diversity in the examined varieties. All markers used in this study were highly informative and the polymorphic information content (PIC) values were higher than 0.50 in all loci. Hence all markers are considered useful for genetic diversity studies in wheat's populations. The dendrogram separated the populations into two main clades and many subgroups. Azadi variety was simplicifolious. This study confirms the discriminating power of SSR typing and its usefulness for comparison within hard and soft wheat populations.
KEYWORDS: *Triticum sp*., hard and soft wheat, SSR markers, genetic diversity, population genetics.

1. INTRODUCTION

Wheat is a species of agricultural importance as cereal grains in most countries around the world, as well as in Iraq (Slim *et al*., 2019). It is an annual self-pollinating plant belonging to the family *Poaceae* (grasses) and genus *Triticum* (Shewry, 2009). The two most cultivated species of wheat are durum (*Triticum turgidum* Desf.) subsp. *durum*, genome AABB, which is tetraploid (2n = 4x = 28) with 14 pairs of chromosomes, and the soft wheat (*Triticum aestivum* L.), genome AABBDD, which is hexaploid (2n = 6x = 42)) with 21 pairs of chromosomes (Kara and Knaouni, 2017). Wheat provides much of food source to human. The global demand for wheat yields is growing parallel to the steady increasing in the human population (Allen *et al.*, 2017). In addition to significant agronomic features, breeders around the world are working for increased grain yield with better quality (Desheva and Kyosev, 2015). The selection of diverse genotypes is an important step for molecular breeding of wheat (Raj *et al.,* 2017). Microsatellites are an effective tool in diversity studies for identification of the degree of genetic similarity (Salem *et al*., 2015). They are independent of environmental conditions under which phenotypic studies are carried out. SSRs are tandem repeat motifs composed of one to six nucleotides. They are suitable for detecting allele frequency within the population and for assessing population structure (Kumar *et al*., 2016). It has been considered as one of the most effective molecular markers for genetic discrimination within interspecific or intraspecific species. SSR markers have major applications as highly variable and multi-allelic PCR based genetic markers. They are abundant and scattered all over the eukaryotic genomes with a high polymorphism rate (Kesawat and Kumar, 2009). Different studies have reported the use of SSRs to reveal polymorphisms in the wheat population (Khan *et al*. 2015; Zarei., *et al*., 2016; Ya Narantsetseg *et al*., 2017; Salehi *et al* 2018; Yadav and Chand, 2018; El-Fiki and Adly, 2019). The results of these investigations indicated that wheat populations had high genetic diversity that can be used in wheat conservation and breeding programs, as well most SSR markers used showed a high level of polymorphism in wheat. This study was conducted to evaluate genetic diversity and population structures of both soft and hard wheat cultivars using ten polymorphic SSR markers.

2. MATERIALS AND METHODS

2.1 Plant materials collection

Seeds of 10 released varieties have been collected from the Agricultural Research Center of Duhok / Kurdistan Region of Iraq. Five of them were hard wheat varieties; Icarasha, Acsad, Secondroue, Simeto, Berghouata and the other were soft wheat varieties; Noor, Azadi, Tamoz2, Sham4, Adana99. These varieties have been released either by ICARDA or Acsad international research centers. The grains collected from each plant were grown in a separate plastic culture plate filled with a mixture of soil and peat moss during December 2018, at Biology Department Lab / University of Zakho. For genomic DNA extraction, fresh leaf samples were collected from 21 days old seedlings. Healthy leaves were chopped with sterilized scissors and washed in distilled water then with ethanol 70% for two minute to remove any sources of foreign DNA. Leaf samples were ground in the presence of liquid nitrogen, using mortar and pistil to make a fine powder and then the powder transferred to 1.5 ml Eppendorf tubes for DNA extraction.

