International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 6 (5): 429-436, 2011
ISSN 1816-4897 / DOI: 10.3923/ijar.2011.429.436

Genetic Diversity and Qualitative Variation of *Rosa damascena* in Syria

[1]T. Alsemaan, [1]N. Albatal, [2]H. Baydar and [1]K. Almaarri

[1]Department of Horticultural Science, Faculty of Agriculture, Damascus University, P.O. Box 30621, Damascus, Syria

[2]Department of Horticultural Science, Faculty of Agriculture, Suleyman Demirel University, P.O. Box 42737, Isparta, Turkey

Corresponding Author: Khalil Almaarri, Department of Horticultural Science, Faculty of Agriculture, Damascus University, P.O. Box 30621, Damascus, Syria Tel: 00963933489702

ABSTRACT

This study aimed at investigating genetic diversity and qualitative variation within Syrian accessions of *Rosa damascene* in order to determine the best oil bearing one for commercial production. The experiments were conducted at Suleyman Demirel University in Turkey and Damascus University in Syria. Microsatellite technique was used to analyze the genetic diversity of seven accessions of *R. damascena* collected across major and minor rose oil production areas in Syria and one accession Control collected from Isparta province in Turkey. The microsatellite DNA allele counting-peak ratios method (MAC-PR) was used. The accessions were clustered using the un-weighted pair group method for arithmetic averages (UPGMA) by the statistical program marked as Popgene 1.31. Gas chromatography/mass spectrometry (GC/MS) Analysis of Rose oil distilled from each accession was used to compare oil quality within genotypes. The analysis results were statistically analyzed by the program marked as SPSS. Six different genotypes have been obtained from *Rosa damascena* accessions collected from Syria. Two accesions, Almarah1 and Bab Alnayrab, were identical to the Turkish gynotype. GC/MS analysis identified the main components of oil such as: Geraniol (28-31%), Citronellol (26-30%), Nerol (12-14%), Germacrene-D (6-8%), Nonadecane (4-6%) and Linalool (1-3%). In addition, many trace compounds were detected such as: Eicosane, Eugenol, Citral, Hexadecane and Rose oxide. This study showed for the first time the existence of genetic diversity within *Rosa damascena* cultivated in Syria. Almarah1 and Bab Alnayrab accessions are recommended to be used to broaden the production of rose oil.

Key words: *Rosa damascene*, accession, genotype, qualitative variation, rose oil

INTRODUCTION

Rosa damascena Mill. is the most important rose species for rose oil production (Moein *et al*., 2010). In recent years, antioxidant, antibacterial and anticancer activities of *R. damascena* essential oil have been demonstrated (Shahriari *et al*., 2007; Rakhshandeh *et al*., 2008; Gholamhoseinian *et al*., 2008). As *Rosa damascena* was originally introduced from the Middle East into Western Europe, it is thought that its origin and diversity can be found in this region (Babaei *et al*., 2008). In Syria, cultivation and consumption of *Rosa damascena* has a long history especially in Kalamoon Mountains where the so-called "village of *Rosa damascena*" Almarah is located (Alsemaan *et al*., 2011). *Rosa damascena* has been commercially cultivated in two regions

