ASSSESSMENT 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 (Triticum 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 simplisticolous. 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 Knaoumi, 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.

Zarei., et al., 2016; Ya Narantssetseg 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, Tamo2, 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 pestil 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 (4001 Korea) according to the instructions provided by the supplier company (GenetBio). Ten polymorphic SSR microsatellites (Table 1) 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 5 min and hold at 4°C. The PCR products were run on 1% agarose gel which was prepared by dissolving 1 g of agarose in 100 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 were electrophoresed on 8% polyacrylamide gel prepared by dissolving 1g of agarose in 50 ml of 1X TBE solution and 5 ml of 5X TBE to 13.150 ml of deionized water. Then 350 µl of 10% ammonium persulfate with 20 µl TEMED was added. The gel was run for 30 min with 65 volts, then for another 10 min with 80 volts. Then the PCR products were run on a 3% agarose gel for their quality and purity.

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 TREEVIEW (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.

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-CTAGTGTTTCAAAATATTCGTA | 7A-7B | 54°C |
| WMC 20 | (CA) | F-TAAAAACACGGGATCTTCTC R-GTACTCATACATTTCCTCGT | 1A | 54°C |
| WMC 24 | (GA)37 | F-CCGTGGCGGTGTAACCACAAATC R-AGTATTGCGGCGTCTCAAACAA | - | 55°C |
| WMC 25 | (GT)28 | F-GTGGACAAATTTTGTATATGCT R-TACCCCTGATGCTGAATATTGTG | 1A | 52°C |
| WMC 26 | (GT)26 | F-TCGTCGACAGTCAATAATTACT R-TAATGATCAGATCCACACCC | 2B | 52°C |
| WMC 48 | (GA)9 | F-GAGGTGGTCTCGAAATTTTCG TACGTTGCTAGGAGGTATTGC | 4B | 64°C |
| WMC 50 | (GT)10 (GT)16 | F-CTCGCGTACAGGCGCGTACAA R-CACCGACGCTAGCTGGCGCGAA | 3A | 60°C |
| WMC 283 | (CA)19| F-GTGCTGGTGTTTATATCATC R-GACCCCGCTGTAGTCGATAGGA | 4A | 57°C |
| Xgwm 11 | (TA)6 CATA (CA)19| F-AGATAGTCAGACATTTTTTGTG R-GTGATAGTGCTTTGTAGTTCC | 1B | 57°C |
| Xgwm 626 | (CT)5 GT13 | F-GATTAAATGTTGTATTCTTCTC R-TGACTATCACATCAACGCTT | 6B | 52°C |

 Allele sizes of soft wheat varieties (Table 2) in different loci ranged from 74 to 226 bp 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 0.83 for 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 |
|--------|-----------------|-----------------|---------------|-----|----|----|-----|
| WMC 17 | 0.20            | 5.00            | 7.00          | 1.00| 0.84| 0.40| 0.82|
| WMC 20 | 0.38            | 4.00            | 6.00          | 0.80| 0.78| 0.75| 0.75|
| WMC 21 | 0.20            | 5.00            | 5.00          | 1.00| 0.80| 0.00| 0.77|
| WMC 24 | 0.10            | 5.00            | 10.00         | 1.00| 0.90| 1.00| 0.89|
| WMC 25 | 0.20            | 5.00            | 5.00          | 1.00| 0.80| 0.00| 0.77|
| WMC 48 | 0.20            | 4.00            | 6.00          | 1.00| 0.82| 0.80| 0.79|
| WMC 50 | 0.20            | 5.00            | 9.00          | 1.00| 0.88| 0.80| 0.87|
| WMC 283| 0.20            | 5.00            | 9.00          | 1.00| 0.88| 0.80| 0.87|
| Xgwm 11| 0.13            | 4.00            | 8.00          | 0.80| 0.88| 1.00| 0.86|
| Xgwm 626| 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:

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

![Figure 2. Dendrogram for five soft wheat varieties showing the genetic similarity derived from a UPGMA cluster analysis](image-url)
3.2 Population structure of hard wheat varieties (*Triticum durum* Desf.)

Allele sizes of hard wheat varieties (Table 5) 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 81 and 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 phylogenetic 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.
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 | Secondroue | Simeto | Berghouata | 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 Icrasha 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 phylogenetic 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-Tamoz2, 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 phylogenetic 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 Sönmezoglu 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). Rousset 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 Narantsseg 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...
of 0.82 to 0.95. The results of this study will indicate the other varieties. The superiority of Azadi variety Tamoz. The denisov structure in these wheat varieties. These data agree with the results of Kara et al., 2019.

