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MOLECULAR VARIABILITY OF *ROSA DAMASCENA* MILL. GROWING IN TAIF REGION OF SAUDI ARABIA

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Abstract

Approximately 700 bp of the cpDNA maturaseK gene were amplified and sequenced for *Rosa damascena* inhabiting four distant localities of the western Saudi Arabia in order to address their genetic framework and origin. The different samples acquired identical gene sequence. The obtained sequences were compared with their counterparts from greenhouse *R. damascena* samples and other related taxa by using maximum-parsimony, neighbor-joining and maximum-likelihood methods. One tree was executed in which all samples showed sister relationship to greenhouse samples and to *R. moschata*. Some other greenhouse samples and *R. gallica* were shown to be out of this cluster, but they were sister to their clade. It is most likely that the *R. damascena* possibly originated from *R. moschata* and/or hybridization of *R. moschata* × *R. gallica*.

Introduction

Among 200 *Rosa* species, half of them distributed in Asia (Fougère-Danezan *et al.* 2015), *Rosa damascena* stands very important. Flowers of *R. damascena* are economically important for perfumes as well as for ornamental, cosmetic and pharmaceutical purposes (Guimaraes *et al.* 2010). Hybridization and morphological homogeneity make it difficult to identify the species of this genus. It was believed also that *Rosa damascena* was first introduced from Middle East to the western of Europe and this reintroduction happened twice (Beales *et al.* 1998).

Taif is a tourist region in the central west of Saudi Arabia with high altitude and temperate weather all over the year. The city is characterized with high cultivation of the Damask Rose (*Rosa* × *damascena trigintipetala*) which is traditionally called Ward Altaifi. There are two varieties of Damask rose, such as autumn Damask rose (*R.* × *damascena sempervirens*) and Damask rose Kazanlik (*Rosa* × *damascena trigintipetala*). It has been suggested that Ward Altaifi was cultivated in Taif region as Bulgarian "kazanlik" strain brought from Balkans 600 years ago by Turks who occupied Arabia in 14th century (El-Assal *et al*. 2014).

Different molecular studies have been conducted for addressing the phylogenetic and genetic framework of the genus. These studies were based on RAPD data (Wissemann and Ritz 2005, Bruneau *et al.* 2007 and Qiu 2012), microsatellites (Scariot *et al.* 2006) and amplified fragment length polymorphism (AFLP) data (Koopman *et al.* 2008). DNA sequences-based phylogenetic studies were also conducted in *Rosa* (Bruneau *et al.* 2007, Shaw *et al.* 2007, Fougère-Danezan *et al.* 2015). However, variability within Ward Altaifi and its genetic relationship to the closely related species and varieties are poorly investigated. Only a few studies have recently conducted on these points (El-Assal *et al.* 2014, Amer *et al.* 2016). Intraspecific and interspecific hybridization within the genus make the phylogentic resolution of this species more difficult (Fougère-Danezan *et al.* 2015). The present study, therefore, aimed at investigating the genetic variability within Ward Altaifi and to address its phylogenetic origin molecularly.

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Materials and Methods

Twenty five leaf samples were collected from field of 4 distant localities (2 from Hada, 2 from Shafa, 1 from Missan and 1 from Abha). The first three localities found in Taif region at the midwest of Saudi Arabia, while the last locality (Abha) is 500 km south from Taif region. Samples were obtained from plants cultivated in gardens from wild origin and were not obtained from seeds that more likely to have undergone hybridization. Samples were immediately taken to biology laboratory, Faculty of Science, Taif University and preserved in –40ºC until DNA extraction and further laboratory steps were carried out.

After grinding the leaf tissues in liquid nitrogen DNA was extracted according to Doyle and Doyle (1987) cetyltrimethylammonium bromide (CTAB) protocol with some modifications. The plant tissues were incubated with CTAB solution in a water bath at 65ºC overnight with occasional mixing to avoid aggregation of homogenate. Equal volume of chloroform-isoamyl alcohol (24 : 1) was added and the homogenate was well mixed and centrifuged at 10,000 rpm for 5 min. The upper phase was carefully transferred to a new sterile 1.5 ml tube and 600 µl of absolute ethyl alcohol was added. The mixture was mixed gently, centrifuged at 10000 rpm for 5 min and the supernatant was decanted. The precipitate was dissolved in 300 µl TE buffer and treated with 500 µl CTAB. The above mentioned steps were repeated with incubation at 65°C for 30 min. The clear DNA pellet was dissolved in 150 µl and was used for PCR. Amplification of maturase K (matK) gene was conducted with PCR experiments as described by Amer *et al.* (2016) using the same primers (RosaL and RosaH) and the same conditions.

