Assessment of Genetic Relationships in Some Syrian Pistachio Cultivars and Genotypes, *Pistacia vera* L., Based on ISSR Markers

العلاقات الوراثية لبعض الأصناف والطرز المؤنثة من الفستق الحلبي في سورية اعتماداً على معلمات الـISSRs

Najwa Motaeb Alhajjar
Researcher / General Commission for Scientific Agricultural Research / Syria
najwa81hj@yahoo.com

نجوى متعب الحجار
باحث/ الهيئة العامة للبحوث العلمية الزراعية / سورية

Bayan Mohammad Muzher
Researcher / General Commission for Scientific Agricultural Research / Syria
bmuzher@hotmail.com

بيان محمد مزهر
باحث/ الهيئة العامة للبحوث العلمية الزراعية / سورية

Received: 07/02/2021, Accepted: 17/05/2021
DOI: https://doi.org/10.33977/2106-000-005-003
https://journals.qou.edu/index.php/PJTAS

تاريخ الاستلام: 2021/02/07، تاريخ القبول: 2021/05/17
E-ISSN: 2521-411X
P-ISSN: 2520-7431

Abstract

It was clearly observed that the performance of the commercial pistachio genotypes was confusing within each variety according to the accredited substantial criteria of international descriptors. Therefore, the current work aimed to assess the genetic variation among 10 genotypes and cultivars, including 4 Ashouri, 2 Batouri, Ajami, Beid alhamam, and Ras alkharof across fifteen ISSR primers in Sweida Research Center 2019-2020. All of the used primers were polymorphic, which revealed a total of 148 bands, 141 of them were polymorphic (95.27%). The number of bands ranged from 6 to 15, with an average of 9.87 bands for each locus. Genetic similarity among all studied genotypes and cultivars ranged from 0.31 to 0.73. Depending on the UPGMA algorithm and the Dice equation, the cluster analysis divided the studied material into three main clusters. The first and second clusters comprised the following white genotypes: White Batouri, Batouri cultivar, Grahi, Beid alhamam, and Ras Alkharof that are analogous in many morphological characters, and the third cluster contained all other genotypes and cultivars: Ajami, White Ashouri, Ashouri cultivar, Ashouri Abureha, and Ashouri Mawardi. The current results demonstrated the efficiency of the ISSR technique by revealing the genetic variation among *P. vera* genotypes and cultivars and separating all of them into standing apart clusters according to their resembling appearance.

Keywords*: Pistacia vera* L., genotypes, ISSR, genetic similarity, clustering.

الملخص

يعتبر الخلط الوراثي ووجود طرز عدة ضمن الصنف الواحد من أهم التحديات التي تواجه زراعة الفستق الحلبي عند اعتماد المعايير الأساسية في توصيف الأصناف وفق المواصفات الدولية القياسية؛ لذا هدفت الدراسة إلى تقدير التباين الوراثي بين عشرة طرز وأصناف (4 طرز من الصنف (عاشوري)، 2 طراز من الصنف (باتوري) وبقية الأصناف هي: عجمي، بياضي، بيض الحمام، رأس الخروف) من خلال تطبيق (15) مركزاً في تقنية الـ ISSR في مركز البحوث العلمية الزراعية في محافظة السويداء (2019- 2020). كشفت كافة المركزات المستخدمة التعددية الشكلية حيث أعطت (148) حزمة، كان من ضمنها (141) حزمة متعددة شكلياً (95.27%). تراوح عدد الحزم من (6) إلى (15) حزمة، بمتوسط (9.87) حزمة لكل مركز، وتراوحت درجة التشابه الوراثي بين (0.31 و0.73) قسم التحليل العنقودي العينات المدروسة اعتماداً على طريقة (UPGMA) ومعادلة (Dice) إلى ثلاث مجموعات رئيسة، شملت المجموعتين الأولى والثانية الطرز والأصناف بيضاء اللون، والتي تبدي تشابهاً ظاهرياً فيما بينها في بعض الصفات، بينما ضمت المجموعة الثالثة بقية الطرز والأصناف. أثبتت النتائج كفاءة تقنية الـ (ISSR) في كشف التباين الوراثي بين أصناف وطرز الفستق الحلبي، وفصلها إلى مجموعات منفصلة بما يتوافق مع درجة التشابه المظهري لها.

