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GENETIC RELATIONSHIPS AMONG *PISTACIA VERA* L. F1 HYBRIDS AND THEIR PARENTS (P. *VERA*×HERMAPHRODITE GENOTYPES OF *P. ATLANTICA*) USING SSR MARKERS

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ABSTRACT

This research was conducted at the Scientific Agricultural Research center in Sweida province during (2014-2015). Breeding program was assessed in the aim to insert the bisexual phenomena of *P.atlantica* species (3 different hermaphrodite genotypes PA12, PA35, and PA37 as donators of pollen grains) to the commercial cultivars of *P.vera* (Ashouri and Batouri). Genetic relationships among the previous species and their progenies (F1, 6 genotypes of crossing program) was studied using 20 specific SSRs primer pairs, 16 of them were able to detect PCR amplification. Simple Sequence Repeat (SSR) segregation produced 44 putative alleles, out of which 40 were polymorphic (90.91%). Genetic similarity between the hybrids and their parents were closer to their female than to their male parents except for the hybrid HB3,which revealed a genetic distance 0.37 with its female parent (Batouri cultivar FB) and 0.43 with its male parent (PA35 hermaphrodite *P.atlantica* genotype). The UPGMA cluster plots based on Jaccard's coefficient grouped the genotypes into two main clusters. The number of alleles revealed by each SSR analysis ranged from 1 to 8, with a level of expected heterozygosity (He) 0.496, observed heterozygosity (Ho) 0.25, and Marker Index (MI) 19.84. These results suggested the efficiency of SSR markers for distinguishing lineage genetic studies in the *Pistacia* spp. in breeding programs to elicit new cultivars, in particularly the primer pairs Ptms-7, EPVM021, EPVM016, and EPVF019 which may form the platform to detect sex expression in the genus *Pistacia*.

Keywords: *pistachio, P.atlantica,hermaphrodite, SSR, genetic similarity.*

INTRODUCTION

Pistacia vera L. belongs to *Anacardiaceae* family, and it is one of the most economic trees in the world. All species of the genus *Pistacia* are recognized by their high genetic diversity at DNA level (Baghizadeh and Dehghan, 2018). However, Pistachio breeding programs face a lot of complicated obstacles, since it is dioecious species and therefore the potential of male parents is unknown. Pistachio juvenility cycle is somehow too long to determine the sexual genotype in early years after cultivation, because the heritage probability should be

(theoretically) half for each male and female parent. In this prospect, molecular markers could facilitate breeding and allow early seedlings selection, saving time and economic resources (Vendramin *et al.*, 2009). SSR markers have been developed in some species of genus *Pistacia* (Zaloglu *et al.*, 2009; Kafkas *et al*., 2009; Salimi *et al*., 2009).Indeed, all *Pistacia* species are dioecious and their flowers are unisexual except of some exceptional individual specimens of *P. atlantica* and *P. terebinthus* species (Kafkas, 2002; Isfendiyaroglu, 2007; Abdelkader *et al.*, 2009). Our previous study identified two new specific monoecious genotypes of *P. atlantica* (A9 and A10); in the first genotype all the flowers in the raceme are hermaphrodite with only 1-2% 0f female flowers, whereas the other genotype has four patterns of racemes (Alhajjar *et al*., 2011). These exceptional transsexual genotypes are of importance in relation with pistachio breeding programs. Ziya Motalebipour *et al* (2016) indicated that the genome size of pistachio is about 600 Mb with a high level of heterozygosity, subsequently, this information may help in assessing stratigies for sequencing all pistachio genome. Turkeli and Kafkas (2013) assessed the first linkage map in pistachio using an inter-specific cross between *Pistacia vera* L. (siirt cultivar) and monoecious *Pistacia atlantica* Desf. (Pa-18 genotype), using ISSR, SRAP and AFLP markers. In this investigation, breeding program was assessed using three monoecious and hermaphroditic *P. atlantica* genotypes as donators and two *P. vera* female cultivars (Ashouri and Batouri) as receptors. Genetic relatedness of F1 progeny to their parents (*P. vera* × bisexual *P. atlantica*) was studied using 20 SSR markers.

MATERIALS AND METHODS

Plant Material

Three monoecious/hermaphroditic *P. atlantica* genotypes (PA12,PA35 and PA37), two commercial female *P. vrea* cultivars (Batouri FB and Ashouri FA) as receptors were used. Also, 6 F1 hybrids (HA1, HA2, HA3, HB1, HB2 and HB3) as a consequence of cross pollination between the previous species.

Methods

DNA Extraction**:** By using CTAB protocol (Porebski *et al*., 1997).