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2.2 DNA extraction and microsatellites analysis

Total genomic DNA was extracted from ground leaves using DNA extraction kit (4001Korea) according to the instructions provided by the supplier company (GeNetBio). Ten polymorphic SSR microsatellites (Table1) were used in screening all varieties. All extracted DNA samples were checked by Nanodrop instrument for their quality and purity.

2.3 PCR amplification

Reaction was performed using PCR Eppendorf tubes by mixing 12.5 µl of 2x master mix, 1 µl from each forward and reverse primer and 2-3 µl of DNA, the volume was made up to 25 µl by deionized distilled water. The thermocycling program was optimized at initial denaturation at 94°C for 4 minutes followed by 35 cycles of 95°C for 1 minute, 1 minute at annealing temperature (52 to 64°C gradient cycle), 1 minute at 72°C for extension followed by one cycle of final extension at 72˚C for 5min and hold at 4°C. The PCR products were run on 1% agarose gel which was prepared by dissolving 1g of agarose in 100

Table 1. Locus name, motif repeats, sequences, chromosome location and annealing temperature of 10 SSR microsatellite markers used in this study supplied by Macrogen Company / South Korea (Kara *et al*., 2017; Röder *et al*., 1998)

Locus	Motif	Sequences	Location on Chromosomes	Annealing Temp.
WMC 17	(CA)	F-ACCTGCAAGAAATTAGGAAC R-CTAGTGTTTCAAATATGTCGA	7A-7B	54 C^0
WMC 20	(CA)	F-TTAAAAACACGCGGATCTTCTC R-GTACTCACATATTTCTCGGTCT	1A	54 C^0
WMC 21	(GA)37	F-CGCTGCCGTGTAACTCAAAATC R-AGTTAATTGGGCGCTCCAAGAA	-	55 C^0
WMC 24	(GT)28	F-GTGAGCAATTTTGATTATACTG R-TACCCTGATGCTGTAATATGTG	1A	52 C^0
WMC 25	(GT)26	F-TCTGGCCAGGATCAATATTACT R-TAAGATACATAGATCCAACACC	2B	52 C^0
WMC 48	(GA)9	F-GAGGGTTCTGAAATGTTTTGCC R-ACGTGCTAGGGAGGTATCTTGC	4B	64 C^0
WMC 50	(GT)10 (GT)16	F- CTGCCGTCAGGCCAGGCTCACA R- CAACCAGCTAGCTGCCGCCGAA	3A	60 C^0
WMC 283	(CA)19(CA)8	F-CGTTGGCTGGGTTATATCATCT R- GACCCGCGTGTAAGTGATAGGA	4A	57 C^0
Xgwm 11	(TA)6 CATA (CA)19(TA)6	F-GGATAGTCAGACAATTCTTGTG R-GTGAATTGTGTCTTGTATGCTTCC	1B	57 C^0
Xgwm 626	(CT)5(GT)13	F- GATCTAAAATGTTATTTTCTCTC R- TGACTATCAGCTAAACGTGT	6B	52 C^0

ml of 1X TBE buffer, the PH of the buffer was adjusted to 8, for 5 min with 45 volts then for 60 min with 80 volts. Then the PCR products electrophoresed on 8% polyacrylamide gel (PAGE). The polyacrylamide gel was prepared by adding 6.650 ml of acrylamide solution and 5 ml of 5X TBE to 13.150 ml of deionized water. Then 350 µl of 10% ammonium persulfate with 20 µl of TEMED was added. The gel was run for 30 min with 65 volts, then for another 40 min on 120 volts until the dark blue dye run off the bottom. The bands were measured to 25 bp ladder. The amplified DNA bands were visualized by silver staining according to a protocol by Bassam and Gresshoff (2007).

2.4 Data analysis

The resulting data was analyzed using power marker V3.25 software. The genetic relationship parameters calculated according to Nei's (1973 and 1987) statistics. The similarity matrix was used to construct the dendrogram using the unweighted pair group method arithmetic averages (UPGMA) procedure (Sokal and Michener, 1958). The tree viewed by using the TREEVEIW (version 1.66) software.