الكلمات المفتاحية: الفستق الحلبي، الطرز الوراثية، ISSR، التشابه الوراثي، التحليل العنقودي.

INTRODUCTION

The leading world producers of pistachio are Iran, the USA, Turkey, and Syria (Fares *et al*., 2009). Within the genus *Pistacia*, *P. vera* L. is counted as the only comestible and vendible species (Al-Saghir and Porter, 2012). The most important economic cultivars in Syria are Ashouri, which covers over 75% of the pistachio acreage, and Batouri cultivar, which covers about 15% of the cultivated area. In contrast, the remaining acreages are cultivated with other local cultivars such as Olaimi, Bondokii, Nab-jamal, Ajami, and other marginal cultivars. The genetic variance of the *P. vera* L. species is huge, and the locally main cultivars are not pure. Many genotypes belong to the same basic name as Ashouri wardani, Ashouri Abushawka, Ashouri kafer, small and large or common Batouri, white Batouri, and red Batouri. This genetic assortment affects the specific behavior of each cultivar and genotype and creates many difficulties while certifying thorough credence for the normative staple characters. The same problem is presented in other produced countries as in Tunisia, where the most commonly cultivated variety is Matueur, which resembles the Syrian variety Ashouri (Ghorbel *et al*., 1998). This variety includes three main genotypes: Male precocious 25 A, male late 40 A, and female 11 D (Ghorbel and Kchouk, 1996). Relatedly, the inventory and identification of *Pistacia vera* L. in Algeria face taxonomic confusion problems (Kebour *et al*., 2012). Definitely, pistachio production fluctuates from one season to another due to the alternate bearing occurrence and climatic conditions. The agro-morphological

description of the pistachio is a leisurely growing tree. Nonetheless, its longevity exceeds 150 years. Great attention has to be directed towards preserving and evaluating pistachio genetic resources (Alhajjar *et al*., 2017). Furthermore, many genetic genotypes and marginal cultivars with basic characters are being neglected and face a serious risk of being lost. Works on pistachio breeding programs have been increased for the last few years (Alhajjar *et al*., 2016). Positively, there are good prospects for obtaining outstanding cultivars through crossing superior male and female cultivars from different species (Alhajjar *et al*., 2015). Therefore, more efforts have to be prearranged for genetic studies. Despite the revelation of several varieties, the morphological traits remain inconstant criteria that the same cultivar could be expressed in different characters according to the environmental conditions. Under these considerations, the precise description of the cultivars becomes very difficult, and the problem of varietal identification becomes complicated for improvement. For the last few decades, molecular markers have been applied on pistachio and its wild relatives to detect the DNA polymorphism, genetic diversity, and sex determination using either SSR (Alhajjar *et al*., 2017; Alhajjar and Muzher, 2017) or ISSR and RAPD techniques (Ehsanpour *et al*., 2008; Esfandiyari and Davarynejad, 2001; Kamiab *et al*., 2014). Hereafter, inter simple sequence repetition is a semi-arbitrary technique that seems to have the reproducibility of SSR markers for the cause of its longer length of their primers (Noroozi *et al*., 2009). Amplification in this method leads to multi-locus and exceedingly polymorphous outlines (Kafkas and Topaktas, 2003; Kafkas *et al*., 2006). However, the aspects of the recent investigation concern the determination of the genetic polymorphism of a collection of *Pistacia vera* L. Female genotypes based on genetic markers to assess the genetic diversity among some locally Syrian cultivars and genotypes.

MATERIALS AND METHODS

This investigation was carried out at the General Commission for Scientific Agricultural Research, Sweida Research Center in molecular biology laboratory during 2019- 2020.

Plant Material

The study was applied on 10 pistachio cultivars and genotypes, which have been planted in an experimental field since 1998, including 4 Ashouri genotypes, Ashouri Mawardi, Ashouri Abureha, White Ashouri, and common Ashouri, 2 Batouri genotypes (white Batouri, and common Batouri), Ajami, Beid alhamam, Ras alkharof, and Grahi cultivars.