Nucleotides of a chloroplastid DNA segment matK gene were sequenced for all collected samples of *R.* × *damascena trigintipetala*. The same gene fragment of other related taxa was collected from the database and was used for phylogenetic analysis. The phylogenetic analysis was done primarily by maximum-likelihood (ML) method with PAUP* 4.0b10 (Swofford 2002) by heuristic searches with the nearest-neighbor interchange (NNI) branch swapping and 10 random taxon additions. The general reversible model (GTR) and parameters optimized by modeltest 3.0 (Posada and Crandall 1998) was used. Bootstrapping replicates were set to 500. Maximum-parsimony (MP) and neighbor joining (NJ) were also conducted with PAUP* 4.0b10 under bootstrapping replications of 10000 to confirm the robustness of the ML tree topology. The distance option of Tamura-Nei was adjusted for executing the neighbor-joining analysis.

Results and Discussion

In the flowering plants, the chloroplast DNA gene (matK) that encodes maturase like protein is located within trnK intron. It has ideal size of approximately 1500 bp, high substitution rate, high variation level at first and second codon position and low ts/tv ratio. These characters are necessary to construct genetic relationships on the familial and specific levels. The gene is used as a preferred universal DNA barcode for flowering plants (Kumar *et al.* 2016). In *Rosa*, the complete matK gene acquired a sequence length of 1512 bp. By using the primers published by Amer *et al.* (2016), approximately 700 bp of matK gene were amplified and sequenced for all collected samples. After trimming the ambiguous nucleotides from both sides of the obtained sequences, 684 bp were finally analyzed. The sequenced fragment was located between base 123 and base 804. The sequence was identical for samples collected from all localities and exhibited one base substitution compared with the greenhouse samples.

The base composition for these data was as follow: A = 31.23%, C = 18.11%, G = 13.42% and T = 37.24%. Within the obtained sequences, 677 bp were constant and 4 varied. Four of the variable sites were parsimony uninformative and 3 were informative under parsimony criterion. The maximum-parsimony tree exhibited consistency index (CI = 1.00), homology index (HI =

0.00), retention index (RI = 1.00) and rescaled consistency index (RC = 1.00). The obtained ML tree showed a negative log likelihood score -lnL = 926.5 and the best-fit model that explained the datasets was GTR. The three analytical methods produced identical tree topology and executed 65% bootstrap probability at the node clustering samples from all localities (Fig. 1).