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 during durum wheat variety Icarashu and the soft wheat variety Tamoz. The dendrogram in Figure 4 divides the wheat varieties into two distinct groups: Azadi variety formed during b. The frequency ranged from 0.40 to 1.0 while the expected heterozygosity (He) ranged from 0.10 to 0.30. The observed heterozygosity (Ho) 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.

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.

REFERENCES

Ahmad Nariman S., Shadia H. S. Kareem, Kamil M. Mustafa and Dastan A. Ahmad (2017) Early Screening of Some Wheat Cultivars under Drought Stress. Journal of Agricultural Science, Vol. 9, No. 2. 88-103.

Allen, A. M., Winfield, M. O., Burridge, A. J., Downie, R. C., Benbow, H. R., Barker, G. L., Wilkinson, P. A., Coghill, J., Waterfall, C., Davassi, A. and Scoops, G. (2017). Characterization of a Wheat Breeders’ Array suitable for high-throughput SNP genotyping of global accessions of hexaploid bread wheat (Triticum aestivum L.). Plant biotechnology journal, 15(3): 390-401.

Babay E., Chaabane R., Mzid-Abdouleh R., Ben Naceur M. (2015). Diversity of Tunisian bread wheat genotypes revealed by Morpho-agronomical and microsatellite markers. Journal of Plant Molecular Breeding (JPMB). Vol. 2 no. 1: 74-89.

Baloch Faheem Shehzad, Ahmad Alsaleh, Muhammad Qasim Shah, Vaheddin Citi, Luis E. Sáenz de Miera, Muhammad Aasim, Muhammad Azhar Nadeem, Husnu Akta, Hakan Özkın, Rüştü Hatipoğlu (2017). A Whole Genome DArTseq and SNP Analysis for Genetic Diversity Assessment in Durum Wheat from Central Fertile Crescent. PLOS ONE 12(1): e0167821.

Baraccia Gianni (2010). Molecular Markers for Characterizing and Conserving Crop Plant Germplasm Chapter 10 S.M. Jain and D.S. Brar (eds.), Molecular Techniques in Crop Improvement. Springer Science +Business Media B.V. 2009

Bassam, Brant J & Peter M Gresshoff (2007). Silver staining DNA in polyacrylamide gels nature protocols vol2.No.11. 2649.

Desheva, G. & Kyosev, B. (2015). Genetic diversity assessment of common winter wheat (Triticum aestivum L.) genotypes. Environments Journal of Food and Agriculture, 27(3), 283-290.

El-Fiki, A. & Adly, M. (2019). Molecular characterization and genetic diversity in some Egyptian wheat (Triticum aestivum L.) using microsatellite markers. Potravinarstvo Slovak Journal of Food Sciences, 13(1), 100-108.

Hao C., Wang L., Ge H., Dong Y. and Zhang X. (2011). Genetic diversity and linkage disequilibrium in Chinese bread wheat (Triticum aestivum L.) revealed by SSR markers. PLoS One 18(62): e17279.

Kara Karima, Najia Mezghani, Olfa Saddouk Debbahi, Maher Madini, Malika Rached-Kanouni, M’barek Ben Naceur (2016). Assessment of genetic diversity of wheat (Triticum aestivum L.) using agro-morphological characters and microsatellite markers. International Journal of Biosciences vol. 9, No. 4, p. 92-101.

Kara, K., Kanouni, M. R., Debbabi, O. S., & Naceur, M. B. (2017). Genetic diversity of bread wheat genotypes (Triticum aestivum L.) revealed by agromorphological characterististics and microsatellite SSR markers. Int J Res Eng Technol, 6, 178-182.

Kara Karima, Rached-Kanouni Malika and Ben Naceur M’barek (2018). Investigation of Genetic Diversity among Bread Wheat Cultivars (Triticum aestivum L.) Using SSR Markers. 14th LISBON International Conference on Agricultural, Biological, Environmental and Medical Sciences (LABEMS-18) (Lisbon, Portugal) 17-19.

Kesawat, Mahipal Singh, and Basanta Das Kumar. (2009). Molecular Markers: It’s Application in Crop Improvement. Journal of Crop Science and Biotechnology 12 (4): 169–81.

