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Original Article

Molecular Characterization of *Vitex agnus-castus* L. (Verbenaceae) Populations Grown in Aydin, Turkey

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Abstract

In this study, we performed a genetic diversity analysis using RAPD markers for some *Vitex agnus-castus* populations grown in Aydin, Turkey. Total genomic DNA isolation from the leaves of *Vitex agnus-castus* was performed using a commercial kit. Seven RAPD primers (OPA-02, OPA-05, OPA-13, OPA-15, OPA-16, OPA-18, OPA-20) were used to determine genetic diversity among populations. Polymerase Chain Reaction (PCR) was performed with all genomic DNA samples and primers. PCR products were run in agarose gel electrophoresis and visualized under UV light. The amplified products were scored as bands (1) and no bands (0) for all gel images and their matrix files were generated. A total of 36 characters were obtained from the primers. Phylogenetic relationships and genetic distances between the cultivars were calculated by using the PAUP* (Phylogenetic Analysis Using Parsimony and other methods) program. According to PAUP analysis, the closest genetic distances were between Çine pink flower and Çakmar purple flower, and Çakmar pink flower and Çakmar purple flower populations with a value of 0.05556; and the greatest genetic distance was between Çakmar pink flower and Köşk purple flower populations with a value of 0.36111. In the phylogenetic analysis obtained using UPGMA algorithms, the phylogenetic tree consisted of four groups. The results suggest that RAPD markers are useful tools for determining genetic relationships among *Vitex agnus-castus* genotypes.

Keywords: Aydın; molecular characterization; RAPD, Turkey; *Vitex agnus-castus*

Introduction

Verbenaceae family is represented by approximately 100 genus and 3000 species all over the world. Consisted of plants in the form of herbs, briers and trees, and being a member of this family, *Vitex* L. has almost 250 taxa (Hürkul and Köroğlu, 2018). *Vitex agnus-castus* L. is a medicinal plant of *Vitex* L. (Rice-Evans *et al.,* 1997) although its native land is Mediterranean countries; it displays distribution in West Asia and West Africa (Fakir *et al*., 2014). *Vitex agnus castus* has been used as a medicine for women since ancient times. It has also been used for the treatment of acne, catarrh, cholera, diarrhea, ear diseases, fever, headache, heart diseases, hemorrhoids, liver diseases, malaria, nausea, rheumatism, skin diseases and ulcer (Eryiğit *et al*., 2015). In addition, the fruits were formerly used as a substitute for pepper from Italy to Eastern Georgia, a use which is still reflected in the local culture, as some Italian synonyms of the plant ('albero del pepe', 'pepe falso') (Stojkovic´*et al*., 2011).

Thanks to improvements in molecular biology techniques, many highly beneficial DNA markers were developed in order to detect genetic polymorphism. One the most frequently used techniques to develop DNA markers has been known as the Randomly Amplified Polimorphic DNA (RAPD) technique which is based on Polymerase Chain Reaction (PCR) (Bardakçı, 2001). RAPD technique was first described by Williams *et al.* (1990). RAPD-PCR technique is easy to use and features random primers with a length of 9-10 bases (Sesli and Yegenoglu, 2017). This technique provides genetic markers which have been used extensively in many different applications and in different plant species because of its simplicity (Hussein *et al*., 2005).

The aim of this study was to performe a molecular characterization using RAPD markers for some *Vitex agnus-castus* populations grown in the Aydın region of Turkey.

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Materials and Methods

Plant samples and DNA isolations

Leaf samples of *Vitex agnus-castus* populations to be used in this study were collected from Central, Koçarlı, Çakmar, Çine, Germencik, İncirliova, and Köşk districts of Aydın. Flower colors of the collected samples were classified as white, purple, and pink. Collected leaf samples were brought to the laboratory and prepared for genomic DNA isolation. *Vitex-agnus-castus* plant is rich in secondary compounds and volatile oils, and it is hard to acquire pure genomic DNA from these species because these compounds they contain prevent both the acquisition of pure DNA and successful PCR amplification. In order to avoid these disadvantages, genomic DNA isolation was carried out by using a commercial kit (GeneMark) as a preferred genomic DNA isolation method.

PCR amplifications

RAPD primers chosen for PCR amplifications are given in Table 1. Ready mixes were used as an alternative to PCR reaction. Amplification will be carried out by adding 2 µL genomic DNA (1/5 diluted), 1 µL primers, 5 µL master mix (PCR buffer, $MgCl_2$, dNTP, Taq DNA polymerase) and 17 µL dH_2O into the PCR tube. The PCR programs were separately created for each primer based on temperature TM values of the primers which were used in previous studies (Table 2). PCR products were analysed by electrophoresis on 1% agarose gel and the amplified products were detected after staining by ethidium bromide. Because of RAPD-PCR some of the gel photos were shown in Fig. 1 and Fig. 2.