METHODS

DNA Extraction

Samples of young leaves of all investigated genotypes and cultivars of *P. vera* were collected (a half gram of each sample), and DNA extraction was done by using the CTAB protocol (Porebski *et al*. (1997). DNA quantity and quality were estimated using a spectrophotometer (Eppendorf, Germany) by measuring the absorbencies at A260 and A280 nm.

Applying of ISSRs Primers

Fifteen ISSRs primers (Table1) were used, and the amplified reactions were done in a 25 μL volume containing 10X PCR buffer; 100 mM Tris-HCl (pH 8.4), 500 mM KCl. 2 mM of each of the dNTPs, 10 pmol primer, one unit of Taq DNA Polymerase enzyme (*Go taq*) and 50 ng of genomic template DNA. The cycling parameters were as follows: one cycle of 95° for 4 min 35 cycles of 94◦C for one min, annealing temperature for one min ranged between 38- 58°C according to GC/TA percentage of each primer, and 72◦C for one min, followed by 4 minutes at 72◦C for an extension. PCR products were injected in 1.0% agarose gel using gel documentation (VILBER LOORMOT Germany) and then were visualized after exposure to UV rays.

Genetic Analysis

The amplified bands were scored either as present or absent. The genetic similarity between any two genotypes was calculated from the bands across the 15 ISSR markers using the Dice similarity coefficient (Dice, 1945) using the PAST program. Polymorphism percentage was estimated according to the equation: the number of polymorphic bands / the total number of amplified bands × 100. A dendrogram was carried on using the UPGMA method.

Table 1 ISSR primers applied on female pistachio cultivars and genotypes and their repeat motifs

	Primer	Repeat Motif	Tm (GC%)
1	ISS2	(GA)5GC	49.17
2	ISS3	(CA)5 GT	45.75
3	ISS5	(GAA)5	50.17
4	ISS6	(AC)8 CG	66.78
5	ISS7	(AC)8TA	62.22
6	K11	(CA)6 AG	53.79
7	K25	(AG)8 G	63.50
8	K26	(AG)8T	61.09
9	K24A	(GA)8T	61.09
10	K24B	(CA)8T	61.09
11	UBC840	(GA)8TT	62.22
12	A2	(GA)6CC	62.22
13	A4	(AG)10T	68.88
14	A5	(CA)6GT	53.79
15	A6	(CT)10G	70.83

RESULTS AND DISCUSSION

ISSR banding patterns for assessing the polymorphism:

The 15 ISSR primers produced a various number of DNA fragments according to their sequence repeat motifs. The number of amplified fragments throughout all used primers ranged from 6 bands, ISS7 and K26, to 15 bands (ISS6 primer), giving an overall number of 148 bands, out of which 141 bands were polymorphic, and the polymorphism percentage was 95.27% as it is illustrated in Table 2. The recent results were in accordance with Baghizadeh and Dehghan (2018), who used 15 ISSR primers on 20 pistachio genotypes pertaining to four commercial cultivars. The number of total bands was 131 bands, and 124 of them were polymorphic with a polymorphism percentage of 94.6%. Noroozi et al. (2010) studied 31 pistachio cultivars and genotypes using three ISSR markers that amplified 28 bands, 13 of them were polymorphic, giving a polymorphism percentage of 46.42%.

The primer ISS6 amplified 15 bands as all of them were polymorphic (100%), followed by the primer UCB840, which amplified 13 bands, and similarly, all of them were polymorphic (100%). Besides, the primer K24A produced 13 bands, 12 of them were polymorphic 92.31% (Table 2). The primer K24B produced 12 bands (Figure1), where 10 were polymorphic (83.33%). The band size ranged between 209- 1208 bp. Tagizad et al. (2010) applied 10 ISSR primers on 19 pistachio cultivars. The percentage of polymorphism of the used primers ranged between 37- 92%, and the number of amplified bands was amplified 8- 12 bands for each primer. On the other hand, Turhan-Serttas and Ozan (2018) mentioned low bands size compared to our current results that ISSR primers detected 81 bands in a range of 161-188 bp only and polymorphism percentage was 96.3%.