Khan MK, Pandey A, Choudhary S, Hakki EE, Akkaya MS, Thomas G. 2014. From RFLP to DArT: molecular tools for wheat (Triticum spp.) diversity analysis. Genetic Resources and Crop Evolution 61: 1001-1032.

Khan Mohd Kamran, Anamika Pandey, George Thomas, Mahinur S. Akkaya, Seyit Ali Kayis, Yusuf Ozsensoy, Mehmet Hamurcu, Saif Gezgin, Ali Topal and Erdogan E. Hakki (2015). Genetic diversity and population structure of wheat in India and Turkey. AoB PLANTS: pv083–pv083.

Kumar, P., Yadava, R. K., Kumar, S., & Kumar, P. (2016). Molecular diversity analysis in Wheat genotypes using SSR markers. Electronic Journal of Plant Breeding, 7(2), 464-468.

Nei, Masotoshi (1973). Genetic distance between populations. The American Naturalist, 106 (949), 283-292.

Nei, Masotoshi (1987). Molecular evolutionary genetics. Columbia university press. New York. 512 pp.

Raj, R. Sandeep, Yama S. Vyas, Viral Kumar M. Baranda, Madhvi N. Joshi, Shradha Nand Tyagi, and Snehal B. Bagatharia (2017). Ascertainment narrow genetic base in commercial accessions of wheat commonly grown in Gujarat via molecular markers. Electronic Journal of Plant Breeding 8 (2): 558.

Röder, M.S.; Korzun, V., Gill, B.S., Wendehake, K., Pleaschke, J., Bassam, Brant J & Peter M Gressho (2007). Silver staining DNA in polyacrylamide gels nature protocols vol2.No.11. 2649.

Roussel V, Koenig J, Beckert M, Balfourier F. (2004). Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes. Theor. Appl. Genet. 108: 920-930.

Salehi, Marzieh, Ahmad Arzani, Majid Talebi and Asad Rokhazadi (2018). Genetic diversity of wheat wild relatives using SSR markers. Genetika, Vol. 50, No1, 131-141.

Salem, K. F., Röder, M. S., & Börner, A. (2015). Assessing genetic diversity of Egyptian hexaploid wheat (Triticum aestivum L.) using microsatellite markers. Genetic resources and crop evolution, 62(3), 377-385.

Shewry, P. R. (2009). Wheat. Journal of experimental botany, 60 (6), 1537-1553.

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Singh Piyusha and Naveen Kumar Singh (2018). SSR Molecular Marker are efficient tools for finding Genetic Diversity in Bread Wheat. International Journal of Current Microbiology and Applied SciencesSpecial Issue-7 pp. 1098-11053

Slim Amine, Luciana Piarulli, Houda Chennaoui Kourda, Mustapha Rouaissi, Cyrine Robbana, Ramzi Chaabane, Domenico Pignone, Cinzia Montemurro and Giacomo Mangini (2019). Genetic Structure Analysis of a Collection of Tunisian Durum Wheat Germplasm. Int.J.Mol.Sci. 20, 3362.

Sokal. R. R. and C. D. Michener (1958). A statistical method for evaluating systematic relationships, Univ. Kans. Sci. Bull, 28, 1409–1438.

Sönmezöglu Özlem Ates and Begüm Terzi1(2018). Characterization of some bread wheat genotypes using molecular markers for drought tolerance. Physiol Mol Biol Plants: 24(1):159–166.

Suresh S, Chung JW, Cho GT, Sung JS, Park JH, Gwag JG, Baek HJ (2014). Analysis of molecular genetic diversity and population structure in Amaranthus germplasm using SSR markers. Plant Biosyst. 148: 635-644.

Yadav, M. K., & Chand, P. (2018). Assessment of Genetic Diversity among Twenty Indian Wheat (Triticum aestivum L.) Cultivars using Simple Sequence Repeat (SSR) Markers. Int. J. Curr. Microbiol. App. Sci, 7(3), 1708-1717.

Zarei Abbasabad E, Mohammadi SA, Moghaddam M, Jalal Kamali MR. (2016). Analysis of genetic diversity, population structure and linkage disequilibrium in Iranian wheat landraces using SSR markers. Plant Genetic Resources 1: 1-8.

Zhang D, Bai G, Zhu C, Yu J, Carver BF. (2010). Genetic diversity, population structure, and linkage disequilibrium in U.S. elite winter wheat. The Plant Genome 3: 117-127.