RESEARCH PAPER

Characterization of Microsatellite Loci in different Fig (Ficus carica L.) Landraces in Duhok and Erbil Provinces in Kurdistan Region-Iraq.

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A B S T R A C T:
Fig (Ficus carica L.) is one of the underused fruit crops cultivated in Mediterranean countries, which is considered as an important resource for commercial cultivation and breeding. In the current study, simple sequence repeats (SSR) markers were implemented to investigate genetic polymorphism and to assess the phylogenetic relationships among 14 fig landraces in Kurdistan Region-Iraq. Results revealed that, twenty SSR loci produced 73 alleles across all 14 studied samples. The expected heterozygosity was ranged from 0.071 to 0.796. The observed heterozygosity was ranged between 0.071 and 1.0000. Polymorphic information content, Fis and p-value were ranged from 0.067-0.735, 0.0020-1.0000 and 0.0020-1.0000 respectively. The genetic distances among the studied genotypes were ranged between 0.0145 and 0.2372. The UPGMA clustering analysis discriminated all these 14 fig genotypes and classified them completely into seven major genetic clusters namely; C1, C2, C3, C4, C5, C6 and C7 in the phylogenetic tree. Overall, it can be concluded that there were significant genetic diversities among these local fig landraces. The selected SSR markers allowed an unambiguous differentiation between studied fig landraces and proved the reliability of these markers in fingerprinting of fig genotypes. Also, it is noteworthy to mention that the study findings will aid the management of fig genotypes and might help the selection of these landraces for future breeding program in this region.

KEY WORDS: Fig (Ficus carica L.), PCR, SSR, Genetic diversity.
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1. INTRODUCTION:

A diploid species (2n = 26) fig tree (Ficus carica L.), which belongs to the family Moraceae, is considered as one of the oldest traditional fruit that distributed along the Mediterranean basin, east and western Asia (Almajali et al., 2012). This tree, is comprehensively characterized by the presence of latex in all its parts, it is naturally well adapted to dry and harsh climate with hot summer. This is due to developing of large root system, many meters away from the trunk, to obtain water from soil.

Thus, it can be grown in areas with hot and dry environments and the hot resistant feature may make this tree more adaptable to the climate changes and global warming consequences (Sugiura et al., 2007; Perez- Jiménez et al., 2012). A gynodioecious fig plant is present in two sexual forms in nature, the pollinator or male tree (caprifig) and the female tree (domesticated fig), which is the producer of the edible fruit (Chatti et al., 2010; Ikegami et al., 2013). Therefore, the pollinators like wasps (Blastophaga psenes) play significant roles in the biological cycle of Ficus carica (Chatti et al., 2010). This tree is traditionally considered as a medicinal plant, as its organic extracts are well known to have many

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antibacterial, antiviral as well as antioxidant activities (Trichopoulou et al., 2006; Lansky et al., 2008; Lazreg-Aref et al., 2012; Ganopoulos et al., 2015).

The preservation of the genetic diversity of traditional varieties and promoting the conservation of the agro diversity and the genetic resources through international initiatives may have definitely very significant and positive consequences on food production and security (Esselman et al., 2000). Although, fig tree populations exhibit a rich genetic biodiversity, it has not yet been subjected to intensive plant breeding programs, as it needs to be properly identified, fully exploited and classified (Giraldo et al., 2010). In general, the classical identification of cultivars relies mainly on the morphological traits including flesh color, skin color, floral characteristics, parthenocarpy and pollination requirement (Caliskan et al., 2018). These morphometric classifications lead to miss assessing the genetic relatedness among cultivars. As, these phenotypic features might vary through years and geographical regions and might greatly be affected by the interactions between genotype and environment (Chatti et al., 2010). Therefore, discrimination of the morphologically similar cultivars at the molecular level is crucial to assess fig tree biodiversity and genetic resources. As such, molecular techniques provide a precise identification and reliable information for the genetic characterization of germplasm (Caliskan et al., 2012; Ganopoulos et al., 2015).

To date, many DNA-based molecular markers have been developed and being long used for germplasm characterization such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) or microsatellites markers (Stafne et al., 2005) Among these, SSR markers, which are short variable number tandem repeats of nucleotide sequences, have received valuable attentions, and become a marker of choice in most plant species for genetic diversity analysis, and fingerprinting. This is due to its reproducibility, co-dominant, and high level of polymorphism, as well as simplicity of detection through PCR based assays, and its reliability (Gupta et al., 2000; Giraldo et al., 2010).

