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Assessing Genetic Variation of Dog Rose (*Rosa Canina L.*) in Caspian Climate

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Dog rose is one of the important rose species in Iran that the distant past had been considered due to nutritional value and medicinal. Despite its long history of use, due to poor information on the genetic modification of plants has been done resources inheritance. In this study was to assess the genetic diversity. Total of 30 genotypes Dog rose from areas of northern Iran in the Caspian region (provinces of Guilan and Mazandaran) were evaluated using 25 RAPD primers. The number of bands produced total of 202 and for each primer were measured in a bands with an average 8-band .The number of polymorphic bands per primer ranged from 1 to 13 and the bands were in the range of 300 to 3000 bp. Based on the results OPA-04 primer with 13 bands and PRA-1, E-09 and A-04 with 5-band were created maximum and minimum number of amplified fragments. Molecular marker genotypes showed a high degree of polymorphism. Studied genotypes based on RAPD results were divided into 2 groups and 2 subgroups. Most similar in subgroups A2 and B group was the lowest.

Key word: *Rosa canina spp*., RAPD marker, Genetic Variation, Caspian climate.

The genus *Rosa* L., one of the most widespread members of the Rosaceae family, is widely scattered in Eurasia, North America, and Northwest Africa (Davis 1972). *Rosa canina* (known as the dog rose) is a variable scaling wild rose species native to Europe, northwest Africa and western Asia (Lim *et al*.,2005). This plant is native of north Alborz Mountain in Iran that the wild shrub and perennials grows in dry areas, on rocky plains and in the pastures (Omidbeigy, R. 2001). It is a deciduous shrub normally ranging in height from 1–5 m, though sometimes it can scramble higher into the crowns of taller trees. The colors of the petals change from white to pale pink, rarely deep pink. The stems of the species are covered with sharp, small, hooked prickles. The leaves are pinnate, with 5–7 leaflets (Ercisli 2005). Its' fruit attracts and is eaten by birds and mammals. The plant includes high content of certain antioxidants. *R. canina* is also important for conservation of the surface soil and controlling the soil erosion due to its wide spreading and intensive root system and wide crown structure (Ozkan and Bilir 2008).

In some European countries released drugs such as viroma and diviroma in the drug market. Ingredients of this fruit plant to reduce uric acid (Omidbeigy, 2001, Zargari, 1996, Klasterska, 1974). Rosehips have been used for medicinal purposes, and in Iran we have been making rosehip soup for more than one hundred years (Omidbeigy, R. 2001). Dog rose (Rosa sp.) that is grown naturally in many parts of Iran is a plant of high economical value in food and drug

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industry and also have many other fields of usage. Recent molecular dog rose research is assisting traditional morphological and cytological studies by genomic analyses. Direct DNA-based diagnostic assays are informative because the markers are phenotypically impartial and not influenced by environmental effects. The molecular approach opens possibilities for marker assisted selection in the introgression of species derived genes into the cultivated germplasm base (Debener *et al*., 1998, 1999; Rajapakse *et al*., 2001, Yan *et al*. 2005). These methods were used to solve problems related with diverse identification (Hubbard *et al*., 1992 with RFLPs; Torres *et al*., 1993 with RAPDs; Kim and Byrne, 1996, with isozymes), and they are currently used in taxonomy and evolutionary researches. DNA polymorphism suggests direct observations of the plant genotype. One of the methods of performance used for this purpose is Random Amplified Polymorphic DNA (RAPD) (Manners *et al*., 2004). RAPD analysis has been used for recognition of varieties (Mohapatra and Rout, 2005), phylogenetic connection (Nair *et al*., 1999) , preservation and management of genetic resources (Bretting and Widrelechner, 1995). This technique has also been found functional for determining genetic divercity (Matsumoto and Fukui, 1996; Fredrick *et al*., 2002). Evaluate resulted by RAPD are very alike to those of other methods (AFLP and ISSR) and may be directly able to be compared (Nybom, 2004). RAPDs have been extensively applied to study taxa of the Rosaceae. These markers demonstrated to be a powerful procedure for the characterization of genetic deviation in fragrant roses (Prasad *et al*., 2006; Tabaei *et al*., 2006; Kiani *et al*., 2007) and *Rosa canina* (Jurgens *et al*., 2007). Despite their commercial significance, little information is available about the inheritance of important agronomic characters. Genetic diversity has been estimated in rose using AFLP (Baydar *et al*., 2004; Pirseyedi *et al*., 2005) and microsatellite (Babaei *et al*., 2007) markers.