in Syria which are Aleppo and Rural Damascus (Sulayman, 2010). In recent genetic diversity studies using RAPDs and SSRs with Bulgarian Damask roses (Rusanov *et al*., 2005) and AFLPs with Turkish Damask roses (Baydar *et al*., 2004), no variation was revealed among the Damask roses cultivated for oil in these two countries. Thus, all production material in Bulgaria and Turkey consists of only one oil bearing genotype (Babaei *et al*., 2007). Microsatellite analysis showed the existence of multiple genotypes within *Rosa damascena* in Iran (Babaei *et al*., 2007). During the last few decades, the use of molecular markers, revealing polymorphism at the DNA level, has been playing an increasing part in plant biotechnology and their genetics studies. There are different types of markers viz. morphological, biochemical and DNA based molecular markers. These DNA based markers are differentiates in two types first non PCR based (RFLP) and second is PCR based markers (Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphic DNA (AFLP, Simple Sequence Repeats OR Microsatellites (SSR), Simple Nucleotide Polymorphism (SNP) etc.), amongst others, the microsatellite DNA marker has been the most widely used, due to its easy use by simple PCR, followed by a denaturing gel electrophoresis for allele size determination and to the high degree of information provided by its large number of alleles per locus (Kumar *et al*., 2009). The strengths of microsatellites include the codominance of alleles, their high genomic abundance in eukaryotes and their random distribution throughout the genome, with preferential association in low-copy regions (Morgante *et al*., 2002). Because the technique is PCR-based, only low quantities of template DNA (10-100 ng per reaction) are required. Due to the use of long PCR primers, the reproducibility of microsatellites is high and analyses do not require high quality DNA. Although microsatellite analysis is, in principle, a single-locus technique, multiple microsatellites may be multiplexed during PCR or gel electrophoresis if the size ranges of the alleles of different loci do not overlap (Ghislain *et al*., 2004). This decreases significantly the analytical costs. Rose essential oil should be extracted from fresh flowers picked before 8 am in the morning, by steam distillation (Gunes, 2005). Rose essential oil is also called otto of rose and attar of rose (Morris, 2011). The chemical composition of rose oil is complex. It contains more than 300 known compounds. The main chemical components of rose oil can be listed as -citronellol, phenyl ethanol, geraniol, nerol, with traces of other components (Yousefi *et al*., 2009). Baydar *et al*. (2004) indicated that the qualitative variation among Turkish accessions could be caused by ecological conditions which resulted in mutations. In contrary (Babaei *et al*., 2007) expected that qualitative variation might be genetically explained. Sulayman (2010) found that different accessions of Rosa damascena showed different yields and different rose oil qualities. According to the big climatic differences of the areas in which accessions of *Rosa damascene* cultivated in Syria, this investigation was conducted to examine genetic diversity and qualitative variation within them.

MATERIALS AND METHODS

Eight accessions of *Rosa damascena* were examined. Seven of them were collected from commercial production fields in seven sites located in two regions of Syria's. They were (Bab Alnayrab) in Aleppo, (Almarah1, Almarah2, Rankoos, Ernah, Issal Alward and Mesraba) in Rural Damascus. The eighth accession "Control" was collected from the experimental field of Suleyman Demirel University in the province of Isparta in Turkey. Table 1 specifies each area mentioned above (Fig. 1). All accessions have been grown in the experimental field of faculty of agriculture at Damascus University since 2008. Eight robust microsatellite markers were used as linkage groups on the genetic map of rose to differentiate genotypes (Babaei *et al*., 2007). Primer pairs were amplified using the Qiagen PCR multiplex kit (India) after the extraction of DNA from

Table 1: Geographical origins of *Rosa damascena* accessions in Syria and Turkey

Site (Region), Country	Longitude	Latitude	Elevation (m)	Rainfall average (mm)
Almarah (Rural Damascus), Syria	36° 44' 00"	34° 01' 10"	1500	125-75
Ernah(Rural Damascus), Syria	35° 52' 39"	33° 21' 55"	1400	850-700
Bab Alnayrab (Aleppo), Syria	37° 15' 25"	36° 11' 18"	385	350-300
Issal Alward (Rural Damascus), Syria	36° 24' 35"	33° 51' 49"	1679	300-280
Mesraba (Rural Damascus), Syria	36° 24' 07"	33° 32' 51"	825	220-200
Rankoos (Rural Damascus), Syria	36° 23' 09"	33° 45' 28"	1607	650-350
Isparta (Turkey) "The Control"	37° 46' 00"	30° 33' 14"	950	600-500