In the present study, SSR markers were employed to characterize local fig landraces from Duhok and Erbil provinces in Kurdistan region of Iraq. Also, to estimate the genetic diversity and phylogenetic relationships among fig landraces to provide a reliable molecular database for future fig breeding program in this region.

2. MATERIALS AND METHODS

2.1. Sample Collection

In the current study, fresh and young leaves of 14 fig landraces including; rohani bahari, zard payazi, namezi rash, shoshi gawra, kongi doshawi, taqtaq, zard chawsor, zard gawra, zard bahari, namezi shen, rash, shenik, arzani and rohani, were collected from different locations at Duhok ( Malta, Bagera and Balata) and Erbil (Shaqlawa, Hiran and TaqTaq) provinces.

2.2. Genomic DNA Extraction

The genomic DNA was extracted according to the method previously described by Weigand et al., (1993). Briefly, 3g of fresh leave tissues were used from each sample. The tissue was ground to a fine powder using liquid nitrogen. Then the powder was dissolved in pre-heated (60°C) extraction buffer (CTAB) (2gm CTAB (cetyl trimethyl ammonium bromide), 28 ml of 5 M NaCl, 10ml of 1M Tris-HCL, 4 ml of 0.5 M EDTA, the volume was adjust to 100 ml by distilled water) and incubated at 60°C in shaking water bath for 30 min. The mixture was then treated with an equal volume of chloroform/isoamyl alcohol (24:1, v/v) and then centrifuged at 4000g for 30mins. The aqueous phase was transferred to a clean tube and precipitated with 0.66 volume of isopropanol. Precipitated nucleic acid was then hooked out and dissolved in 500-750µl of Tris-EDTA (TE)-buffer (1ml of 1Mtris-HCl (Ph8.0) 0.2ml of 0.5M EDTA to 100ml with dH2O.

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2.3. Purification of Extracted Genomic DNA

The extracted DNA sample from previous step was transferred to sterile Eppendorf tube, mixed with an equal volume of phenol/chloroform isoamyl (25:24:1, v/v) and centrifuged at 12000g for 15min. The aqueous layer was transferred to a new sterile Eppendorf tube, 0.1 volume of sodium acetate and 2 volumes of absolute ethanol were added, mixed gently and centrifuged for 5min at 12000g. The supernatant was removed and the pellet dried out for 5mins at room temperature. The pellet was then re-suspended in 350-500µl (depending on pellet size) of TE buffer and stored at -20°C for further downstream applications.

2.4. PCR Amplification of SSR Markers

A total of 20 SSR primers, which were previously developed by Khadari et al., (2004), Zavodna et al., (2005), and Vignes et al., (2006), were used for PCR amplifications and genetic variabilities of respective trees. The PCR amplification was performed in 25µl reaction volume containing 12.5µl of 1X one Taq quick-load master mix, 1µl of each primer including forward and reverse (6pmol/µl), 7.5µl of nuclease free water and 2µl of (25-50ng) genomic DNA. Reaction tubes were placed in a thermal cycler (Applied Biosystems-2720) to carry out DNA amplification. The PCR cycle parameters were as follows; initial denaturation at 94°C/45s for 1 cycle, denaturation at 94°C/45s, annealing 50°C - 56°C/45s, extension at 72°C/2-3min for 30 cycles and final extension at 72°C /10 min for 1 cycle. The PCR products were confirmed by electrophoresis on agarose gel 2% (w/v), 100bp ladder were used to verify the product size, stained with ethidium bromide and visualized by UV transilluminator.

2.5. Data scoring and analysis

Gel electrophoresis images were used to score and analyze SSR-data. Number of alleles per locus, observed (Ho) and expected heterozygosity (He), polymorphic information content (PIC), Fis, P-value, Fixation index genetic distances and phylogenetic tree, all were statistically analyzed by using Power marker V3.0 and Fig Tree V1.3.1 software programs (Rambaut, 2009).