North Alborz Mountain located in Caspian climate at north of Iran that is one of the most important dog rose location growths. In this respect it is vital to make selection studies from the naturally grown dog rose populations. With the purpose of facilitate selection researches, in joining with morphological studies Polymerase Chain Reaction (PCR) based molecular markers are widely used at molecular level. The progression of PCR based molecular marker methods has led to increase the use of molecular marker technologies for crop improvement and facilitate selection researches. In this respect Randomly Aamplified Polymorphic DNA (RAPD) method has drawn much attention in a wide variety of organisms. The aim of this research was to discover distant cultivars to be used in an ongoing *Rosa Canina L*. hybridisation programme in the North Alborz Mountain located in Caspian climate at north of Iran.

MATERIALS AND METHODS

Plants and growth conditions

Plant materials of *Rosa canina* were collected at September-October of 2011 from three sites and ten altitude (totally 30 samples) in north of Iran (Mazandaran Province). Rainfall and temperature of contour maps showed that the average rainfall and temperatures are in the same (Fig.1-Table 1). Temperatures ranged from 20°C to 25°C. Leaves samples were placed in ice during transport to the laboratory of Islamic Azad University Tonekabon Branch (IAUTB). Reference specimens were placed in the (IAUTB) herbarium.

DNA extraction and RAPD analysis

Fresh leaves collected of mature plants was frozen in liquid nitrogen and stored at –80°C. DNA was extracted using the method described by Khan *et al*. (2004). To reduce the effects of secondary metabolites in the process of extracting and prevent of DNA degradation and subsequent inhibition of the PCR reaction, we used of the protocol reported by Cheng *et al*. (1997). Co-precipitated RNA was removed, adding 0.5 units of RNAse per sample. DNA extracted was dissolved in TE, for quality assessment and verified spectrophotometerically after dilution of 12.5 ng/ìl in ddH_2O.

Twenty five primers 10 bases in length (GENEray biotechnology Co.) were chosen (Table 2). The selection was made from a pool of primers that gave strong and consistent amplification. Polymerase chain reaction (PCR) was performed using thermal cycler (Bio RAD, MyCycler) and was carried out in 15 ìL reactions containing 4 ìL of plant genomic DNA, 7.5 ìL Ready to use master

mix (Cinnagen Co., Tehran, Iran), 1 µL (with concentration0.5 ìM) of primer and 2.5 µL ddH_2O. Conditions applied were as follows: one cycle of 95°C for 5 min, 40 cycles of 1 min at 94°C, 2 min at 34°C and 2 min at 72°C. Cycling was concluded with a final extension at 72°C for 10 min. PCR amplification products were electrophoresed in 1.5% agarose gels. DNA was stained with ethidium bromide and photographed under UV light in a gel documentation system (UVIdoc, UK).

Data analysis

All the samples were scored for presence or absence of RAPD fragments with UVIsoft (version 12.6), and the data were entered into a binary data matrix as discrete variables ("1" for presence and "0" for absence of a homologous band) with Excel (version 2003). Jaccard's coefficient of similarity was calculated with Popgen software (version 1.44), and the species were grouped by cluster analysis using based on Nei's unweighted pair-group of Arithmetic Means Averages (UPGMA) method with SPSS (version 13).

RESULTS AND DISCUSSION

Through of RAPD primer bank, 25 primers were suitable for analysis that were entered in analysis and were created in total 202 different bands for all genotypes. Mean a band for each primer was 8- band. Selective primers resulted in 202 bands were scored, among which 77 bands (36.44%) were polymorphic.The number of polymorphic bands per primer ranged from 1 to 13 and the bands were in the range of 300 to 3000 bp (Fig. 2). Amplified bands are presented in the Table 2. Based on the results OPA-04 primer with 13 bands and PRA-1, E-09 and A-04 with 5-band were created maximum and minimum number of amplified fragments. The lowest frequency among the polymorphic locus was 0.027 in locus of A-07-3 and OPO-18-5 were observed and the OPA-04-11 showed the highest frequency rate of 0.66. Mean of Amplify Frequency by RAPD Primers are shown in Fig 3. This value for each set of primers were by the mean of amplification frequency all of locus those related to primer.