3. RESULTS

Ten used SSR markers were highly polymorphic with amplification bands in all 10 varieties of wheat. Figure 1 shows an example of a polyacrylamide gel profile generated using WMC24 primer.

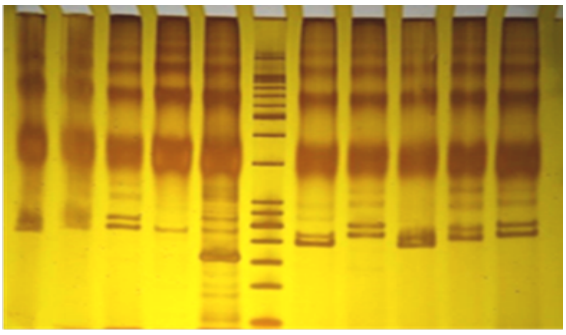

Figure 1. polyacrylamide gel profile in two populations of wheat *Triticum aestivum* L. and *Triticum durum* Desf. using (WMC24) primer. L: Represents the DNA ladder, V1: Icrasha; V2: Acsad; V3: Noor; V4: Azadi; V5: Secondroue; L: Ladder V6:Simeto; V7: Tamoz 2; V8: Berghouata; V9: Sham4; V10: Adana 99

3.1. Population structure of soft wheat varieties (*Triticum aestivum* L.)

Allele sizes of soft wheat varieties (Table 2) in different loci ranged from 74 to 226bp in different loci. Table 3 shows the genetic diversity in the five soft wheat varieties based on 10 SSR markers. Allele frequency ranged from 0.10 for WMC24

and Xgwm626 loci to 0.38 at WMC20 locus with a mean of 0.19. In this species, a total number of 75 alleles with an average of 7.5 have been detected. WMC24 and Xgwm626 loci scored the highest number of alleles (10) while the least score of five was detected at WMC21 and WMC25 loci. To obtain a reliable data analysis, the value of the availability which is the number of observed alleles per number of individuals sampled was calculated. This value was found to be high in this population with an average of 0.96.

This average indicated that the number of null alleles (not amplified) was only in two samples (Azadi variety at Xgwm11 and Tamoz2 at WMC20 loci). Heterozygosity at a locus is an indicator of the genetic variability. Observed heterozygosity (Ho) and the genetic diversity or expected heterozygosity (He) were calculated and found that the expected heterozygosity (He) in this group ranged from 0.78 in WMC20 locus to 0.90 in WMC24 and Xgwm626 loci with an average of 0.85. The Ho was ranged between 0.4 in WMC17 locus to 1.0 in Xgwm626, Xgwm11 and WMC24 loci, with average of 0.66. The polymorphic information content (PIC) values for overall genetic variability also calculated for all primers (Tables 3). The highest PIC value of 0.89 observed in Xgwm626 and WMC24 loci and the lowest value of 0.75 in WMC20 with an average of 0.83for all loci.

Table 2. Shows allele's size in soft wheat varieties *(Triticum aestivum* L.)

Primers	Noor	Azadi	Tamoz 2	Sham4	Adana 99
WMC 17	185/205	182/182	193/203	206/206	212/212
WMC 20	112/127	112/112	?/?	117/132	118/133
WMC 21	74/74	76/76	75/75	81/81	84/84
WMC 24	149/164	134/144	136/151	130/142	135/150
WMC 25	119/119	121/121	142/142	143/143	138/138
WMC 48	116/116	118/134	115/133	115/131	118/134
WMC 50	95/95	96/108	107/118	110/123	113/125
WMC 283	86/98	88/88	93/102	92/106	97/110
Xgwm 11	212/226	?/?	196/214	197/218	200/220
Xgwm 626	115/130	93/105	129/142	134/148	135/145

Table 3. Shows the genetic diversity in five soft wheat varieties based on 10 SSR markers