3. RESULTS AND DISCUSSION

The results revealed that 73 alleles were produced across all studied genotypes (Table 1). The number of alleles (richness of genotypes) varied from one primer to another. The highest number of alleles was 7 detected at FinsQ6HEX-L locus, while the lowest number of alleles was 2 which were found with primers (FinsK9HEX-L, MFC1, FinsQ5FAM-L, FinsI12FAM-L and MFC7). Major allele frequencies ranged between 0.3571 and 0.9643 detected at MFC5 and FinsQ5FAM-L loci, respectively with a mean of 0.5946. The number of alleles obtained in the present investigation was lower than that obtained by Achtak et al., (2009) who used microsatellite markers as reliable tools for Fig cultivar identification, whereas it was higher than those detected by Ikegami et al., (2009) and Chatti et al., (2010). The total number of alleles obtained in this study was slightly lower than that obtained by Perez-JiMénez et al., (2012). The high number of alleles might be due to increasing of heterozygosity levels in modern cultivars during selection program (Khadari et al., 2004). However, type and number of genotypes should also be taken into consideration in any comparison.

In addition, the observed heterozygosity was ranged between 0.071 and 1.0000. The highest observed heterozygosity was detected with primer FinsU9NED-L and the lowest observed heterozygosity was obtained with primer FinsQ5FAM-L. The lowest observed heterozygosity was lower than that reported by Ikegami et al., (2009), while the highest observed heterozygosity was found to be higher than that obtained by Ikegami et al., (2009). Also, the values were higher than those detected by Saddoud et al., (2007), Saddoud et al., (2011), and Perez-JiMénez et al., (2012), whereas they were comparable with those reported by Saddoud et al., (2005). Additionally, the expected heterozygosity was ranged from 0.071 (FinsQ5FAM-L) to 0.796 (MFC4). Higher values of expected heterozygosity were reported by Ikegami et al., (2009). In contrast, lower values were obtained by Khadar et al., (2004) and Perez-JiMénez et al., (2012). Also, values were slightly higher than those detected by Essid et al., (2015).
Furthermore, the mean of expected heterozygosity, obtained in the present study, was higher than observed heterozygosity. This might be due to the much more diverse genetic background within these genotypes (Liang et al., 2015). Gene diversity is a parameter which assumed as the fundamental genetic variability of a species or a population which act as a tool for stability of natural communities or ecosystem as well as increase productivity and invasion resistance (Urrestarazu et al., 2012 and Takahashi et al., 2018).

The highest polymorphic information content was 0.735 obtained with MFC4 locus while the lowest information content was 0.067 found at FinsQ5FAM-L locus. Both, the highest and lowest PIC were lower than PIC obtained by Baraket et al., (2011) and Saddoud et al., (2011). Further, the lowest Fis value was -0.8571 (FinsI12FAM-L), while the highest Fis was 1.0000 (FinsM5HEX-L, FinsP8NED-L, FinsJ10NED-L, FinsH5HEX-L, FinsA1NED-L, MFC3, MFC7, MFC6 and MFC8). The negative values of Fis might refer to the similar distribution of migration and mutation to genetic variation in the material. It is also noteworthy that the presence of null alleles (F) in such kind of investigations, leads to higher Fis values (Urrestarazu et al., 2012). In addition the negative Fis and F produced as a result of excess heterozygosity. The lowest and highest P-values were ranged between 0.0020 and 1.0000, respectively.

Additionally, 11 out of 20 SSR primers had PIC values higher than 0.5. PIC value depicts the richness of the SSR markers and their capability in detection of variability among genotypes depending on their genetic relationships (Kumari et al., 2018). The PIC values in the current study might indicate that these loci have a high polymorphism and could be exploited in genetic diversity analysis. Contrastingly, PIC values less than 0.5 indicate low polymorphism of the locus (Botstein et al., 1980). The presence of high polymorphism in SSR markers due to the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Baraket et al., 2011). Fixation index values were ranged between -0.3022 (FinsI12FAM-L) and 1.0000 (FinsH5HEX-L, MFC8, MFC5 and MFC3). Negative fixation index has been shown with seven SSR loci and positive fixation index found with 13 SSR markers. The presence of positive F value indicates an excess of observed homozygotes while the negative F value depicts an excess of observed heterozygotes (Ganopoulos et al., 2015).

Differences between studies might be due to the differences in sample size, differences in the type of genotypes, number of SSR loci used in each study as well as the differences in genetic diversity present within genotypes (Kumari et al., 2018).