Table 2 The number of total amplified and polymorphic bands, polymorphism percentage and band size (bp)

	No. of amplified bands	No. of polymorphic bands	Polymorphism %	Band size bp
ISS2	8	8	100	237-715
ISS3	9	8	88.89	405-1150
ISS5	10	10	100	249-660
ISS6	15	15	100	247-921
K25	7	7	100	322-1021
A4	9	9	100	350-663
A5	8	8	100	495-1138
A6	12	12	100	209-592
K11	13	12	92.31	212-974
ISS7	6	5	83.33	414-693
K26	6	6	100	416-895
K24A	13	12	92.31	248-567
K24B	12	10	83.33	372-1208
UBC840	13	13	100	225-1017
A2	7	6	85.71	298-818
Total	**148**	**141**	**95.27**	
Average	**9.87**	**9.4**		

Genetic Similarity

The percentage of genetic similarity ranged from 0.31 Beid alhamam and Grahi cultivars to 0.73 Ash. Mawardi and Ash. Abureha genotype, also between white Ashouri and the comparative Ashouri cultivar. Within Ashouri's genotypes, the average percentage of polymorphism was 0.638. The polymorphism percentage between white Batouri and the comparative Batouri cultivar was 0.64. Ghrahi cultivar occasionally missed up with Batouri cultivar with a genetic similarity of 0.61 with the reasonable Batouri as seen in Table 3. Compared to previous studies, Fares et al. (2009) referred to a high percentage of a coefficient similarity that reached 0.857 between Meknessy and Red Aleppo (Ashouri) cultivars and 0.750 between Kermezi and Kerman cultivars using ISSR markers. Mahmoodnia and Malekzadeh (2017) indicated that genetic similarity percentages ranged between 25- 78% across 12 ISSR primers carried out on 56 male and female pistachio genotypes. However, Amirebrahimi et al. (2017) referred to an adjacent genetic similarity among 56 male and female pistachio genotypes by 12 ISSR primers ranging between 0.25- 0.78.