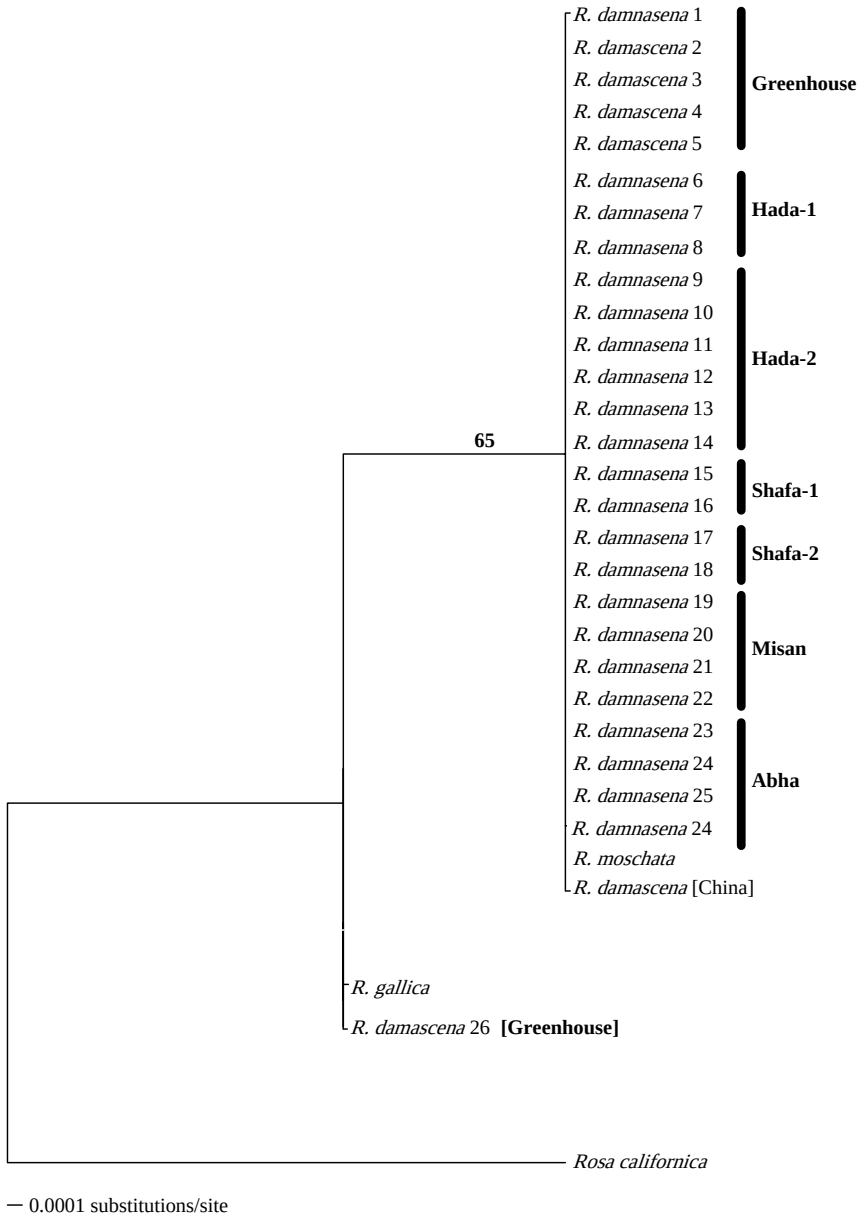

Fig. 1. A neighbor joining tree constructed from 684 bp matK gene fragment sequenced in this study. The value at the node refers to the bootstrapping of maximum-parsimony, neighbor joining and maximum likelihood analyses.

Samples cultivated in greenhouse at Taif region were recently studied by Amer *et al.* (2016). The authors used the same gene fragment and concluded that the greenhouse *R.* × *damascena trigintipetala* was characterized into 2 varieties with different origins. In the present study, together with the greenhouse *Rosa*, *R.* × *damascena trigintipetala* grouped in one clade with *R. moschata*. Out of this calde, one sample of greenhouse clustered with *R. gallica*.

The calculated pairwise genetic distance (Table 1) was zero between *R. moschata* and all samples of *R.* × *damascena trigintipetala* (D = 0.000). The genetic distance together with the tree topology indicated, therefore, that *R.* × *damascena trigintipetala* from all localities could be originated from *R. moshata* while the greenhouse samples could be originated from either *R. moschata* or *R. gallica*.

Table 1. Pairwise genetic distances among the different varieties of *R. damascena* populations. The distances were calculated from matK data sequenced in this study.

Taxon	*R. moschata*	*R. gallica*	*R. damascena* (current samples)
R. moschata	-		
R. gallica	0.0015	-	
R. damascena (current samples)	0.0000	0.0015	-

In greenhouse samples, a transversion was found in the second position of C_{220} to G_{220}. This non-synonymous mutation changed leucine amino acid into valine. This mutation discriminated *R.* × *damascena* into two varieties: the greenhouse variety which was closely related to *R.* × *damascena gori* and *R. chinensis semplerfornis* (as described by Amer *et al*. 2016) while the other variety is identical to *R. moschata* as found by the present study. It could be assumed that greenhouse variety was introduced to Saudi Arabia from Turkey or China, while the second is the cultivated variety commonly found in Taif. Thus, it is inferred that *R.* × *damascena* cultivated in Taif and other Saudi localities originated from the European *R. moschata*. Iwata *et al.* (2001) used the internal transcribed spacer of ribosomal DNA to identify the triparental origin of *R.* × *damascena*. *R. moschata*, *R. gallica* and *R. fedschenkoana* contributed the original hybridization to produce such Damask variety. The authors also applied *psb*A-*trn*H spacer sequences and indicated that the four oldest Damask varieties had chloroplasts derived only from *R. moschata*. The present study confirms Iwata *et al.*'s finding where the genetically identical *R.* × *damascena* originated from both *R. moschata* and *R. gallica* with the first being common and more confident. Iwata's team reasoned that the original hybrid stemmed from *R. moschata* as seed parent and *R. gallica* as pollen parent. This hybrid (*R. moschata* × *R. gallica*) was then crossed with *R. fedtschenkoana*, giving rise to Damask roses. However, the gene for recurrent bloom is expressing itself only in Autumn Damask.

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