Data and phylogenetic analysis

After PCR analyses, "1" was given if there were DNA bands on the gels and "0" was given if there were no bands; DNA bands were scored in this way, and RAPD analyses were carried out on polymorphic bands by applying monomorphic bands. Genetic relations of local *Vitex agnus-castus* samples which were used in the research were analyzed by using PAUP 4.0b10 program (Swofford, 2002); UPGMA phylogenetic tree of the same program was drawn based on arithmetic means of family trees, and genetic distance matrix between populations was created.

Results and Discussion

Because of RAPD-PCR analysis, 36 characters were acquired in total. 15 of these characters were stable, 8 characters did not give information in terms of parsimony, and 13 characters gave information in terms of parsimony. Genetic distance matrix between UPGMA tree and *Vitex agnus-castus* populations were created by using PAUP 4.0b10 phylogenetic analysis program. UPGMA tree consisted of 4 groups. Group 1 consisted of two sub-groups in itself. Subgroup 1 consisted of Çine purple flower and Koçarlı white flower populations; and sub-group 2 consisted of Çakmar white flower, Germencik purple flower, Erbeyli white flower populations. Group 2 consisted of two subgroups in itself. Sub-group 1 consisted of Çine white flower, Aydın purple flower, and Aydın white flower. Populations gathered from Aydin within this group have emerged in one branch. Subgroup 2 consisted of Çine pink flower, Çakmar purple flower and Çakmar pink flower. Populations of Çakmar within this group have emerged in one branch. Group 3 consisted of only İncirliova purple flower; and group 4 consisted of only Köşk purple flower (Fig. 3). According to PAUP analysis, the closest genetic distances were between Çine pink flower and Çakmar purple flower, and Çakmar pink flower and Çakmar purple flower populations with a value of 0.05556; and the greatest genetic distance was between Çakmar pink flower and Köşk purple flower populations with a value of 0.36111 (Table 3). RAPD markers can be used to detect genetic variability of plants, to study population genetics, systematic analyses, and genetic relationships (Aydın, 2004; Nayak *et al*., 2003).

Table 1. Primers used in the RAPD-PCR reactions and their Tm degrees

Primers	DNA Sequences (5'-3')	Tm
OPA-15	5'-TTCCGAACCC-3'	32 °C
OPA-20	5'-GTTGCGATCC-3'	32 °C
OPA-02	5'- TGCCGAGCTG-3'	34 °C
OPA-13	5'- CAGCACCCAC-3'	34 °C
OPA-16	5'-AGCCAGCGAA-3'	32 °C
OPA-18	5'-AGGTGACCGT-3'	32 °C
OPA-05	5'-AGGGGTCTTG-3'	32 °C

Table 2. Cycles program for RAPD-PCR reactions

Step	Heat/Time	Cycles
1. step	94 °C /2 min	1 Cycle
2. step	94 °C /1 min.	35 Cycles
3. step	32-34 °C /1 min	35 Cycles
4. step	72 °C /1 min	35 Cycles
5. step	72 °C /10 min.	1 Cycle