Marker	Allele Frequency	Genotype Number	Allele Number	Ava	He	Ho	PIC
WMC17	0.20	5.00	7.00	1.00	0.84	0.40	0.82
WMC20	0.38	4.00	6.00	0.80	0.78	0.75	0.75
WMC21	0.20	5.00	5.00	1.00	0.80	0.00	0.77
WMC24	0.10	5.00	10.00	1.00	0.90	1.00	0.89
WMC25	0.20	5.00	5.00	1.00	0.80	0.00	0.77
WMC48	0.20	4.00	6.00	1.00	0.82	0.80	0.79
WMC50	0.20	5.00	9.00	1.00	0.88	0.80	0.87
WMC283	0.20	5.00	9.00	1.00	0.88	0.80	0.87
Xgwm11	0.13	4.00	8.00	0.80	0.88	1.00	0.86
Xgwm626	0.10	5.00	10.00	1.00	0.90	1.00	0.89
Mean	**0.19**	**4.70**	**7.50**	**0.96**	**0.85**	**0.66**	**0.83**

3.1.1 Genetic relationships between soft wheat varieties: Genet distances were calculated for these soft wheat varieties to estimate t extent of their divergence. Table (4) shows the lowest genetic distan (0.610) was found between Tamoz 2 and Sham4 and the highe genetic distance (0.722) was found between Azadi variety and Shar variety. The average genetic distance among the varieties was equal 0.668 The results of the phylogenetic dendrogram based on the gene analysis of distance matrix is displayed in Figure 2. The dendrogra separated the five soft wheat varieties into two main groups. The fi group consists of Azadi variety. The second group was divided in three sub accessions consists of the rest varieties. The highe similarity value was observed between Tamoz2 and sham4 varieti while the highest distance was between Azadi and Sham4 varieti Azadi variety was different from the rest varieties and formed a uniqu leave.

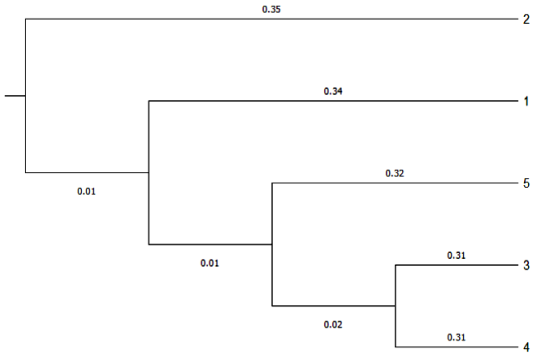

Figure 2. Dendrogram for five soft wheat varieties showing the genetic similarity derived from a UPGMA cluster analysis

3.2 Population structure of hard wheat varieties (*Triticum durum* Desf.)

Allele sizes of hard wheat varieties (Table5) at different loci ranged between 73bp to 234 bp. The genetic diversity in the five hard wheat varieties is shown in Table 6. Allele frequency ranged from 0.10 for WMC25, WMC48 and Xgwm626 loci to 0.40 for WMC21 locus with an overall mean of 0.21. The total number of the detected alleles was 81and the average value was 8.1. The highest number of alleles (10) was scored at WMC25, WMC48 and Xgwm626 loci, while the lowest score of four alleles was at WMC21 locus.

Table 4. Shows the genetic distance for the five soft wheat varieties based on 10 SSR microsatellite markers, the varieties are: 1-Noor, 2-Azadi, 3-Tamoz2, 4-Sham4, 5-Adana99

Primers	Icrasha	Acsad	Secondroue	Simeto	Berghouata
WMC 17	200/214	189/198	180/180	175/175	198/198
WMC 20	117/131	112/125	112/127	113/128	113/127
WMC 21	75/75	73/73	75/75	74/74	78/78
WMC 24	146/160	152/152	103/118	120/133	121/129
WMC 25	122/136	118/133	126/137	135/148	140/153
WMC 48	123/143	130/145	132/147	120/135	127/144
WMC 50	94/105	93/102	121/128	103/113	108/108
WMC 283	80/91	80/92	87/93	88/98	88/102
Xgwm 11	218/234	216/230	204/218	202/218	208/224
Xgwm 626	115/128	91/105	121/135	125/136	132/144

