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# ASSESSMENT OF GENETIC VARIATION AMONG *PISTACIA ATLANTICA* DESF. REGARDING SEXUAL GENOTYPES (MALE, FEMALE AND HERMAPHRODITIC) USING ISSRS. MARKERS

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## ABSTRACT

All *Pistacia* species are dioecious, male and female flowers are born on separated trees. Our recent studies identified new hermaphroditic genotypes of *P. atlantica* with different structure of racemes and flowers at the south of Syria. Therefore, the current research aimed to assess genetic variation among 11 genotypes (3 female, 5 hermaphroditic, 3 male) across fifteen ISSRs primers in Sweida Research Center (2018-2019). All of the primers were able to detect the polymorphism, which revealed 214 bands, 205 of them were polymorphic (95.79%). The number of bands for each primer ranged from 6 to 33, with an average 14.27 bands for each Primer. Genetic similarity among all studied genotypes ranged from (0.27) between hermaphroditic genotype (PA52) with female genotype (FA3) as well as between MA3 male genotype and FA2 female genotype, while the highest genetic similarity was 0.77 between two hermaphroditic genotypes (PA37and PA52). Cluster analysis grouped all studied genotypes into three main clusters according to their sexual structure; the first cluster contained all of the hermaphroditic genotypes and the second cluster comprised of all male genotypes, while the third cluster included all female genotypes. The results demonstrated the importance and the efficiency of ISSR technique by revealing the genetic variation among *P. atlantica* genotypes and separating all of them into detached clusters according to their sexual structure. Farther more, some primers were able to detect common bands in each sexual structure which might help to understand the mechanism of sexual inheritance within the studied species.

**Key words:** *hermaphroditic genotypes, genetic similarity, P. atlantica, ISSR technique*.

## INTRODUCTION

*Pistacia atlantica* Desf. belongs to the genus *Pistacia* and *Anacardiaceae* family as dioeciously trees (karimi *et al*., 2009). The native origin for the genus *Pistacia* is the dry and semi-dry lands in the Iranian Turonian canton in Iran, Turkey and Syria (Padulosi *et al*., 1996). In deed the researches about this species is modicum, and the information of environmental and morphological trends was not clearly studied

among the populations (Belhadj *et al*., 2007). *P. atlantica* Desf. species is considered as one of the most important genetic rootstocks for commercial cultivars of *P.vera* and provide a great gene-pool for genetic improvement and breeding programs, since this species tolerate the vital and non-vitals conditions due to their deep root system which activate the uptake process of mineral elements. Special individuals as hermaphroditic patterns of different racemes and flowers structure was found naturally in Sweida governorate (Alhajjar *et al*., 2011). Kafkas (2002) mentioned that all species of the genus *Pistacia* are dioecious and this phenomenon has a negative effect on both of yield and nut's quality, and hermaphroditic genotypes were found in Yunt Mountains in Manisia governorate. Isfendiyaroglu (2007) referred to hermaphroditic flowers in some *P. atlantica* genotypes in Izmir region. Furthermore, Gercheva *et al*. (2008) reported that the monoecious phenomenon in *Pistacia atlantica* supposed to generate in the second generation, hence studying the mono and bi-sexual individuals and their hybrids using molecular markers contribute to clarification of inheritance mechanism of this peculiarity in the genus. The importance of these genetic hermaphroditic genotypes is an economic indicator through its benefits in breeding programs with *Pistacia vera* species in the aim to transfer this sexual behavior into the traditional pistachio cultivars. In the same domain, Abdelkader *et al*. (2009) mentioned conformable hermaphroditic genotypes of *Pistacia atlantica* in Morocco. Breeding programs between hermaphroditic *Pistacia atlantica* genotypes "as pollen donors" and two pistachio cultivars (Ohadi and Siirt) was carried out in Turkey (Kafkas *et al*., 2005). Turkeli and Kafkas (2013) assessed the first genetic linkage map depending on the hybrids between Siirt cultivar and the hermaphroditic *P.atlantica* genotype PA18. Biotechnology is an important technique in breeding programs once ISSRs. (Inter simple sequence repeat) is well-thought-out as one of the most important molecular markers in genetic studies, and it depends on semi-arbitrary sequences for specific microsatellite loci, it is also an easy and rapid method that doesn't need preceding knowledge of the studied genome with high repetitive frequency and high percentage of genetic variance (Kebour *et al*., 2012). The current study aimed to clarify the genetic relationships among hermaphroditic, female, and male *Pistacia atlantica* genotypes using ISSRs markers which contribute to understand the genetic variance that pretend to be associated to the sexual inheritance mechanism in the genus *Pistacia* to highpoint the breeding and hybridization programs.