SSR markers: Extracted DNA was PCR-amplified using 20 SSR primer pairs which were previously cloned and sequenced in *P. vera* according to (Ahmad *et al.*, 2003; Vendramin *et al.*,2010)

Data Analysis

The amplified alleles were scored either as present (1) or absent (0).Genetic similarity (GS) between any two genotypes was calculated depending on Jaccard's similarity coefficient (Jaccard, 1908). A dendrogram was constructed using the unweighted pair group method using arithmetic averages UPGMA (Sneath and Sokal, 1973). Number of alleles, observed heterozygosity (Ho) according to (Wunch and Hormaza, 2007)., expected heterozygosity (He) according to (Lorenzo *et al*., 2007), and Marker Index (MI) were also determined

to estimate the efficiency of SSR technique(Powell *et al*., 1996). The software used through this study was Microsoft EXCEL, SPSS17, and Past.

RESULTS AND DISCUSSION

Levels of polymorphism

Twenty SSR primer pairs were applied, 16 of them were able to detect the polymorphism, and revealed 44 alleles across all genotypes, 40 alleles were polymorphic (90.91%). These results were in agreement with Arabnezhad *et al.* (2011) as they used 18 SSR primer pairs developed from *P. khinjuk* genome. All primer pairs produced 1-8 putative alleles each with an average 2.75 alleles per locus, and the highest number of putative alleles were revealed in the progeny HB3 (FB×PA35), Table (1). Baghizadeh *et al.* (2010) reported that in SSR population analysis, four SSR primers produced 11 alleles among 31 pistachio genotypes with an average value of 2.75 alleles. The genomic EST-SSRs primer pairs (EPVM021, EPVM016, EPVF019, and EPVM056) gave sufficient polymorphism in all F1 genotypes and their parents of the two studied *Pistacia* species (*P. vera* L. and bisexual genotypes of *P. atlantica*) and were more effective in comparison with those constructed by (Ahmad *et al.*, 2003) except Ptms-7 primer pairs, Figure (1). However, some of investigated loci (EPVM021 and EPVF019) revealed specific variation between individuals within the certain locus, which reflect the importance of these loci in selection programs. However, Primers (Ptms-11, Ptms-14, Ptms-42) revealed monomorphic alleles, whereas the other primer pairs revealed polymorphic alleles. The progeny HB3 (FB×PA35) was recognized by 7 alleles using EPVM021 primer pairs, 3 of which were unique alleles (904, 748 and 633 bp, respectively) (Table 1). Using EPVM021 primer, the allele 147 bp was mutual between all *P. atlantica* bisexual genotypes and F1 genotypes (HA1, HA2, HA3, HB2 and HB3) except the hybrid HB1 (FB×PA12) which was matched with its male parent PA12. EPVM016 primer was able to detect heterozygosity in the hybrid HB3, which were 488 bp from FB3 and 550 from PA35 monoecious *P. atlantica* genotype. Heterozygosity was also detected in HA1, HA2, HA3, HB1 and HB3 hybrids using EPVF019. Also, Ptms-7 primer was able to detect heterozygosity in all studied genotypes, and it labeled the HB1 (FB×PA12) by 3 alleles. Allele size ranged between 76 bp in Ptms-11primer to 904 bp in EPVM021 primer. Vendramin *et al.*, (2010) indicated that the allele size ranged from 206 to 609 bp in pistachio, while it ranged from 213 to 766 bp in *P.integerrima*, and 219-766 bp in *P.terebinthus*.

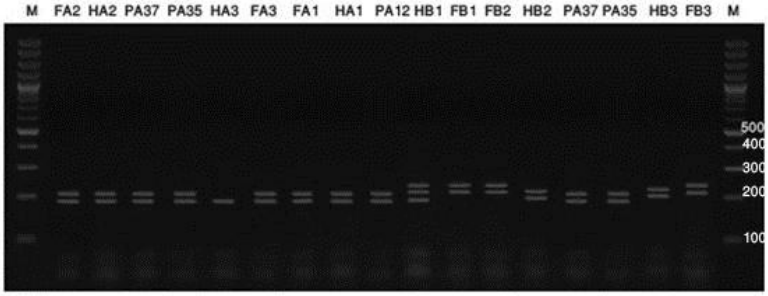

Figure (1). Putative alleles produced by using Ptms-7 SSR primer pair on studied *Pistacia* spp. genotypes (FB1, FB2 and FB3: Batouri FB; FA1, FA2 and FA3: Ashouri FA). M: 100 bp ladder

Table (1): Total number of alleles produced by 16 SSRs primer pairs, number of polymorphic alleles, polymorphism percentage and allele size (bp)