Table 5. Shows allele's size of the hard wheat population

varieties	Noor	Azadi	Tamoz 2	Sham 4	Adana 99
Noor	0.000				
Azadi	0.694	0.000			
Tamoz2	0.660	0.688	0.000		
Sham4	0.675	0.722	0.610	0.000	
Adana 99	0.675	0.670	0.639	0.650	0.000

Table 6. Shows the genetic diversity in five hard wheat varieties based on 10 SSR markers

Marker	Allele Frequency	Genotype Number	Allele Number	Availability	He	Ho	PIC
WMC17	0.30	5.00	6.00	1.00	0.80	0.40	0.77
WMC20	0.20	5.00	7.00	1.00	0.84	1.00	0.82
WMC21	0.40	4.00	4.00	1.00	0.72	0.00	0.67
WMC24	0.20	5.00	9.00	1.00	0.88	0.80	0.87
WMC25	0.10	5.00	10.00	1.00	0.90	1.00	0.89
WMC48	0.10	5.00	10.00	1.00	0.90	1.00	0.89
WMC50	0.20	5.00	9.00	1.00	0.88	0.80	0.87
WMC283	0.20	5.00	8.00	1.00	0.86	1.00	0.84
Xgwm11	0.30	5.00	8.00	1.00	0.84	1.00	0.82
Xgwm626	0.10	5.00	10.00	1.00	0.90	1.00	0.89
Mean	**0.21**	**4.90**	**8.10**	**1.00**	**0.85**	**0.80**	**0.83**

The value of the availability which is the number of observed alleles per number of individuals sampled was found to be high in all loci with an average of 1.0. This average indicated that there was no null allele sample in all loci. The expected heterozygosity's (He) in this group of wheat ranged from 0.84 for WMC20 and Xgwm11 loci to 0.90 for WMC25, WMC48 and Xgwm626 loci with an average of 0.85. The Ho value ranged from 0.4 for WMC17 locus to 1.0 for most of the other loci with an average of 0.66. The polymorphic information content (PIC) values for overall genetic variability was also calculated for all primers (Tables 6). The highest PIC value of 0.89 was observed in and WMC25, WMC48 and Xgwm626 loci and the lowest value of 0.67 in WMC21 with an average of 0.83 for all loci.

3.2.1 Genetic relationships between hard wheat varieties: The calculated genetic distance for the hard wheat varieties is shown in Table 7. The lowest genetic distance (0.45) was found between Icrasha and Secondroue and the highest genetic distance with a value of 0.60 was found between Acsad and Icrasha as well between Acsad and Secondroue varieties. The overall average of genetic distance among these varieties is equal to 0.52. Results of the phylogenic dendrogram based on a genetic analysis of distance matrix are shown in Figure 3. The dendrogram separated the five hard wheat varieties into two main groups. The first group consists of Icrasha, Berghouata and Secondroue varieties. The second group consists of Simeto and Acsad. The highest similarity value was observed between Icrasha and Secondroue varieties while the highest distance was between Acsad and Berghouata varieties.

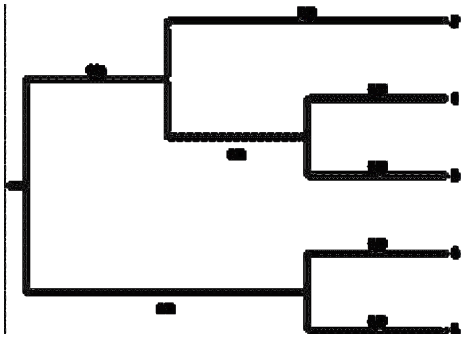

Figure 3. Dendrogram for five hard wheat varieties showing the genetic similarity derived from a UPGMA cluster analysis