## MATERIALS AND METHODS

This investigation was carried out at the General Commission for Scientific Agricultural Research, Sweida Research Center - molecular biology laboratory during 2018-2019.

Eleven sexual *Pistacia atlantica* genotypes were investigated; 5 hermaphroditic genotypes (PA12, PA13, PA35, PA37, PA52), 3 female genotypes (FA1, FA2, FA3), and 3 male genotypes (MA1, MA2, MA3).

Samples of young leaves of all sexual structure genotypes of *P. atlantica* were collected and DNA extraction was done by using CTAB protocol depending on Porebski *et al*. (1997). DNA quantity and quality were estimated using spectrophotometer (Eppendorf, Germany) by measuring the absorbencies at A260 and A280 nm.

Fifteen ISSRs primers were used and the amplified reactions were done in a 25$\mu L$ volume containing 10X PCR buffer; 100 mM Tris-HCl (pH 8.4), 500 mM KCl. 2 mM of each of the dNTPs, 10Pmol primer, one unit of Taq DNA Polymerase enzyme (*Go taq*) and 50 ng of genomic template DNA. The PCR products were detected by electrophoresis on 1% agarose gel and then it was visualized after exposing to UV rays using gel documentation (VILBER LOORMOT Germany)

The amplified bands were scored either as present (1) or absent (0). Genetic similarity between any two genotypes was calculated from the bands across the 15 ISSR markers using Jaccards' similarity coefficient (Jaccard, 1908). Polymorphism percentage was estimated according to the equation: the number of polymorphic bands / the total number of amplified bands × 100. A dendrogram was constructed using UPGMA method. The software programs used through this study were Microsoft EXCEL and Past program.

## RESULTS AND DISCUSSION

### Levels of polymorphism and discriminating of the assay

The number of amplified bands ranged between 6 bands using primer ISS7 to 33 bands using ISS5 primer with an average 14.27 bands for each applied primer in all the different sexual structures of *Pistacia atlantica.* The total number of amplified bands was 214 that 205 bands of them were polymorphic figuring polymorphism percentage of 95.79%. Figures (1) illustrate the amplified bands using primers K24. The number of generated bands in the current study was widely higher in comparison to literature studies that Noroozi *et al*. (2009) obtained only 28 bands of which 13 bands were polymorphic with an average of 9.3 bands for each primer. Fares *et al*. (2009) mentioned that ISSRs markers detected a higher percentage of polymorphism (26 bands). Kebour *et al*. (2012) indicated to 111 bands throughout 6 ISSR primers applied on *P. vera* cultivars of which 60 were polymorphic bands (54.04%). The polymorphism percentage for each primer ranged between 72.73% using K25 primer to 100% in ISSRs primers; K24B, K24A, K26, A6, A5, ISS6, ISS5, ISS3, and ISS2. Band's size ranged between 200-1238bp. The primer ISS6 detected the highest bands number (33 bands) of the size ranged between 440-1203 bp, whereat all of them were polymorphic and the number of unique bands among them was 21 unique bands. In addition, the primer K11 amplified 27 bands where only one band which was monomorphic with a polymorphism percentage 96.30% and the number of unique bands were 11 bands (table- 1). Turhan-Serttas and Ozan (2018) mentioned low bands size in comparison with our current results that ISSR primers detected 81 bands in a range of 161-188 bp only and polymorphism percentage 96.3%. The band size 1079 bp was shared between the hermaphroditic genotype PA35 and the two male genotypes (PM1, PM2) in the primer K11, while

the band size 628 bp was shared just in all female genotypes using the same primer. Likewise, the primer ISS5 amplified the band (565 bp) among all male *P. atlantica* genotypes, whereas this band was absent in all other genotypes (female and bisexual genotypes). Accordingly, these primers may primarily use for detecting responsible sexual genes. This result is corresponding with Ehsanpour *et al*. (2008) while referring to the possibility of sexual determination in pistachio male and female cultivars using ISSR primers whereat they use 9 primers and 2 of them (AC)CG and (AC)8TA were capable to detect each sexual mechanism behavior for male and female patterns.