Polymorphism information content (PIC) in the total population of 0.21131 in primer P-443 to 0.41876 for OPAA-10 was varied with the mean equal 0.32651for all primers. Nei Gene diversity (H) is one of the important indicators in determining allele diversity. H values for each primer obtained by averaging the H values are calculated for all primers. Value of H estimated in each primer with the PIC matches, so that the primer OPAA-10 has the highest amount of PIC and equal to H (0.41731), in contrast, primer P-443 which has the lowest PIC, the lowest value H (0.21052) are also indicated. Cluster analysis was performed using UPGMA and Jaccard's similarity coefficient was calculated, with the highest correlation Cophenetic coefficient (0.902). Dendrogram (Fig. 4) obtained from RAPD primers been cutting at 0.68 similarity coefficient and genotype were grouped in two main groups A and B.

A group has two sub-groups of A1 and A2 were the most common genotype in subgroup A2. Group A1 consisted of Dohezar (altitudes 816, 981 and 1259 m), Janet Rudbar (altitude 1534 m) and Javaherdeh (altitudes 1416, 1427 and 1644 m) was. A2 group was included of Dohezar (altitudes

Table 1. *Rosa canina* genotypes and their locations used in RAPD analysis

Genotype-Altitude	No.	Genotype-Altitude	No.	Genotype-Altitude	No.
Dohezar -816 m	G1	Jennatroudbar-1481 m	G11	Javaherdeh-1317 m	G21
Dohezar -935 m	G2	Jennatroudbar-1518 m	G12	Javaherdeh-1375 m	G22
Dohezar -945 m	G3	Jennatroudbar-1534 m	G13	Javaherdeh -1416 m	G23
Dohezar-957 m	G4	Jennatroudbar-1561 m	G14	Javaherdeh -1467 m	G24
Dohezar-996 m	G5	Jennatroudbar-1583 m	G15	Javaherdeh-1472 m	G25
Dohezar-981 m	G6	Jennatroudbar-1598 m	G16	Javaherdeh-1528 m	G26
Dohezar-1096 m	G7	Jennatroudbar-1603 m	G17	Javaherdeh-1533 m	G27
Dohezar-1182 m	G8	Jennatroudbar1607 m	G18	Javaherdeh-1601 m	G28
Dohezar-1193 m	G9	Jennatroudbar- 1623 m	G19	Javaherdeh-1627 m	G29
Dohezar-1259 m	G10	Jennatroudbar-1635 m	G20	Javaherdeh-1644 m	G30

935 ,945 ,957 and 1096 m), Janet Rudbar (altitudes 1418, 1518, 1561, 1598, 1603, 1607 and 1635 m) and Javaherdeh (the heights of 1317, 1375, 1467, 1528, 1601 and 1627 m). B group was the smallest groups that were in it of Dohezar (altitudes 996, 1182 and 1193 m) and Janet Rudbar (altitudes 1583 and 1623 m).

The low genetic diversity in Dog rose species is an issue of concern which may interfere further them improvement. In this research also, due to limitation of subspecies, genotypes in the dendrogram derived was less, despite, this result do not detracts the efficiency of RAPD markers. Because research conducted in the Rose family, as well has shown the ability this method to identify the subspecies (Emadpour *et al*., 2009, Kiani *et al*., 2007). Molecular markers, especially, RAPD markers have the potential for recognition of clusters and characterization of genetic variation within the species and cultivars. This method is also beneficial in *Rosa canina L.* breeding programs and provides a major input into preservation biology. RAPD results have optimized conditions with the potential to be employed for phylogenetic relationships and taxonomic classification. Research results of some researchers in relation RAPD shown that domestication, selection and breeding have caused a decrease in genetic variation (S. Aras, 2003; McGrath *et al*., 1999; Fernandez *et al*., 2001). On the other hand this phenomenon could have a great impact on several aspects of plants response to different environmental conditions. Therefore, in order to avoid such problems in future, it seems necessary to estimate genetic diversity between Dog rose species and or cultivars. According to research results, now we know that plant improvement is based on the information about the genetic relationships among accessions and within and between species. This study reports results are in agreement with Jurgens *et al*. (2007) and Aras (2003).