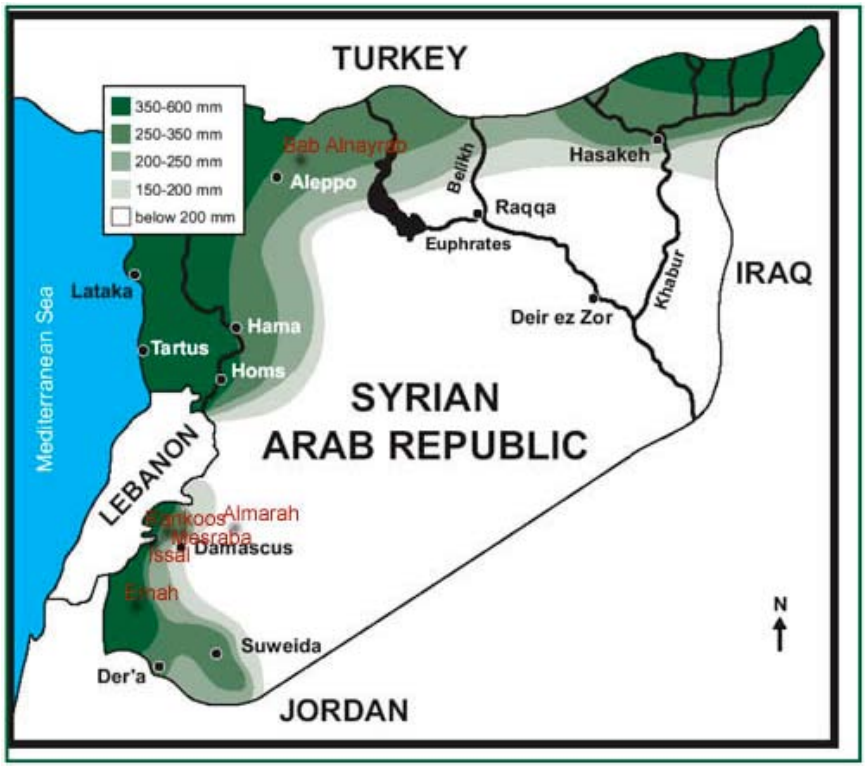

Fig. 1: Agro-ecological Zones in Syria

the young leaves (Baydar *et al*., 2004). Fluorescent amplification products were detected using an ABI Prism 3700 DNA Analyzer (Applied Biosystems) and all samples were genotyped in accordance with reference alleles for each locus as described by Babaei *et al*. (2007) using Genotyper 3.5 NT (Applied Biosystems). The microsatellite DNA allele counting-peak ratios method (MAC-PR) provided by Genotyper software which calculates ratios between the peak areas for each two alleles in which they occurred together was used. The accessions were clustered using the unweighted pair group method for arithmetic averages (UPGMA) by the statistical program marked as Popgene 1.31. Rose oil from each accession was obtained through Clevenger. Then, 20 µL of essential oil was analyzed using gas chromatography/mass spectrometry GC/MS in three replications following these parameters: injection temperature: 240°C, Flow: 10 psi, Ionization Mode: EI (70 eV), Oven Program: 60°C up to 240°C. Gas: Helium, Column: Cp W AX 52 CB m* 10.32 mm, 1.2 µm and Library: Wiley, Nist, Tutor. The analysis results were analyzed statistically using LSD

(Lowest Significant Difference) by the statistical program marked as SPSS. Duncan test was also used to compare mean averages. GC/MS software was used to draw the chromatograms for each studied accession (Fig. 3).