The obtained SSR data where further analyzed to construct genetic distances among the selected genotypes. The lowest genetic distance was 0.0145 between rash, zard bahari, shoshi gawra and zard chawser (Table2). This is indicate that they are genetically similar, have similar alleles and share the same common ancestor (Esselman et al., 2000). Thus, these genotypes might have some common morphological characters as well such as, leaf shape, fruit color, and fruit shape. In contrast, the highest genetic distance (0.2372) was found between rash and zard payazi, suggesting less similarity and have different alleles. Genetic distance information is useful for evaluation of the diversity at genetic level among genotypes which could aid and simplify the selection process in breeding program, preservation and introducing of new accessions before elimination of the redundant genotypes (Govindaraj et al., 2015).

The genetic distance results were further analyzed and used to construct phylogenetic relationships between the studied fig landraces implementing the NTSYS programme and UPGMA clustering method (Nei, 1972) as shown in (Figure 1). The results revealed that all 14 local varieties were clustered into seven main genetic clades namely; C1, C2, C3, C4, C5, C6 and C7. Within the fourth main genetic group two sub-clusters could be found, the first sub-cluster represented zard gawra, while the second sub-group represented rash and zard bahari genotypes. Grouping of genotypes in separate clusters usually relies on their evolutionary paths and their geographical distribution. Furthermore, such kind of classification and phylogeny, which depend
totally on the genetic background of the genotypes through stable genetic markers, are crucial to eliminate blind selection for the purpose of breeding program and/or preservation of types (Gregory, 2008).

4. CONCLUSIONS

In conclusion, it can be stated that SSR markers were successfully used for genotyping and for investigating a collection of local fig genotypes in this region. Also, the SSR markers were found to be reproducible, as well as to be very informative, useful and reliable tool to distinguish and discriminate between phenotypically and genetically related landraces.

Lastly, the estimation of genetic diversity and genetic relationships of landraces might assist to understand the genetic differentiation of these traditional genotypes in the region and to draw a smart delineation of these specific gene pools (genotypes).

Table (1) Results of SSR analysis, name of primer, number of alleles, major allele frequency, observed heterozygosity (Ho), expected heterozygosity (He), polymorphic information content (PIC), P-value, inbreeding coefficient (Fis) and fixation index (F).