Table 7. Genetic distance for the 5 hard wheat varieties based on 10 SSR microsatellite markers, the varieties are: 1-Icrasha 2- Berghouata 3-Secondroue 4-Simeto 5-Acsad

Variety	Icrasha	Bergh ouata	Secon droue	Simeto	Acsad
Icrasha	0.000				
Bergh ouata	0.550	0.000			
Secon droue	0.450	0.575	0.000		
Simeto	0.550	0.600	0.575	0.000	
Acsad	0.600	0.575	0.600	0.575	0.000

3.3 Population structure analysis of both wheat species

The genetic diversity in both wheat species is shown in Table 8. Allele frequency ranged from 0.10 to 0.30 with a mean of 0.17. The total number of detected alleles was 140 and its average was 14.1. The largest number of 19 alleles was estimated for WMC24 locus, while the least score of seven was at WMC21 locus. The value of the availability was found to be high in all loci with an average of 0.98. This average indicates that there were only a few null allele samples in all loci. The expected heterozygosity (He) ranged from 0.82 in WMC21 to 0.95 in WMC25 locus with an average of 0.90. The observed heterozygosity (Ho) ranged from 0.40 in WMC17 locus to 1.0 in Xgwm626 and Xgwm11 loci with an overall average of 0.73. The polymorphic information content (PIC) value for overall genetic variability was also calculated for all primers (Tables 8). The highest PIC value of 0.94 was observed for WMC24 locus and the lowest value of 0.84 indicated for WMC20 locus with an average of 0.90 for all loci.

Table 8. Shows the genetic diversity in 10 wheat varieties based on 10 SSR. Markers

varieties	Icarasha	Acsad	Second roue	Simeto	Bergh ouata	Noor	Azadi	Tamoz2	Sham4	Adana 99
Icarasha	0.00									
Acsad	0.55	0.00								
Secondroue	0.45	0.58	0.00							
Simeto	0.55	0.60	0.58	0.00						
Berghouata	0.60	0.58	0.60	0.58	0.00					
Noor	0.60	0.63	0.60	0.53	0.65	0.00				
Azadi	0.67	0.61	0.64	0.64	0.61	0.69	0.00			
Tamoz2	0.47	0.61	0.47	0.61	0.61	0.67	0.69	0.00		
Sham4	0.55	0.60	0.60	0.60	0.65	0.68	0.72	0.61	0.00	
Adana 99	0.60	0.63	0.60	0.60	0.65	0.68	0.67	0.64	0.65	0.00

3.3.1 Genetic relationships between all wheat varieties: The calculated genetic distances for all 10 wheat varieties in both species are shown in Table 9. The lowest genetic distance (0.45) was found between Icrasha and Secondroue followed by 0.47 between Tamoz2 and Icarasha and Tamoz2 and Secondroue. The highest genetic distance with a value of 0.72 was found between Azadi and Sham4. The average genetic distance among all varieties was equal to 0.61. The results of the phylogenic dendrogram based on a genetic analysis of distance matrix are shown in Figure 4. The first group consists of Icrasha, Berghouata and Secondroue varieties. The second group consists of Simeto and Acsad. The highest similarity value was observed between Icrasha and Secondroue varieties while the highest distance was between Acsad and Berghouata varieties. dendrogram discriminates the ten wheat varieties into five groups. The first group consists of Azadi variety, which is considered as simplicifolious and different from all other varieties. The second group consists of Adana99 variety, which also can be considered as a unique variety. The highest similarity value, which reflects small genetics distance, was observed between Icrasha and Secondroue varieties while the highest distance was between Azadi and Secondroue varieties.