RESULTS AND DISCUSSION

Genetic diversity: In this investigation, six different genotypes have been obtained from *Rosa damascena* accessions collected from Syria by using microsatellite marker analysis. Two of them were identical to the gynotype collected from Turkey (province of Isparta), which supports. All markers detected polymorphisms among the accessions. For all studied accessions, the MAC-PR method showed the allelic configurations at six loci (RhE2b, RhD221, RhAB73, RhB303, RhAB40, RhP50) (Table 2). The dendrogram of Cluster analysis shows six different genotypes. The biggest group consists of the three accessions of (Bab Alnayrab, Almarah1 and Isparta) confirming that they are identical. The Genotypes used for rose oil production are genetically related and perhaps the same genotype is used in several countries such as Turkey, Bulgaria and Iran. That supports (Baydar *et al*., 2004; Rusanov *et al*., 2005; Babaei *et al*., 2007; Kiani *et al*., 2009). This genotype is being used for attar production in Syria (Rural Damascus and Aleppo) (Alsemaan *et al*., 2011). Despite All other genotypes identified in dendrogram represent different genotypes, a close genetic relation within them was found. Figure 2 shows that there is a close genetic relation (90%) between

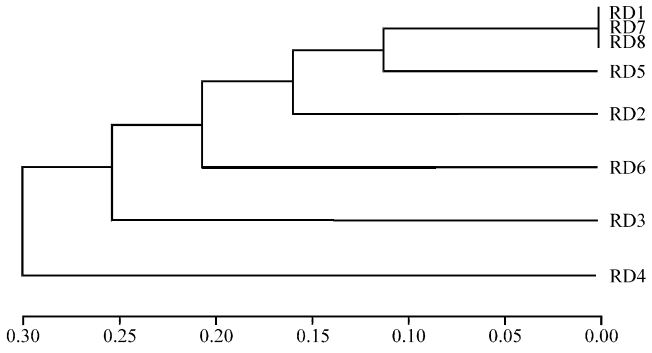

Fig. 2: Genetic relation tree using UPGMA (Popgene 1.31)

Table 2: Allelic configurations of MAC-PR analyses

		Markers					
		RhP50	RhAB40	RhB303	RhAB73	RhD221	RhE2b
		Linkage group					
Genotype	No. of accessions	3	4	Not determined	7	4	6
RD1 bab alnayrab							
RD7 almarah1	4	209 217 223 226	349 371 404 420	232 232 232 232	210 222 228 260	119 125 127 128	168 170 177 180
RD8 isparta "control"							
RD2 almarah2	1	211 217 217 223	349 363 371 396	232 232 232 241	210 213 213 228	127 128 128 130	168 168 180 189
RD3 ernah	1	211 211 223 223	326 354 396 396	219 219 232 232	213 213 228 228	119 125 127 129	180 180 189 189
RD4 mesraba	1	217 223 226 226	374 374 404 404	219 219 232 238	222 225 228 228	122 125 145 146	168 168 174 189
RD5Issal alward	1	209 211 223 223	343 371 396 404	219 232 232 232	210 213 222 228	119 125 127 127	168 177 180 189
RD6 rankoos	1	200 209 220 223	343 371 433 433	219 232 232 238	213 213 228 240	122 127 127 129	168 180 180 189