Table 2. Sequences of random primers used to amplify *Rosa canina* spp. genomic DNA.

No.	Primers	Primer sequence RAPD	Amplified bands per primer	Polymorphic bands per primer	polymorphism	Tm(°C) %
1	OPAA-10	TGGTCGGGTG	12	9	75	31.52
2	OPA-04	AATCGGGCTG	13	7	54	30.91
3	OPAD-10	AAGAGGCCAG	12	6	50	24.24
4	BC-302	CGGCCCACGT	11	3	27	40.92
5	OPM-10	TCTGGCGCAC	8	4	50	32.77
6	OPV-10	GGACCTGCTG	7	2	29	24.51
7	PRA-1	GTGACGTAGG	5	1	25	13.82
8	OPZ-10	CCGACAAACC	9	3	33	25.75
9	P-166	GTGACGGACT	10	5	50	16.83
10	B-12	CCTTGACGCA	6	2	33	27.94
11	P-232	CCGCTTGTTG	10	2	20	28.4
12	E-09	CTTCACCCGA	5	2	40	25.95
13	A-04	AATCGGGCTG	5	1	20	30.91
14	A-07	GAAACGGGTG	6	1	17	25.75
15	OP-B12	CCTTGACGCA	7	2	28	27.94
16	OP-C11	AAAGCTGCGG	8	4	50	32.28
17	OP-C19	GTTGCCAGCC	6	3	50	30.93
18	OP-D20	ACCCGGTCAC	9	3	33	28.38
19	OP-Q11	TCTCCGCAAC	8	2	25	26.24
20	A-12	TCGGCGATAG	6	2	33	28.17
21	B-389	CGCCCGCAGT	9	4	44	41.64
22	P-443	GCCGTGATAG	6	2	33	21.3
23	F-12	ACGGTACCAG	12	5	42	19.65
24	OPO-03	CTGTTGCTAC	6	2	33	9.45
25	OPO-18	CTCGCTATCC	6	1	17	20.48