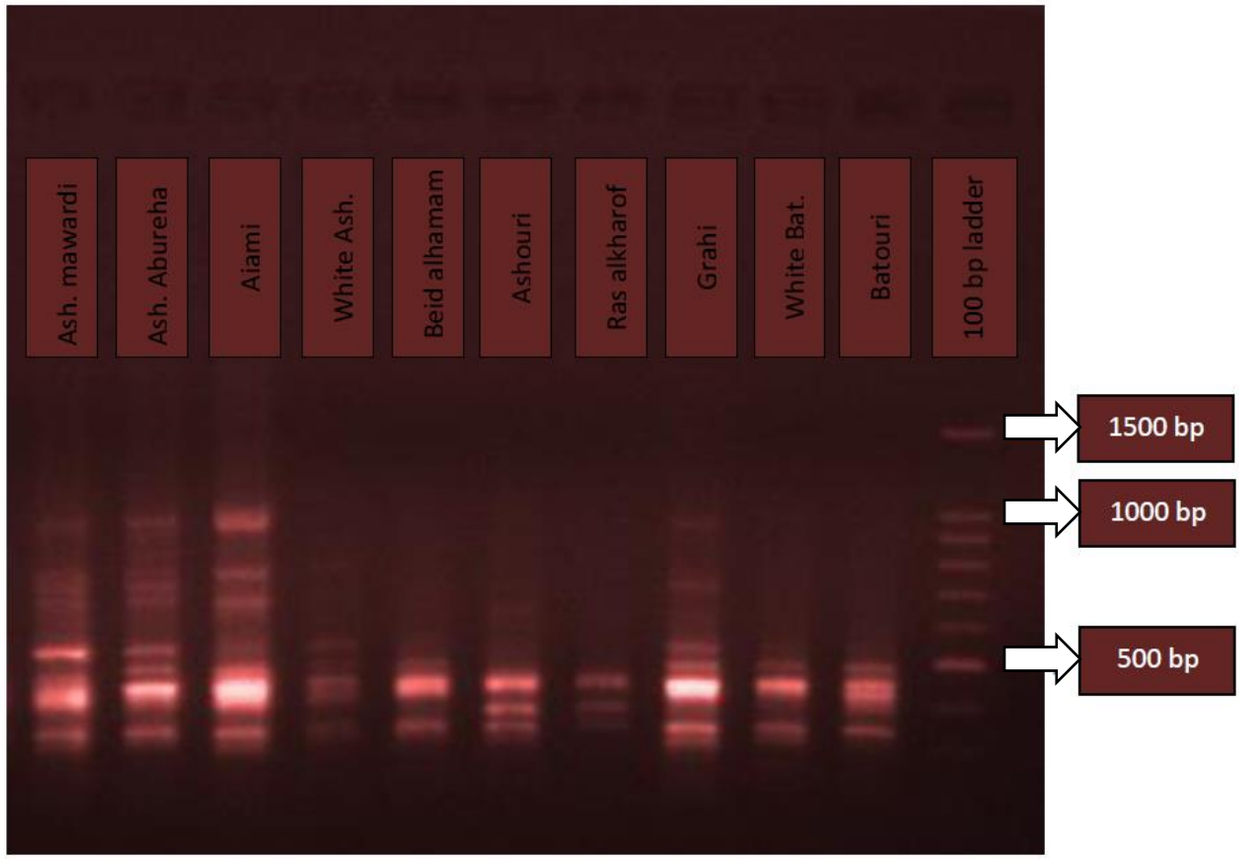

Figure 1 PCR amplified products using Primer K24B

Table 3 Genetic similarity amongst pistachio studied cultivars and genotypes

	Ash. mawardi	Ash. Abureha	Ajami	White Ash.	Beid alhamam	Ashouri	Ras alkharof	White Bat.	Batouri	Grahi
Ash. mawardi	1									
Ash. Abureha	0.73	1.00								
Ajami	0.55	0.56	1.00							
White Ash.	0.69	0.64	0.65	1.00						
Beid alhamam	0.40	0.47	0.43	0.53	1.00					
Ashouri	0.54	0.50	0.57	0.73	0.48	1.00				
Ras alkharof	0.42	0.37	0.45	0.56	0.53	0.61	1.00			
White Bat.	0.40	0.40	0.40	0.47	0.43	0.45	0.45	1.00		
Batouri	0.46	0.43	0.45	0.52	0.40	0.46	0.52	0.64	1.00	
Grahi	0.37	0.36	0.40	0.37	0.31	0.37	0.41	0.48	0.61	1.00

Cluster Analysis

Depending on the UPGMA algorithm and Dice equation, the cluster analysis divided the studied cultivars and genotypes into three main clusters. The first cluster comprised the white genotypes of the largest nut's size, and is divided into two main sub-clusters. The first sub-cluster encompassed white Batouri and comparative Batouri cultivar, while the second sub-cluster included Grahi cultivar. The second main cluster comprised Beid Al-Hamam and Ras Al-Kharof genotypes which are also analogous in many morphological characters and similarly have white nuts with genetic similarity 0.53, as seen in Figure 2. The third cluster was detached into 3 sub-clusters; the first sub-cluster contained Ajami cultivar, whereas all Ashouri genotypes were located in the second and third sub-clusters, white Ashouri and Ashouri cultivar were located together, and Ashouri abureha and Ashouri

mawardi were in another sub-cluster. Undeniably, the third cluster comprised all cultivars and genotypes of red hull nut (Ajami and all Ashouri genotypes) except the white Ashouri genotype due to its resemblance to Ashouri cultivar in most accredited parameters. Baghizadeh and Dehghan (2018) applied 15 ISSR markers on 20 pistachio samples to appraise genetic diversity. Their results improved that ISSR data clearly discriminated the cultivars in terms of their genetic characterization and divided the studied samples into four main clusters.