| locus            | Sequence(5'→3')                  | Range of allele size(bp) | No. of alleles | Major Allele Frequency | Ho    | He  | PIC   | p-value | Fis     | F(Null) |
|------------------|----------------------------------|--------------------------|----------------|------------------------|-------|-----|-------|---------|---------|---------|
| FinsK9HEX-L      | F:ACGACCTTAAACCCCTTTCAG         | 350-400                  | 2              | 0.7857 0.429 0.349 0.280 | 1.000 | -0.238095 -0.1189 |
|                  | R:TCGAGTCACGAAAACACAAA          |                          |                |                        |       |     |       |         |         |         |
| MFC2             | F:GTCCCTGGATGCTGCTTCAAT         | 200-320                  | 6              | 0.5357 0.357 0.685 0.629 | 0.0020 | 0.488189 0.3055 |
|                  | R:TCGGAGCCTTTTGTTGTTCAAT        |                          |                |                        |       |     |       |         |         |         |
| MFC1             | F:ACTGACGTGAAACACACTTG          | 250-420                  | 2              | 0.7500 0.500 0.389 0.305 | 0.5010 | -0.300000 -0.1423 |
|                  | R:TCGAGATGAAAGAAACCAAG          |                          |                |                        |       |     |       |         |         |         |
| FinsQ5FAM-L      | F:CACTGACGGAGGTTGCTTACG         | 200-350                  | 2              | 0.9643 0.071 0.071 0.067 | 1.0000 | -0.000000 -0.0094 |
|                  | R:CCTCAATGTTAATGGTCTCAAG        |                          |                |                        |       |     |       |         |         |         |
| MFC4             | F:CCCAATTTTATTACATACCTT         | 180-400                  | 6              | 0.3571 0.714 0.796 0.735 | ND    | 0.106529 -0.0169 |
|                  | R:TTCCTCAACATATATACAGG          |                          |                |                        |       |     |       |         |         |         |
| FinsQ6HEX-L      | F:TTCCTCAATAATACCTCCTAA         | 110-400                  | 7              | 0.5357 0.929 0.685 0.629 | 0.7310 | -0.373984 -0.2331 |
|                  | R:CATGAAATCCTCCTCCTCAT          |                          |                |                        |       |     |       |         |         |         |
| Fins12FAM-L      | F:AGGTGGAATGAGGGAGGAGTT         | 190-280                  | 2              | 0.5357 0.929 0.516 0.374 | 0.0070 | -0.857143 -0.3022 |
|                  | R:AAACATCTTCTTGGCTTTG           |                          |                |                        |       |     |       |         |         |         |
| FinsM5HEX-L      | F:ATGAAATGGAGAATCTCGGA          | 185-200                  | 3              | 0.5714 0.000 0.593 0.501 | ND    | 1.000000 0.9999 |
|                  | R:CATGCCCCCTACCTTGAACAC         |                          |                |                        |       |     |       |         |         |         |
| FinsP9NED-L      | F:GACCTCTAACAATCCTCAA           | 160-190                  | 4              | 0.5714 0.000 0.624 0.553 | ND    | 1.000000 0.9999 |
|                  | R:CCTCCCCCTTCTCTAGCTTCA         |                          |                |                        |       |     |       |         |         |         |
| FinsJ10NED-L     | F:GAACCTCTACAACCTCCTCAA         | 170-180                  | 3              | 0.7143 0.000 0.466 0.406 | ND    | 1.000000 0.9984 |
|                  | R:CTTATTTCTCTTGGCTTTCA          |                          |                |                        |       |     |       |         |         |         |
| Fins7THHEX-L     | F:GAATCTGGAGTTGGAATAAC          | 180-400                  | 3              | 0.5000 0.500 0.553 0.424 | 0.7840 | 0.099010 0.0343 |
|                  | R:AAAGATCCTGCTGCTCAGCG          |                          |                |                        |       |     |       |         |         |         |
| FinsH5HEX-L      | F:GAACCTCTGAGACACACTTT          | 300-340                  | 3              | 0.4286 0.000 0.667 0.567 | ND    | 1.000000 1.0000 |
|                  | R:CATGCTTGAACACACCTT            |                          |                |                        |       |     |       |         |         |         |
| FinsA1NED-L      | F:AAACCCCGACTCCATT              | 280-295                  | 3              | 0.7143 0.000 0.466 0.406 | ND    | 1.000000 0.9984 |
|                  | R:GAAGACCTTTAGTGATGACAGG         |                          |                |                        |       |     |       |         |         |         |
| FinsNHXHEX-L     | F:AGGGCTGAGATGTTGAAGTTT         | 190-400                  | 3              | 0.5000 1.000 0.638 0.541 | 0.0020 | -0.603524 -0.2727 |
|                  | R:TAATGGTTTGGTTGGTGGCAT          |                          |                |                        |       |     |       |         |         |         |
| MFC3             | F:GATTTCTTATGTTTATGTTT          | 150-400                  | 5              | 0.5000 0.571 0.675 0.598 | 0.0320 | 0.157895 0.0739 |
|                  | R:GAGGATACCAACACCAAC            |                          |                |                        |       |     |       |         |         |         |
| MFC7             | F:CAACATCAAAAATGTTACCG          | 190-230                  | 6              | 0.5714 0.000 0.656 0.605 | ND    | 1.000000 1.0000 |
|                  | R:AGCGAAGACAGCTCACAAGGC         |                          |                |                        |       |     |       |         |         |         |
| MFC6             | F:CTTTGCTGTGCTGCTCAGGG          | 340-345                  | 2              | 0.7857 0.000 0.280 0.280 | ND    | 1.000000 0.9901 |
|                  | R:GCCTAATGGTAAAACAC             |                          |                |                        |       |     |       |         |         |         |
| MFC8             | F:GTGGGCTGCTGCTCTTAATAT         | 230-250                  | 4              | 0.7857 0.000 0.381 0.348 | 0.0020 | 1.000000 0.9933 |
|                  | R:TATTTATGTGCTGTCTATTG          |                          |                |                        |       |     |       |         |         |         |

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Conflict of Interest

The author has no conflicts of interest to disclose.
Table (2) Genetic distance value obtained with SSR markers through selected samples for this study.