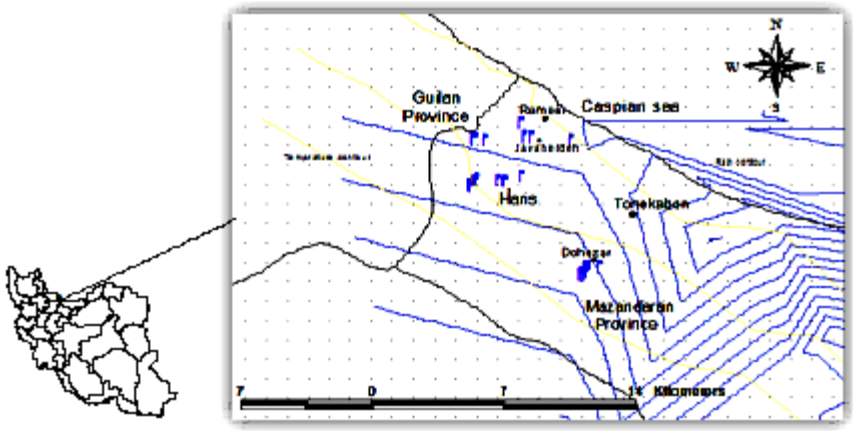

Fig. 1. *Rosa canina L.* collected samples from north of Iran

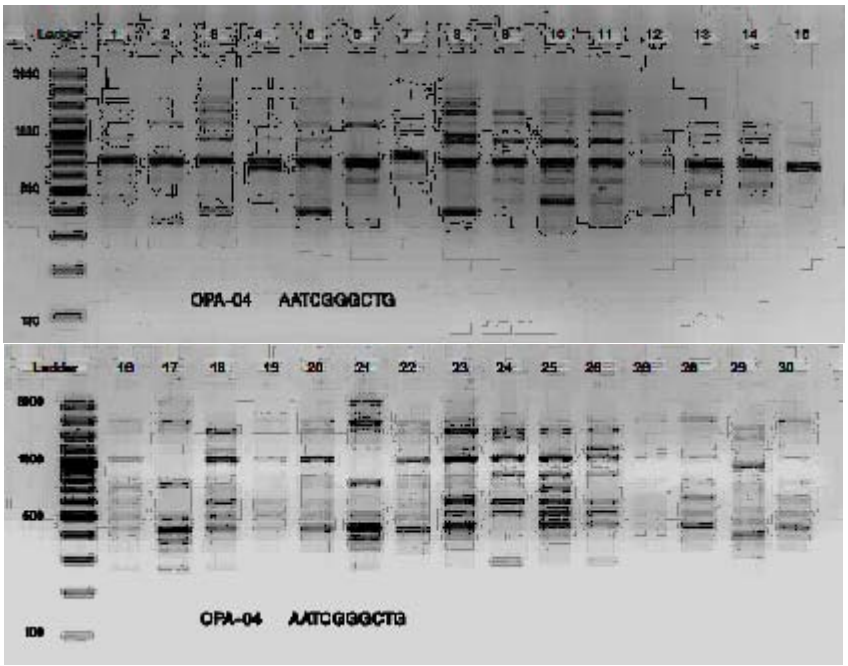

Fig. 2. Banding pattern of amplified genomic DNA for *Rosa canina spp.* with using OPA-04 RAPD primer

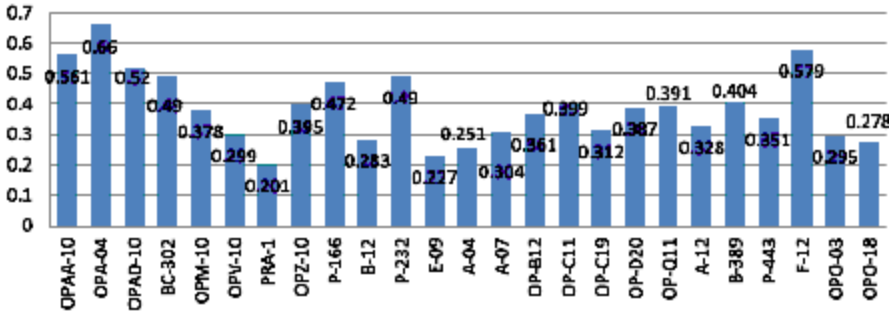

Fig. 3. Mean of Amplify Frequency by RAPD Primers

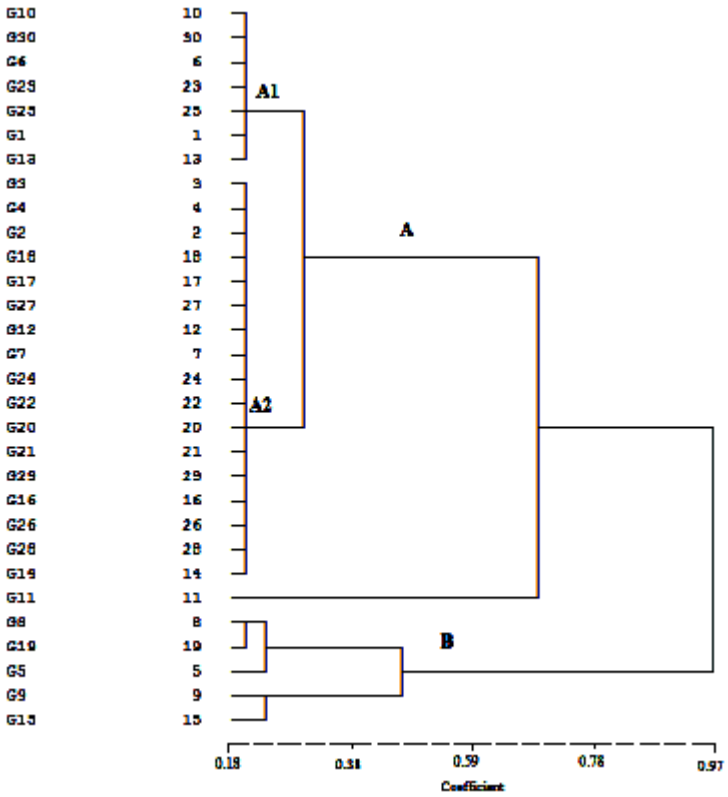

Fig. 4. Dendrogram obtained by cluster analysis based on presence/absence matrix. The numbers on the left side correspond to different genotypes

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