Table 3: Percentage of essential oil components for each investigated accession

Compound (Retention time)	RD1 Bab Alnayrab	RD2 Almarah2	RD3 Ernah	RD4 Mesraba	RD5 Issal Alward	RD6 Rankoos	RD7 Almarah1	RD8I sparta Control	LSD 99%
Rose oxide (25.6)	0.28	0.22	0.27	0.20	0.18	0.23	0.38	0.20	-
Linalool (37.6)	2.44a	2.02b	2.08b	2.01b	1.79b	1.98b	2.45a	2.44a	0.31
beta-Caryophyllene (41.6)	0.80	0.85	0.84	0.90	0.82	0.77	0.73	0.74	-
Citronellyl Acetate (45.0)	0.71b	0.92a	0.95a	0.7b	0.73b	0.72b	0.67b	0.70b	0.21
alpha-humulene/ -selinene 46.2)	0.42	0.35	0.35	0.38	0.41	0.35	0.35	0.35	-
Hexadecane (46.7)	0.10	0.14	0.15	0.15	0.16	0.16	0.11	0.12	-
Germacrene D/G-Muurolene (47.1)	7.48	7.72	7.67	7.05	8.00	7.82	6.95	6.92	-
Linalyl Propionate (47.6)	0.38	0.39	0.39	0.41	0.32	0.36	0.43	0.42	-
Citral (50.0)	0.53b	0.55b	0.73a	0.51b	0.47b	0.50b	0.55b	0.52b	0.18
Geranyl acetate (50.7)	2.27	2.42	2.30	2.31	2.40	2.28	2.09	2.08	-
Citronellol (51.2)	28.96a	28.02a	27.97a	28.05a	28.32a	29.02a	26.32b	26.30b	1.33
Nerol (53.4)	12.57b	12.42b	12.41b	12.75ab	12.59b	12.63b	13.31a	13.30a	0.46
Phenyl Ethyl Acetate(54.7)	0.81	0.85	0.91	0.87	0.94	0.80	0.90	0.85	-
Geraniol (56.0)	28.75b	28.77b	28.65b	28.74b	28.66b	28.36b	30.75a	30.76a	1.01
Nonadecane(58.5)	5.17a	5.40a	4.15b	4.04b	4.06b	4.15b	5.41a	5.42a	0.82
9-Nonadecene (59.1)	0.22	0.22	0.31	0.21	0.28	0.20	0.28	0.22	-
PEA (60.1)	2.18	2.11	2.12	2.17	1.92	2.10	2.14	2.13	-
Eicosane (63.9)	0.62bc	0.66b	0.67b	0.52bc	0.54bc	0.48c	0.88a	0.87a	0.19
Methyl Eugenol(65.4)	1.02	1.00	1.08	1.01	1.08	1.03	0.96	0.99	-
Heneicosane (69.2)	3.50	3.50	3.56	3.21	3.29	3.25	3.12	3.20	-
Eugenol (73.6)	0.48a	0.47a	0.46a	0.44a	0.48a	0.44a	0.33b	0.33b	0.06

- : No significant difference. Numbers followed with different letters are significantly different at 99% confidence

Issal Alward genotype and the accessions mentioned before. While Mesraba genotype showsed the furthest genetic relation to them (70%). That supports (Sulayman, 2010).

Qualitative variation: Table 3 shows that six components, representing 76-89% of the oil, were characterized. Geraniol (28-31%), Citronellol (26-30%), Nerol (12-14%), Germacrene-D (6-8%), Nonadecane (4-6%) and Linalool (1-3%), were found to be major constituents, which supports (Baydar *et al*., 2004; Loghmani-Khouzani *et al*., 2007) It also shows the qualitative variation within the accessions investgated (Fig. 3). No significant differences in their content of Heneicosane, Methyl Eugeno, PEA, 9-Nonadecene, Phenyl Ethyl Acetate, Geranyl acetat, Linalyl, Propionate, Germacrene D, Gamma-Muurolene, Rose oxide, beta-Caryophyllene, alpha-humulene, **β**-selinene and Hexadecane were found. In contrary, Almarah1, Bab Alnayrab and Isparta accessions were significantly superior to others in their content of Linalool, Nonadecane, Geraniol, Nerol and Eicosane, While Erna accession was significantly superior in its content of Citral and Citronellyl Acetate to all other accessions. The genetic relations found might explain the qualitative variation noticed within the accessions, which supports (Sulayman, 2010). But, the qualitative variation between Bab Alnayrab accession on one hand and Almarah1 and Isparta accessions on the other hand could not be explained genetically. Otherwise, agro-ecological variation should be considered. The high percentages of main components of Rose oil are considered as a positive quality parameter. Contradictory, another positive quality parameter is the low content of Eugenol and Citronellol which was noticed in Almarah1 and Isparta accessions. The high content of Eugenol in rose oil may make it inedible. Besides, as Citronellol percentage is higher, as the rose oil