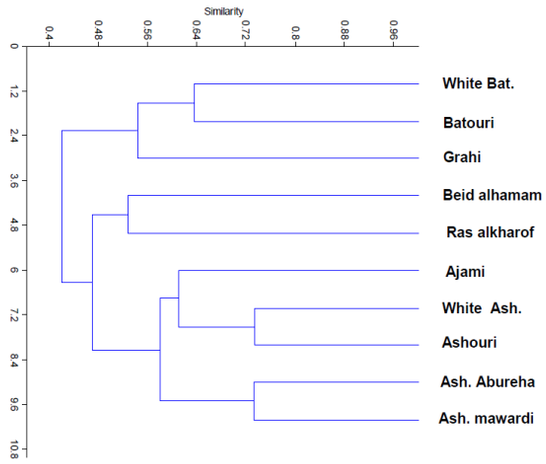

Figure 2 cluster analysis using UPGMA algorithm and Dice equation

Unique Bands

Excluding the primers A24A and A2, all other primers detected 33 positive, unique bands, as illustrated in Table 4. The highest number of unique bands was detected in the white Batouri genotype (9 positives). Followed by Ashouri Mawardi, 6 positives were detected by different primers. The genotypes Grahi and Beid alhamam were diagnosed by 5 positive unique bands. Expressly, in Grahi genotype, the primer ISS6 generated 3 unique bands (360, 678, and 796 bp). Both of the genotypes, white Ashouri and Ashouri Abu reha were not recognized by any unique bands. Five unique bands were scored by primer A6 with molecular weight (475 bp in Grahi, 335 bp in Ash. Mawardi, 300 bp in White Batouri, 215 bp Ajami, and 209 bp in Ras alkharof). Besides, five positive unique bands were recorded by primer ISS6 with molecular weight (921bp in White Batouri, 868 bp in Beid alhamam, and 796 – 678- 360 bp in Grahi).

Table 4 The over-all positive unique bands (bp) scored by ISSR primers in pistachio

Primer	Unique bands	Ash. Mw	Ash. Abur	Aja.	W. Ash.	Beid Alha.	Ash.	Ras Alkh.	W. Bat.	Bat.	Gra.
ISS2	2					569			716		
ISS3	4	1150 415				440	425				
ISS5	1					660					
ISS6	5					868			921		796 678 360
K25	3	861					424		1022		
A4	3	379		663					563		
A5	2			1029						1138	
A6	5	335		215				209	300		475
K11	1	558									
ISS7	1										513
K26	1								442		
K24A	-										
K24B	2								784 372		
UBC840	3					486		511	424		
A2	-										
Total	33	6	-	3	-	5	2	2	9	1	5

CONCLUSION

All the detected primers were effective in clarifying the genetic polymorphism and the unique bands. The cluster analysis classified all investigated cultivars and genotypes according to their resemblance. The current investigation persisted in the importance of molecular markers in identifying the genetic platform for each pistachio cultivar, mainly those more productive genotypes, to insight the knowledge of their genetic base and their relativeness in the aim to assess the genetic identification for all Syrian pistachio cultivars and genotypes.

