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Revision of *Crataegus monogyna* Jacq.(Rosaceae) populations using RAPD marker in Iran

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Abstract

Crataegus monogyna, belongs to Rosaceae family and known as common hawthorn or single-seeded hawthorn. *Crataegus monogyna* is often divided into several infraspecefic taxa and or two or more species. Two varieties of this species is represented in Iran, *Crataegus monogyna* var. *monogyna* from northwest and *Crataegus monogyna* var. *lasiocarpa* from Hyrcanian region. In this survey 10 populations of *C. monogyna* have been studied using RAPD marker for classification of taxonomic relationship. Clustering analysis of RAPD data showed that a population is very differ from others and so It has long shoot thorn (about 4 cm cv. Type species with up to 2.5 cm) . It is reported as a new variety " *Crataegus monogyna* var. *mazandaranica* "for the first time from Ira

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Abbreviations:b 1,b 3, b4, b 5, b 6, b 7 and b 10= *C. monogyna* var.*lasiocarpa*
b 8 and b 9= *C. monogyna* var. *monogyna* , b 2= *C. monogyna* (new taxon)

Introduction

DNA markers are divided into four classes: 1) hybridization-based, 2) PCR-based, 3) PCR followed by hybridization, and 4) DNA sequence based (Angaji, 2011; Kole, 2013).

RAPD stands for 'Random Amplified Polymorphic DNA'. It is a type of PCR reaction, Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR)has been one of the most commonly used molecular techniques to develop DNA markers.

RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. Low expense efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate.(Bardakci,2000).

Crataegus monogyna, known as common hawthorn or single-seeded hawthorn. Other common names include May, Mayblossom, Maythorn, Quickthorn, Whitethorn, Motherdie, and Haw. Shrub or tree up to 10 m tall. Twigs glabrous or villous; thorns up to 2.4 cm long, more or less stout. Phenology is February to June and fruiting from June to October. Its distribution is from Europe to west Asia and north Africa.(Christensen,1992)It has been introduced in many other parts of the world where it can be an invasive weed.*Crataegus monogyna* is often divided into several infraspecefic taxa and or two or more species.It is known to hybridize with many other *Crataegus* species and it is evident that *C. monogyna* represented a good example of so-called 'compilo-species".

According to the monograph of *Crataegus*, Christensen believed that two varieties of this species is represented in Iran, *Crataegus monogyna* var. *monogyna* from northwest and *Crataegus monogyna* var. *lasiocarpa* from Hyrcanian region.(Christensen, 1992) In flora of Iran and flora Iranica *C. monogyna* is synonymous with *C. microphylla* Koch. (Khatamsaz, 1991; Reidle, 1969)In this survey 10 populations of *C. monogyna* have been studied using RAPD marker for clarifying taxonomic relationship.

Material and method

Preparing plant specimens

Plant material of 10 populations were collected from different localities that are desposited in herbarium of Islamic Azad University North Tehran Branch(IAUNT) shown in table 1.

DNA extraction

The total DNA was extracted from young leaves by a method proposed by Shayan Method using MBST kit (Shayan *et al.* 2007).

The MBST DNA extraction kit contains sufficient reagents for 50 µl DNA preparations and components: Lysis buffer, Binding buffer, Proteinase K, Wash buffer, Elotion buffer(Elotion buffer consists of 10 mM Tris-HCL pH 7.4 and 1 mM EDTA pH 8.0) and MBST –column.

For extraction, briefly, the leaves were first hemogenized in 300 µl lysis buffer and the proteins were degraded with 20 µl Proteinase K for 45 min at 60°c. After addition of 580µl binding buffer and incubation for 15 min at 70°c, 440 µl ethanol (%100) was added to the solution.

The complete volume was transferred to the MBST-column. The MBST-column was first centrifuged and washed twice with 500 µl Wash buffer. Finally, DNA was eluted from the carrier with Elution buffer. The quality of DNA was determined using agarose gel (1%) electrophoresis.

RAPD amplification

Amplification of RAPD fragments was performed according to Jayoti method.

A total of 4 different random 10-mer primers were used for RAPD analysis. The sequences of the selected primers are presented in Table 2. Polymerase chain reaction (PCR) was conducted with 50 µl reactions containing 6 µl genomic DNA, 10 µl primer, 9 µl H2O and 25 µl Ampliqon Master Mix (including Tris-HCL pH 8.5, (NH4)2SO4, 2 mM MgCl2, 0.2% tween 20, 0.4 mM dNTPs, 0.2 units/µl Ampliqon Tag DNA polymerase). The PCR reactions was carried out in a thermo cycler (Perkin Elmer, Massachusetts, USA) with following conditions, i.e. denaturation at 97°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, primer annealing at 52°C for 1 min and primer extension at 72°C for 2 min. A final extension step was carried at 72 °C for 4 min. (Jayoti *et al*,2010).