Table 1. The total number of amplified bands, polymorphism percentage, number of unique bands and band's size (bp)

| **Primer** | **No. of amplified bands** | **No. of polymorphic bands** | **Polymorphism %** | **Band size bp** |
|---|---|---|---|---|
| **ISS2** | 8 | 8 | 100 | 1003-418 |
| **ISS3** | 9 | 9 | 100 | 1176-426 |
| **ISS5** | 33 | 33 | 100 | 1203-440 |
| **ISS6** | 15 | 15 | 100 | 1055-365 |
| **K25** | 11 | 8 | 72.73 | 855-246 |
| **A4** | 13 | 12 | 92.31 | 723-274 |
| **A5** | 18 | 18 | 100 | 1238-367 |
| **A6** | 12 | 12 | 100 | 715-394 |
| **K11** | 27 | 26 | 96.30 | 1079-200 |
| **ISS7** | 6 | 5 | 83.33 | 699-419 |
| **K26** | 13 | 13 | 100 | 766-252 |
| **K24A** | 8 | 8 | 100 | 615-348 |
| **K24B** | 16 | 16 | 100 | 850-241 |
| **UBC840** | 15 | 14 | 93.33 | 993-343 |
| **A2** | 10 | 8 | 80 | 944-388 |
| **Total** | **214** | **205** | **95.79** | |
| **Average** | **14.27** | **13.67** | | |


Figure-1: Amplified bands across using K11 ISSR primer.
Genetic similarity. M: DNA molecular weight 100bp ladder

The results showed that the lowest percentage of genetic similarity was 0.27 between the hermaphroditic genotype PA52 and the female genotype FA3, also between the male genotype MA3 with the female genotype FA2. The highest percentage of genetic similarity was 0.77 between the two hermaphroditic genotypes PA52 and PA37. The average of genetic similarity between female and male *P.atlantica* genotypes was 0.326, whereas it was 0.395 between female and hermaphroditic genotypes, and 0.412 between male and hermaphroditic genotypes, which means that the genetic similarity between the hermaphroditic genotypes and both of male and female genotypes was somehow equivalent. Genetic similarity among each of sexual structure was as following: 0.588 among hermaphroditic genotypes, 0.655 among female genotypes and 0.568 among male genotypes (table- 2). In comparison with previous studies Mahmoodnia and Malekzadeh (2017) indicated to genetic similarity percentage ranged between 25- 78% across 12 ISSR primers on 56 male and female pistachio genotypes. Turhan-Serttas and Ozcan (2018) indicated to a high genetic similarity 0.9333 between two *Pistacia lentiscus* genotypes.

Table 2. Genetic similarity using 15 ISSR primers among all sexual genotypes of *Pistacia atlantica*

| | **FA 1** | **FA 2** | **FA 3** | **PA1 2** | **PA1 3** | **PA3 5** | **PA3 7** | **PA5 2** | **MA 1** | **MA 2** | **MA 3** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **FA1** | 1.00 | | | | | | | | | | |
| **FA2** | 0.71 | 1.00 | | | | | | | | | |
| **FA3** | 0.59 | 0.66 | 1.00 | | | | | | | | |
| **PA1 2** | 0.49 | 0.51 | 0.54 | 1.00 | | | | | | | |
| **PA1 3** | 0.47 | 0.39 | 0.46 | 0.66 | 1.00 | | | | | | |
| **PA3 5** | 0.37 | 0.33 | 0.46 | 0.53 | 0.64 | 1.00 | | | | | |
| **PA3 7** | 0.35 | 0.34 | 0.32 | 0.54 | 0.54 | 0.49 | 1.00 | | | | |
| **PA5 2** | 0.32 | 0.31 | **0.27** | 0.47 | 0.52 | 0.44 | **0.77** | 1.00 | | | |
| **MA1** | 0.39 | 0.42 | 0.39 | 0.47 | 0.46 | 0.51 | 0.44 | 0.41 | 1.00 | | |
| **MA2** | 0.29 | 0.29 | 0.32 | 0.42 | 0.49 | 0.45 | 0.41 | 0.43 | 0.65 | 1.00 | |
| **MA3** | 0.28 | **0.27** | 0.28 | 0.31 | 0.35 | 0.33 | 0.34 | 0.36 | 0.52 | 0.55 | 1.00 |