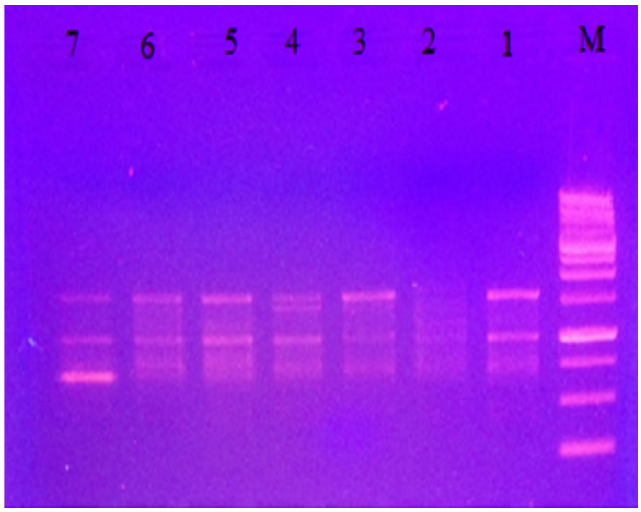

Fig. 1. Gel image of RAPD-PCR bands amplified with OPA-20

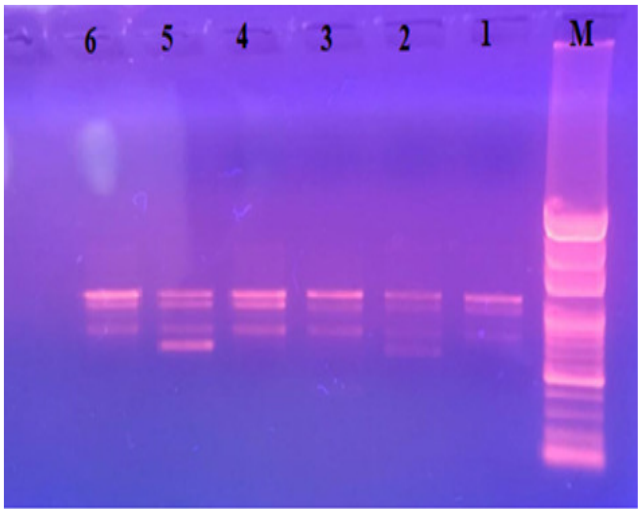

Fig. 2. Gel image of RAPD-PCR bands amplified with OPA-05

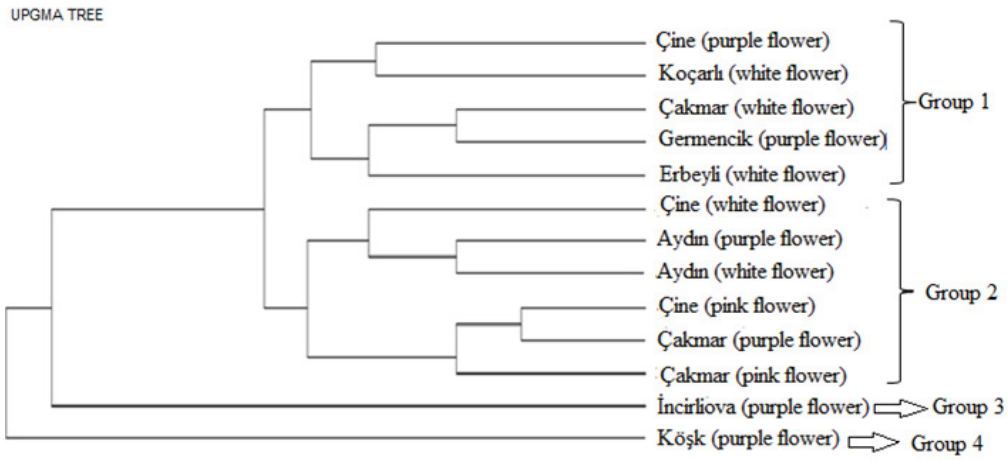

Fig. 3. The UPGMA tree generated using RAPD data of *Vitex agnus-castus* populations

Table 3. Pairwise genetic distance matrix obtained from PCR with RAPD primers

Populations	1	2	3	4	5	6	7	8	9	10	11	12	13
Çine purple flower	-	0.22222	0.11111	0.16667	0.19444	0.11111	0.11111	0.11111	0.30556	0.13889	0.22222	0.30556	0.12121
Çine White flower	8	-	0.16667	0.11111	0.13889	0.11111	0.11111	0.22222	0.30556	0.19444	0.22222	0.30556	0.30303
Çine pink flower	4	6	-	0.11111	0.19444	0.16667	0.05556	0.16667	0.30556	0.13889	0.22222	0.30556	0.18182
Çakmar pink flower	6	4	4	-	0.19444	0.16667	0.05556	0.16667	0.30556	0.19444	0.11111	0.36111	0.21212
Aydın purple flower	7	5	7	7	-	0.08333	0.13889	0.13889	0.27778	0.11111	0.19444	0.27778	0.21212
Aydın white flower	4	4	6	6	3	-	0.11111	0.11111	0.30556	0.13889	0.22222	0.25000	0.18182
Çakmar purple flower	4	4	2	2	5	4	-	0.11111	0.30556	0.13889	0.16667	0.30556	0.18182
Çakmar White flower	4	8	6	6	5	4	4	-	0.25000	0.08333	0.11111	0.19444	0.12121
İncirliova purple flower	11	11	11	11	10	11	11	9	-	0.16667	0.19444	0.27778	0.21212
Germencik purple flower	5	7	5	7	4	5	5	3	6	-	0.13889	0.22222	0.15152
Erbeyli white flower	8	8	8	4	7	8	6	4	7	5	-	0.30556	0.15152
Köşk purple flower	11	11	11	13	10	9	11	7	10	8	11	-	0.33333
Koçarlı white flower	4	10	6	7	7	6	6	4	7	5	5	11	-

In the past studies using RAPD-PCR technique including *Desmodium* (Rahman *et al*., 2017), *Calendula* (Baciu *et al*., 2013) *Vigna mungo* (Arulbalachandran *et al*., 2010), *Velezia* (Poyraz *et al*., 2012), *Hordeum vulgare* (Olgun *et al*., 2015), *Prunus* (Casas *et al*., 1999), *Malus* (Botez *et al*., 2009), *Pyrus* (Monte-Corvo *et al*., 2000), *Olea europaea* ssp. *oleaster* (Sesli and Yegenoğlu, 2017), and *Brassica napus* (Özbek and Gıdık, 2013) were reported with respect to genetic diversity and phylogenetic analysis of many plant species and populations.

Conclusions

In conclusion, this study comprises several innovations for our country with its many aspects. There are hardly any studies in the literature which aim to detect molecular features of *Vitex agnus-castus* and its types which show distribution in Aydın area. From this point of view, detection of especially molecular features of *Vitex agnus-castus* genotypes grown in the region shall contribute to further studies in terms of science and economics. It will also provide solutions to problems by revealing phylogenetic relations between chasteberry genotypes and genetic distances. In addition to this, it has been aimed to evaluate the potential of *Vitex agnus-castus* genotypes grown in Aydın region to detect and identify quality features, to make contributions to chasteberry cultivation, and to preserve gene resources.

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Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

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