Table 9. Shows the genetic distance of the 10 wheat varieties based on 10 microsatellite alleles 1-Icarasha, 2-Acsad, 3-Secondroue, 4-Simeto, 5-Berghouata, 6-Noor, 7-Azadi, 8-Tamoz2, 9-Sham4, 10- Adana 99

Marker	Allele Frequency	Genotype Number	Allele Number	Availability	He	Ho	PIC
WMC17	0.15	10.00	13.00	1.00	0.91	0.40	0.90
WMC20	0.28	8.00	10.00	0.90	0.85	0.89	0.84
WMC21	0.30	7.00	7.00	1.00	0.82	0.00	0.80
WMC24	0.10	10.00	19.00	1.00	0.95	0.90	0.94
WMC25	0.10	10.00	15.00	1.00	0.93	0.50	0.92
WMC48	0.10	9.00	16.00	1.00	0.93	0.90	0.93
WMC50	0.15	10.00	16.00	1.00	0.93	0.80	0.92
WMC283	0.20	10.00	12.00	1.00	0.90	0.90	0.89
Xgwm11	0.22	9.00	15.00	0.90	0.91	1.00	0.90
Xgwm626	0.10	10.00	17.00	1.00	0.94	1.00	0.93
Mean	**0.17**	**9.30**	**14.00**	**0.98**	**0.90**	**0.73**	**0.90**

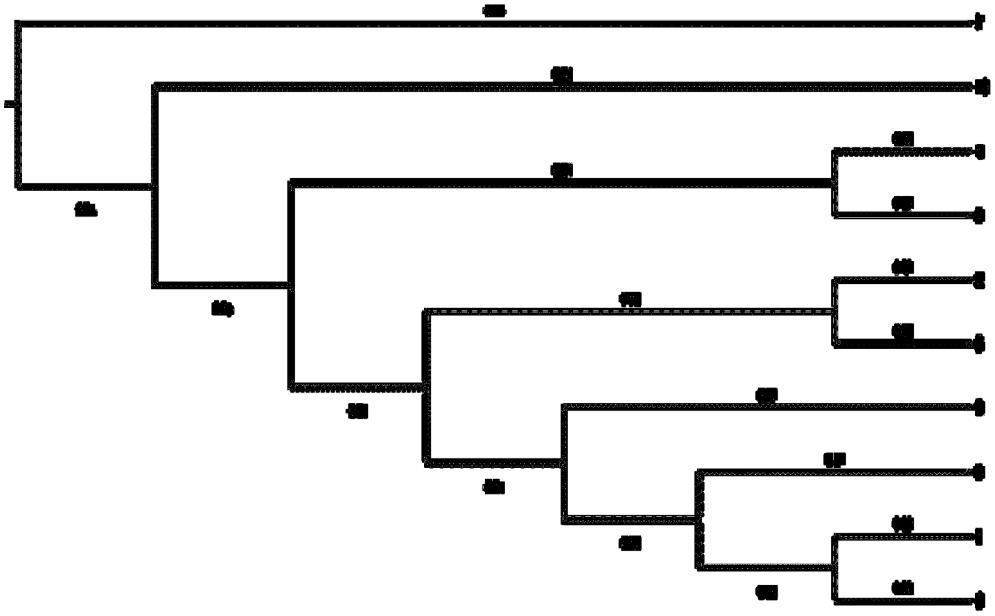

Figure 4. Dendrogram for 10 wheat varieties showing the genetic similarity derived from a UPGMA cluster analysis (1-5 hard wheat varieties 6-10 soft wheat varieties) 1-Icarasha, 2-Acsad, 3-Secondroue, 4-Simeto, 5-Berghouata, 6-Noor, 7-Azadi, 8-Tamoz 2, 9-Sham4, 10-Adana 99