References

- *Alhajjar, N.M., Hamed, F. & Muzher, B.M. (2017). Genetic similarity among pistachio (Pistacia vera L.) female genotypes and cultivars planted in Sweida province using SSR technique. Damascus Journal of Agricultural Science, 33(1), 239- 256.*
- *Alhajjar, N.M. & Muzher, B.M. (2017). Identification of male genotypes in Pistacia vera L. species using SSR markers. International Journal of Environment, 6(2), 1-12.*
- *Alhajjar, N.M., Muzher, B. M. & Hamed, F. (2016). Assessing genetic relationships between Pistacia vera L. Hybrids and their parents (P. vera × monoecious genotypes of Pistacia atlantica) using SSR markers. Jordan Journal of Agricultural Science, 12(1), 148- 157.*
- *Alhajjar, N. M., Muzher, B. M. & Hamed, F. (2015). The effect of pollen grains of Pistacia vera and Pistacia atlantica (unisexual and hermaphrodite) on quality parameters of Ashouri and Batouri pistachio cultivars. Jordan Journal of Agricultural Science, 11(1), 15- 25.*
- *Al-Saghir, M.G. & Porter, D.M. (2012). Taxonomic revision of the genus Pistacia L. (Anacardiaceae). American Journal of Plant Science. 3, 12- 32.*
- *Amirebrahimi, F.F., Meimand, M.M., Karimi, H.R., Malekzadeh, K. & Yajabadipour, A. (2017). Genetic diversity assessment of male and female pistachio genotypes based on ISSR markers. J Plant Mol Breed, 5(1), 31- 39.*
- *Baghizadeh, A. & Dehghan, E. (2018). Efficacy of SCoT and ISSR markers in assessment of genetic diversity in some Iranian pistachio (Pistacia vera L.) cultivars. PHJ., 1(1), 37- 43.*
- *Dice, L. R. (1945). Measures of the amount of ecological association between species. Ecology, 26(3), 297- 302.*
- *Ehsanpour, A.A., Tavassoli, M. & Arab, L. (2008). Sex determination of Pistaia vera L. using ISSR markers. Malays. Appl. Biol., 37(2), 25-28.*
- *Esfandiyari, B. & Davarynejad, G.H. (2011). Data to sex determination in Pistacia species using molecular markers. Euphytica. DOI 10.1007/s10681-011-0527-6.*
- *Fares, K., F. Guasmi, L. Touil, T. Triki & Ferchichi, A. (2009). Genetic diversity of pistachio tree using Inter-Simple Sequence Repeat markers ISSR supported by morphological and chemical markers. Biotechnology, 8(1), 24-34.*
- *Ghorbel, A., Ben Salem-Fnayou, A., Chatibi, A. & Twey, M. (1998). Genetic resources of Pistacia in Tunisia. In: Towards a comprehensive documentation and use of Pistacia genetic diversity in Central and West Asia, North Africa and Europ. Padulosi, S. and Hadj-Hassan, A. (eds.). IPGRI Report of the IPGRI Workshop, 14-17 December, (P: 62-71).*
- *Ghorbel, A. & Kchouk, M. L. (1996). Genetic resources of horticultural crop in Tunisia. Second Meeting of the WANA Working Group on Horticultural Crop. International Plant Genet.Resour. Inst., Aleppo, Syria.*
- *Kafkas, S. & Topaktas, M. (2003). Chromosome numbers of Four (Anacardiaceae) species. Journal of Horticultural Science Biotechnology., 78, 35–38.*
- *Kafkas, S., Ozkan, H. B., E., Acar, I., Atli, H.S. & Koyoncu, S. (2006). Detecting DNA polymorphism and genetic diversity in a wide germplasm: comparison of AFLP, ISSR, RAPD markers. American Society for Horticultural Science, 131, 522-529.*
- *Kamiab, F., Ebadi, A., Panahi, B. & Tajabadi, A. (2014). RAPD analysis for sex determination in Pistacia vera L. Journal of Nuts, 5(1), 51-55.*
- *Kebour, D., Boutekrabt, A. & Mefti, M. (2012). Using Inter Simple Sequence Repeat (ISSR) markers to study genetic polymorphism of pistachio (Pistacia vera L.) in Algeria, African Journal of Biotechnology, 11 (29), 7354- 7360.*
- *Mahmoodnia, M. & Malekzadeh, K. (2017). Genetic diversity assessment of male and female pistachio genotypes based on ISSR markers. J. Plant Mol. Breed, 5(1), 31-39.*
- *Noroozi, Sh., Baghizadeh, A. & Javaran, M.J. (2010). Study on genetic diversity of some Iranian pistachio (Pistacia vera L.) cultivars using RAPD, ISSR and SSR markers: Amplification study. Biotechnology, 4(3), 120-125.*
- *Noroozi, Sh., Amin, B. & Javaran, M. J. (2009). The genetic diversity of Iranian pistachio (Pistacia vera L.) cultivars revealed by ISSR markers. BioDiCon, 2(2), 50- 56.*
- *Porebski, S., Bailey, G.L. & Baum, B.R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components.Plant Molecular Biology Reporter, 15 (1): 8-15.*
- *Tagizad, A., Ahmadi, J., Haddad, R. & Zarrabi, M. (2010). A comparative analysis of ISSR and RAPD markers for studying genetic diversity in Iranian pistachio cultivars. Iranian Journal of Genetics and Plant Breeding, 1(1), 6-16.*
- *Turhan-Serttas, P. & Ozcan, T. (2018). Intraspecific variations studied by ISSR and IRAP markers in Mastic tree (Pistacia lentisecus L.) from Turkey, Trakya University. Journal of Natural Science, 19(2), 147-157.*