| Sample | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1      | 0.0000 | 0.0299 | 0.0774 | 0.0149 | 0.0443 | 0.0299 | 0.2002 | 0.0149 | 0.0443 | 0.0606 | 0.0299 | 0.0299 | 0.0451 | 0.1537 |
| 2      | 0.0299 | 0.0000 | 0.0774 | 0.0457 | 0.1069 | 0.0299 | 0.2002 | 0.0149 | 0.0443 | 0.0924 | 0.0924 | 0.0606 | 0.1086 | 0.1210 |
| 3      | 0.0774 | 0.0774 | 0.0000 | 0.0953 | 0.0919 | 0.0774 | 0.1489 | 0.0625 | 0.0919 | 0.1102 | 0.0774 | 0.0774 | 0.0937 | 0.1060 |
| 4      | 0.0149 | 0.0457 | 0.0953 | 0.0000 | 0.0602 | 0.0457 | 0.2230 | 0.0308 | 0.0602 | 0.0774 | 0.0457 | 0.0457 | 0.0614 | 0.1727 |
| 5      | 0.0443 | 0.1069 | 0.0919 | 0.0602 | 0.0000 | 0.0751 | 0.2147 | 0.0602 | 0.0896 | 0.0751 | 0.0145 | 0.0443 | 0.0293 | 0.2372 |
| 6      | 0.0299 | 0.0299 | 0.0774 | 0.0457 | 0.0751 | 0.0000 | 0.2002 | 0.0149 | 0.0443 | 0.0299 | 0.0606 | 0.0299 | 0.0606 | 0.1210 |
| 7      | 0.2002 | 0.2002 | 0.1489 | 0.2230 | 0.2147 | 0.2002 | 0.0000 | 0.1853 | 0.2147 | 0.2002 | 0.2002 | 0.2002 | 0.2189 | 0.1924 |
| 8      | 0.0149 | 0.0149 | 0.0625 | 0.0308 | 0.0602 | 0.0149 | 0.1853 | 0.0000 | 0.0602 | 0.0457 | 0.0457 | 0.0457 | 0.0614 | 0.1388 |
| 9      | 0.0443 | 0.0443 | 0.0919 | 0.0602 | 0.0896 | 0.0443 | 0.2147 | 0.0602 | 0.0000 | 0.1069 | 0.0751 | 0.0145 | 0.0751 | 0.1354 |
| 10     | 0.0606 | 0.0924 | 0.1102 | 0.0774 | 0.0751 | 0.0299 | 0.2002 | 0.0457 | 0.1069 | 0.0000 | 0.0606 | 0.0606 | 0.0606 | 0.1876 |
| 11     | 0.0299 | 0.0924 | 0.0774 | 0.0457 | 0.0145 | 0.0606 | 0.2002 | 0.0457 | 0.0751 | 0.0606 | 0.0000 | 0.0299 | 0.0148 | 0.2227 |
| 12     | 0.0299 | 0.0606 | 0.0774 | 0.0457 | 0.0443 | 0.0299 | 0.2002 | 0.0457 | 0.0145 | 0.0606 | 0.0299 | 0.0000 | 0.0299 | 0.1537 |
| 13     | 0.0451 | 0.1086 | 0.0937 | 0.0614 | 0.0293 | 0.0606 | 0.2189 | 0.0614 | 0.0751 | 0.0606 | 0.0148 | 0.0299 | 0.0000 | 0.2227 |
| 14     | 0.1537 | 0.1210 | 0.1060 | 0.1727 | 0.2372 | 0.1210 | 0.1924 | 0.1388 | 0.1354 | 0.1876 | 0.2227 | 0.1537 | 0.2227 | 0.0000 |

1= arzani, 2= Kongi doshawi, 3= Mamezi rash, 4= Mamezi shen, 5= rash, 6= rohani, 7= Rohani bahari, 8= shenk, 9= Shoshi qawra, 10= Taq taq, 11= Zard bahari, 12= Zard chawsor, 13= Zard gawra, 14= Zard payazi.
Figure (1) Phylogenetic relationships among 14 Fig genotypes based on SSR marker, using Power Marker V3.0, Fig Tree v.1.3.1, proportion of shared alleles and UPGMA clustering.

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