4. DISCUSSION

Molecular markers have revolutionized and modernized our ability to characterize genetic variation and to rationalize genetic selection, being effective and reliable tools for the analysis of genome architectures and gene polymorphisms in crop plants (Barcaccia, 2010). Many studies have calculated genetic diversity and phylogenic relationships among wheat genotypes (Khan *et al*., 2015; Baloch *et al*., 2017). Similarly, various methods have been used for surveying population structures (Khan *et al*., 2014). Analysis of population structure is essential for collections, conservation, and sustainable utilization of gene bank accessions (Suresh *et al*. 2014). In both species, allele sizes detected by these primers ranged between 73 to 234bp. Similar results were scored by Sönmezoğlu and Terzi (2018). The number of detecting alleles over all loci across the two populations ranged from 7 to 19, with an average of 14 alleles per locus (Table 7). Roussel *et al.,* (2004) used 42 SSR markers to analyze 559 French wheat accessions, reporting an average of 14.5 alleles per locus which is very close to the results of this study. Other studies in different wheat collections as well have reported averages close to these findings (Zhang *et al*., 2010; Hao *et al*., 2011; Bafghi *et al*., 2014; Salehi *et al*., 2018; Slim *et al*., 2019). Kara *et al.,* (2016) reported an average of 3.2. A similar pattern of few alleles per locus was also detected by Babay *et al*., (2015). The high number of alleles per locus indicates a broad genetic base of these varieties. The major allele frequency which refers to how common an allele is in a population in the examined loci ranged between 0.19 in the soft wheat varieties to 0.21 in the hard wheat varieties with a great diversity between loci in both populations. Ya Narantsetseg *et al*., (2017) and Salehi *et al*., (2018) observed similar estimates. Gene diversity often referred to as expected heterozygosity (He) which is considered as one of the common indicators in population genetics (Nei, 1987). The presence of a considerable level of genetic differentiation in populations is depicted by the value of gene diversity. The estimated (He) value for the 10 varieties of both species was 0.85 which indicates a high degree of variations in both populations. This forms a good base for future wheat breeding program. The primers used in this study were able to discriminate between all the ten varieties. Similar variations have been reported by Salehi *et al*., (2018). The informativeness of SSRs was calculated using the polymorphism information content (PIC). The (PIC) values of the analyzed microsatellite markers, WMC24 locus had the highest PIC values followed by WMC48 and Xgwm626 primer and the lowest value of 0.84 was presented by WMC20 primer. These results suggest that all markers used in this study were highly informative; because the (PIC) values were higher than

0.50 in all loci, therefore, they can be considered as useful markers for genetic diversity studies in wheat populations to be grown in this region and other places (Singh and Singh 2018). Genetic distance is a measure of the genetic divergence between species or within a species. This distance may measure time from a common ancestor or degree of differentiation (Nei, 1987). Populations with many similar alleles have small genetic distances. This indicates that they are closely related and have a recent common ancestor. Genetic distance is also used for understanding the origin of biodiversity. The microsatellites used in this study were efficient in discrimination between varieties within each species of soft and hard wheat rather than between the two species (*Triticum aestivum* L. and *Triticum durum* Desf.). The dendrogram in Figure 4 also demonstrates the ability of the microsatellites used in this study to detect significant quantities of genetic diversity in these wheat varieties. These data agree with the results of Kara *et al*., (2018).

The close genetic relationships observed between these two species can be explained by having common ancestor parents in their pedigree. For example, the close genetic relationship between durum wheat variety Icarasha and the soft wheat variety Tamoz. The dendrogram in Figure 4 divides the wheat varieties into two distinct groups: Azadi variety formed simplicifolious leave which is substantially different from all the other varieties. The superiority of Azadi variety for drought tolerance was also indicated by Ahmad *et al*. (2017), to screen common bread wheat varieties in Kurdistan, at germination and early growth stage.

5. CONCLUSIONS

In conclusion, 75 alleles were detected in soft wheat varieties and 81 alleles in hard wheat varieties. Allele frequency ranged from 0.10 to 0.30. The observed heterozygosity (Ho) ranged from 0.40 to 1.0 while the expected heterozygosity (He) revealed a high level of genetic diversity in tested varieties and ranged between 0.82 to 0.95. The results of this study will provide information for future breeding programs and may be useful for the evaluation and conservation of wheat genetic resources.

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