Detection of PCR products

The amplified products were detected using agarose gel electrophoresis (1% gel in 1X TAE buffer), stained with ethidium bromide and visualized with UV transilluminator and photographed using gel documentation system.

Data analysis

The RAPD bands were scored as "1" for presence and "0" for absence across all *Crataegus* populations for each primer. The data was analyzed for genetic diversity with the help software SPSS version 20 and genetic distances were calculated on the basis of average linkage method Coeffic. (fig.1).

Results

Cluster analysis of RAPD data showed that in phenoline 25 the populations of *C. monogyna* is divided into two main clusters, population b2 is a distinct taxon from other populations, this taxon has long thorn (about 4 cm) and so it is very different from genetic markert point of view. It is reported as a new variety by the name of *Crataegus monogyna* var. *mazandaranica* for the first time from Iran.(Fig.1 and fig.2).

Table 1. Localities of 10 populations of *Crataegus monogyna*.

Taxon	Locality
b1	Tehran: City park, 1250m, Zehzad 12212 – IAUNT
b2	Mazandaran: Chalus road , vali Abad, 1775 m,Sharifnia & Seyedipour 12213 - IAUNT
b3	Qazvin : Taleghan road , above of Taleghan dam , 1650 m , Sharifnia & Seyedipour12214 - IAUNT
b4	Qazvin : Ghaziklayeh village, 1912m , Sharifnia & Seyedipour 12215 IAUNT
b5	Qazvin : Samgh Abad village, 1610 m , Sharifnia & Seyedipour 12216 IAUNT
b6	Ghazvin : Taleghan road , Khuznan village , 1600 m , Sharifnia & Seyedipour 12217 IAUNT
b7	Qazvin : Ghaziklayeh village , 1620 m , Sharifnia &Seyedipour 12218 IAUNT
b8	W.Azerbaijan : Ghasmloo valley 1400m, Sharifnia & Seyedipour 12219 IAUNT
b9	W.Azerbaijan :16 km Piranshahr to Sardasht , 1400 m , Sharifnia & Seyedipour 12220 IAUNT
b10	Mazandaran :70 km S. of Amol, Larijan village, 2300m, Sharifnia & Seyedipour 12221 IAUNT

In flora of Iran *C. monogyna* is synonymous with *C. microphylla* but Christensen and Sharifnia and her colleagues are accepted *C. monogyna* for flora of Iran.

(Khatamsaz,1991; Christensen,1992; Christensen & Zeilinsky, 2008 and Sharifnia *et al* 2013) The populations b8 and b9 from northwest of Iran belong to *C. monogyna* var.*monogyna* and other populations(i.e. b1, b3, b4, b5, b6, b7 and b10) belong to *C. monogyna* var. *lasicarpa*.(Fig. 1).

A new key to the Iranian *Crataegus monogyna* varieties.

1-Thorns of shoot up to 4 cm leaves often small.....*C. monogyna* var. *mazandaranica* Sharifnia& Seyedipour.

1-Thorns of shoot up to 2.5 cm..... 2.

2- leaf blades villous in vein axil and major veins .....*C. monogyna* var.*monogyna*

2- leaf blades more and less densely volous.....*C. monogyna* var.*lasiocarpa*.

Table 2. The sequence of four primers.

RAPD Primers:		GC content(%)
OPB-10	5'-CTGCTGGGAC-3'	70
OPE-08	5'-TCACCACGGT-3'	60
OPM-17	5'-TCAGTCCGGG-3'	70
OPO-15	5'-TGGCGTCCTT-3'	60

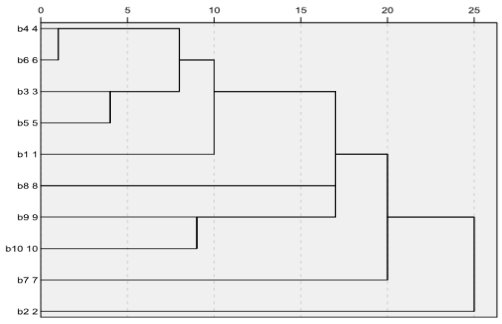

Fig. 1. Phenogram of Crataegus monogyna populations using RAPD bands. Abbreviation of populations are the same as table 1.

Fig . 2. *Crataegus monogyna* var.*mazandaranica*

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