No.	**Primer**	**alleles No.**	**Polymorphic Alleles**	**Polymorphism%**	**Alleles Size bp**
1	Ptms-3	2	2	100	129-138
2	Ptms-7	4	4	100	182-202-222-242
3	Ptms-9	2	2	100	116-125
4	Ptms-11	1	0	0	76
5	Ptms-14	1	0	0	108
6	Ptms-31	2	2	100	121-129
7	Ptms-33	2	2	100	176-184
8	Ptms-40	3	3	100	192-213
9	Ptms-42	1	0	0	183
10	Ptms-45	2	2	100	154-163
11	EPVF021	7	6	88	101-147-307-439-491-633-748-904
12	EPVF013	2	2	100	633-690
13	EPVF016	2	2	100	488-550
14	EPVF056	3	3	100	300-410-520
15	EPVF019	8	8	100	667-532-377-318-202-196-161-112
16	EPVM058	2	2	100	249-273
	SUM	44	40	90.91	
	AVE	2.75	2.5		

Genetic similarity

Genetic similarity ranged from 0.24 between both *P. vrea* female cultivars; Ashouri and Batouri (FA and FB) with PA37 monoecious *P. atlantica* genotype, to 0.96 between two F1 progenies HA1 (FA×PA37) and HA2 (FA×PA12). Genetic similarity between the two studied cultivars of *P. vera* (Ashouri and Batouri) was 0.79. The genetic distances between all bisexual genotypes of *P. atlantica* and *P. vera* female cultivars (Batouri and Ashouri) were 0.282 and 0.264 respectively.

Genetic distances between the hybrids and their parents were closer to their female parents (FA and FB) than to their male parents (PA12, PA37 and PA35) except for the hybrid HB3 (FB×PA35) which revealed genetic distances 0.37 with FB and 0.43 with PA35. Pazouki *et al.* (2010) reported that genetic similarity within *Pistacia* spp. ranged from 0.03 to 0.8.

Clustering and genetic relationships among all studied genotype

The pattern of cluster analysis based on Jaccard's coefficient and UPGMA algorithm clustered the genotypes into two main clusters; the first cluster divided into three sub clusters, the first one branched into two groups, the first group contained all F1 hybrids 'between female Ashouri cultivar and all P. atlantica bisexual genotypes and the second group contained the hybrid HB1 (FB×PA12). The second sub-cluster divided into two groups; the first one contained female cultivars (FA and FB), and the second one contained only the progeny HB2 (FB×PA37). Whereas the progeny HB3 (FB × PA35) was
clustered in the third sub cluster. All P. atlantica bisexual genotypes were grouped in the second cluster which divided into two sub-clusters, the first one contained both of PA12 and PA37, while PA35 genotype was located in the second sub cluster Figure (2)

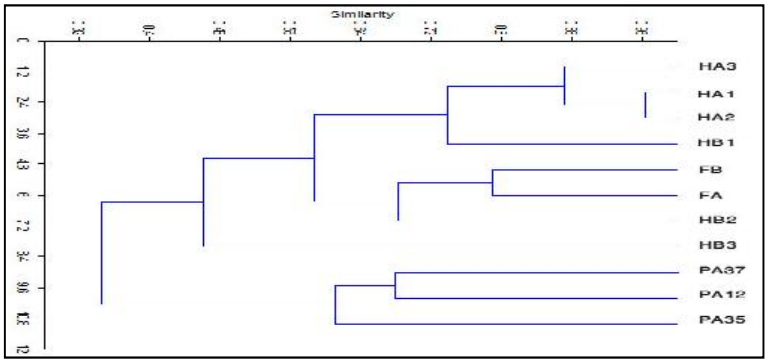

Figure (2): cluster analysis for F1 progenies and their parents using Jaccard's coefficient

To evaluate the efficiency of SSR microsatellite markers for polymorphism detection; Observed and Expected Heterozygosity (Ho and He), in addition to Marker Index (MI) were calculated. Observed heterozygosity Ho was appeared in 3 of 16 SSR loci giving an average value 0.25. Expected heterozygosity depending on allele frequency was 0.496 in all polymorphic tested loci. Marker Index (MI) was19.84. Kolahi-Zonoozi et al. (2010) indicated to a value of Observed Heterozygosity (Ho) 0.490, while the Expected Heterozygosity (He) was 0.345 in their study by using 12 primer pairs.

CONCLUSION

Depending on this investigation, it be concluded that SSR marker is an informative technique which revealed high ability to differentiate individuals and played an important role as genetic marker for lineage studies, and inheritance of sex expression as some of studied primers showed, and may form the platform to detect the sex expression in the genus Pistacia. Since hybrids (HB1, HB2 and HB3) revealed specific loci using different SSR primer pairs (particularly the hybrid HB3), they may form an essential platform for further breeding programs concerning hermaphroditism in P. vera species, in addition to introduce the male parent PA35 for this purpose. On the other hand, advances approach should be continued at different DNA levels using different informative molecular markers for sexual determination in pistachio.

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