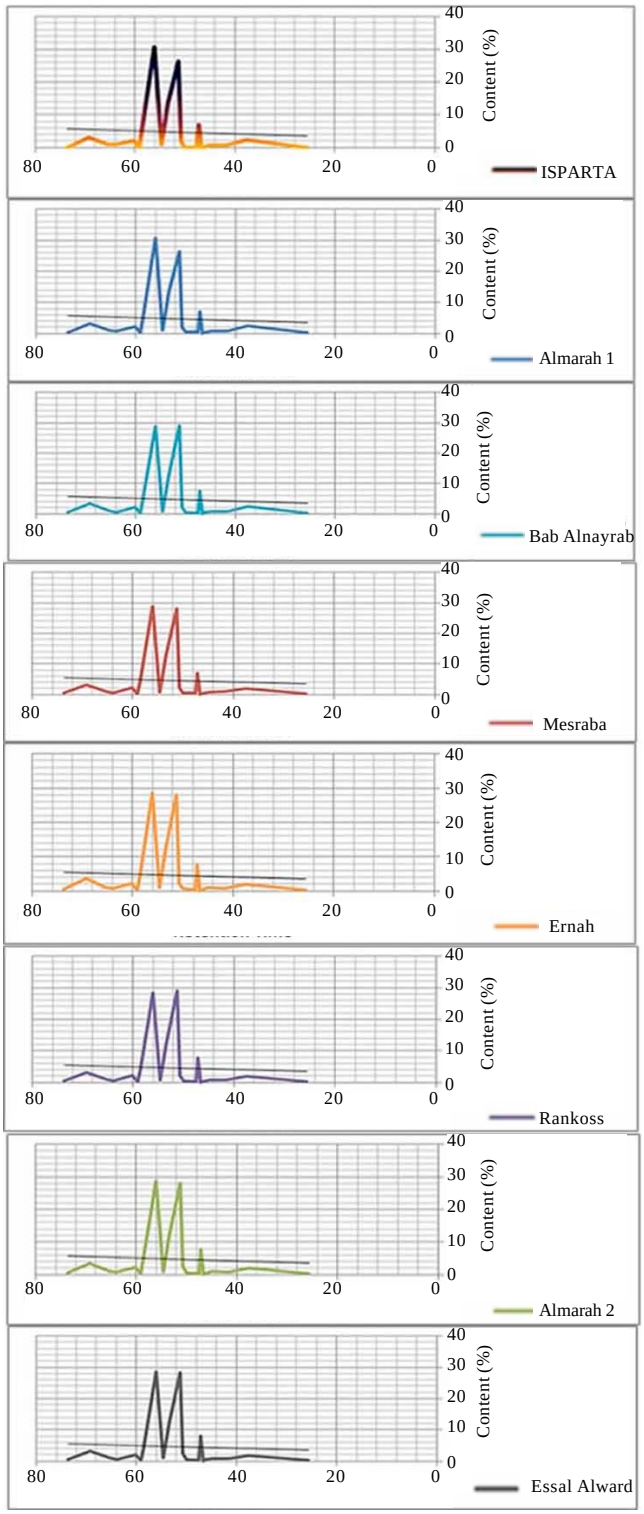

Fig. 3: Chromatograms of GC/MS analysis for all the accessions

decomposition is faster (Yousefi *et al*., 2009). It was remarkable that the content of Rose oxide was high in all the accessions, which could be attributed to either insufficient distillation machines or the delayed flower harvest (Moein, 2010).

CONCLUSION

This study showed for the first time the existence of genetic diversity within *Rosa damascena* cultivated in Syria. GC/MS analysis indicated that different genotypes may have qualitative differences in composition of essential oil. Almarah1 and Bab Alnayrab accessions are recommended to be used to broaden the production of rose oil.

ACKNOWLEDGMENTS

The authors would like to thank Prof Souheil HADDD for his collaboration. Also, we acknowledge Prof Adel SAFAR the minister of agriculture in Syria for his support, General Commission of Biotechnology in Syria for its laboratories, Institute of Rose and Aromatic Plants in Isparta (Turkey) for providing leaf material of damask roses. This work was financed by Damascus University, General Commission of Biotechnology in Syria, Suleyman Demirel University and the higher commission for scientific research in Syria.