Cluster analysis

The cluster analysis using UPGMA method divided all genotypes into three main clusters according to their sexual structure; the first cluster comprised all hermaphroditic genotypes which were distributed into three sub-clusters, the first sub-clusters contained two bisexual genotypes (PA12, PA13) and the second one only contained the bisexual genotype PA35, while the other two hermaphroditic genotypes PA37, PA52 were localized in the third sub-cluster. The second cluster comprised all male genotypes that divided into two sub-clusters, the first one comprised both of MA1 and MA2, while the third male genotype was lonely situated in the second sub-cluster. The third main cluster contained all female genotypes and it was also divided into two sub-clusters, the first one contained only the female genotype FA3, whereas the second sub-cluster comprised both of the other female genotypes (FA1 and FA2) as it is shown in figure (2).

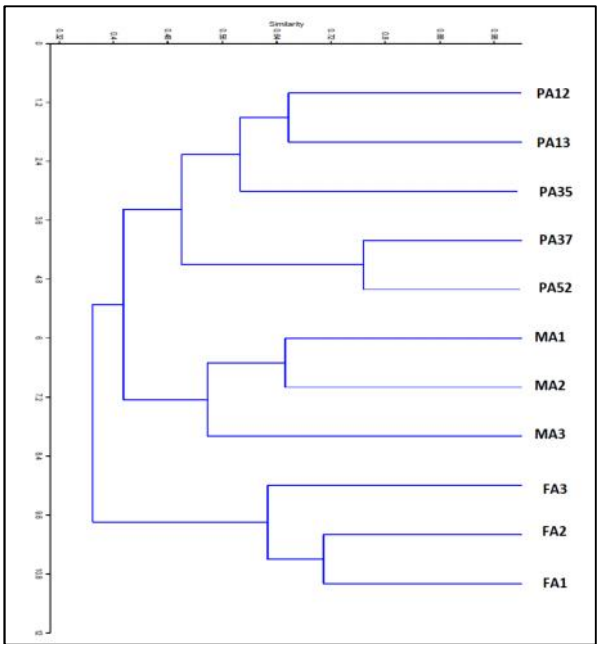


Figure- 2: cluster analysis according to Jaccard coefficient

## Genotyping identification by unique DNA markers

Unique DNA markers obtained by ISSR primers were used in the current study to characterize the different sexual *P. atlantica* genotypes. All applied primers were able to detect positive unique bands except the primer A2. The overall number of all unique bands in all genotypes was 73. The hermaphroditic genotype PA37 as well as the male genotype MA2 presented the highest number of unique bands upward to 11 bands, followed by the male genotype MA3 (10 unique bands), while the lowest number of unique bands was 3 in the male genotype MA1.

## CONCLUSION

In conclusion all the detective primers were of high efficiency of illuminating the genetic polymorphism and the unique bands. The most substantial point that the cluster analysis detached all investigated genotypes according to their sexual structure as male, female and hermaphroditic genotypes. Consequently, this index considers as a preliminary exponent assist in determining sexual responsible genes which might contemplate as an introductory sight in studying the sexual genetic mechanism in the genus *Pistacia*. The current investigation persisted in the importance of farther molecular studies using higher number of primers that aids to profound comprehension of genetic variance which is might connected to sexual inheritance that facilitates breeding programs and opened new vision in genetic studies within the genus *Pistacia*.

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