REFERENCES

Alsemaan, T., N. Albatal and KH. Almaarri, 2011. Micropropagation of *Rosa damascene*. Arab. Univ. J. Agric. Sci., Vol. 19.

Babaei, A., S.R. Tabaei-Aghdaei, M. Khosh-Khui, R. Omidbaigi, M.R. Naghavi, G.D. Esselink and M.JM.D. Smulders, 2007. Microsatellite analysis of Damask rose (*Rosa damascena* Mill.) accessions from various regions reveals multiple genotypes. BMC Plant Biol., 10: 7-12.

Babaei, A., S.R. Tabaei-Aghdaei, M.R. Naghavi, M. Khosh-Khui, R. Omidbaigi and M.H. Assareh, 2008. *Rosa damascena* (Rosaceae) characters and their heritability analysis in Iran. Iran. J. Bot., 14: 75-80.

Baydar, N.G., H. Baydar and T. Debener, 2004. Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. J. Biotechnol., 111: 263-267.

Ghislain, M., D.M. Spooner, F. Rodriguez, F. Villamon and C. Nunez *et al*., 2004. Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. Theor. Applied Genet., 108: 881-890.

Gholamhoseinian, A., H. Fallah, F. Sharifi-Far and M. Mirtajaddini, 2008. Alpha mannosidase inhibitory effect of some Iranian plant extracts. Int. J. Pharmacol., 4: 460-465.

Gunes, E., 2005. Turkey rose oil production and marketing: A review on problem and opportunities. J. Applied Sci., 5: 1871-1875.

Kiani, M., Z. Zamani, A. Khalighi, R. Fatahi and D.H. Byrne, 2009. Microsatellite analysis of Iranian Damask rose (*Rosa damascena* Mill.) germplasm. Plant Breed., 129: 551-557.

Kumar, P., V.K. Gupta, A.K. Misra and D.R. Modi, 2009. Potential of molecular markers in plant biotechnology. Plant Omics J., 2: 141-162.

Loghmani-Khouzani, H., O. Sabzi Fini and J. Safari, 2007. Essential oil composition of *Rosa damascena* mill cultivated in central Iran. Scientia Iranica, 14: 316-319.

Moein, M., F. Karami, H. Tavallali and Y. Ghasemi, 2010. Composition of the essential oil of *Rosa damascena* Mill. Iranian J. Pharma. Sci., 6: 59-62.

Morgante, M., H. Hanafey and W. Powell, 2002. Microsatellites are preferentially associated with nonrepetitive DNA in plant genome. Nat. Genet, 30: 194-200.

Rakhshandeh, H., N. Vahdati-mashhadian, K. Dolati and M. Hosseini, 2008. Antinociceptive effect of *Rosa damascena* in Mice. J. Biol. Sci., 8: 176-180.

Rusanov, K., N. Kovacheva, B. Vosman, L. Zhang, S. Rajapakse, A. Atanassov and I. Atanassov, 2005. Microsatellite analysis of *Rosa damascena* Mill. Accessions reveals genetic similarity between genotypes used for rose oil production and old Damask rose varieties. Theor. Applied Genet., 111: 804-809.

Shahriari, S., N. Yasa, A. Mohammadirad, R. Khorasani and M. Abdollahi, 2007. *In vivo* antioxidant potentials of Rosa damascene petal extract from Guilan, Iran, comparable to α-tocopherol. Int. J. Pharmacol., 3: 187-190.

Sulayman, W.E.D., 2010. Ecological and chemical study of *Rosa damascena* Mill. in regions of (Al-Kalamon, Orneh, Aleppo). Master's Thesis, Damascus University, Syria.

Yousefi, B., S.R. Tabaei-Aghdaei, F. Darvish and M.H. Assareh, 2009. Flower yield performance and stability of various *Rosa damascena* Mill. Landraces under different ecological conditions. J. Food Eng., 121: 333-339.