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Microsatellite analysis of *Rosa damascena* Mill. accessions reveals genetic similarity between genotypes used for rose oil production and old Damask rose varieties

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Abstract Damask roses are grown in several European and Asiatic countries for rose oil production. Twenty-six oil-bearing *Rosa damascena* Mill. accessions and 13 garden Damask roses were assayed by molecular markers. Microsatellite genotyping demonstrated that *R. damascena* Mill. accessions from Bulgaria, Iran, and India and old European Damask rose varieties possess identical microsatellite profiles, suggesting a common origin. At the same time, the data indicated that modern industrial oil rose cultivation is based on a very narrow genepool and that oil rose collections contain many genetically identical accessions. The study of long-term vegetative propagation of the Damask roses also reveals high somatic stability for the microsatellite loci analyzed.

Introduction

Some varieties of *Rosa damascena* Mill. (Damask roses) are of great importance for rose oil production, others are widely cultivated as garden roses. Crude distillation of roses for the oil probably began in Persia in the late 7th century AD, and later in the 14th century, spread into the provinces of the Ottoman Empire. Old Damask rose varieties (e.g., 'Quatre Saisons') have been extensively used for European rose improvement. As garden roses, the Damask roses were introduced from the Middle East into western Europe and re-introduced later in the 16th century (Saakov and Rieksta 1973; Beales et al. 1998). In Bulgaria, the cultivation of Damask rose varieties for production of rose oil was initiated in the 16th century after the expansion of the Ottoman Empire (Topalov 1978). The main present oil rose plantations in Turkey (Isparta Region) were established in the 1880s, using plant material from Bulgaria (Baydar et al. 2004). At present, the Isparta Province of Turkey (cultivation of *R. damascena*, *R. gallica*, and *R. centifolia*) and the Kazanluk Valley of Bulgaria (cultivation of *R. damascena*) are the main centers for both rose oil and rose water production in the world. *R. damascena* plants are also being planted in Iran and India for production of rose petals and rose water (Staikov and Kalaijiev 1980).

Several different types of DNA marker have been successfully applied for rose genotyping (Rajapakse et al. 1992; Debener et al. 1996; Jan et al. 1999; Debener et al. 2000; Crespel et al. 2002; Esselink et al. 2004), to study the domestication process (Martin et al. 2001), as well as to study genetic relationships among different rose species and varieties (Rajapakse et al. 1992; Ben-Meir and Vainstein 1994; Ballard et al. 1995; Millan et al. 1996; Debener et al. 1996; Martin et al. 2001; Baydar et al. 2004; Nybom et al. 2004). Furthermore, DNA markers have also been used for the development of genetic linkage maps in roses (Debener and Mattiesch 1999; Rajapakse et al. 2001). Highly polymorphic microsatellite markers have been developed for *R. hybrida* (Esselink et al. 2003) and *R. wichuraiana* (Zhang et al. 2002). The use of a set of robust microsatellite markers for variety identification and reference collection management was proposed (Esselink et al. 2003).

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Genotyping of Damask roses has been reported in several recent DNA marker studies. Applying RAPD markers to old varieties of *R. damascena* and closely related rose species, Iwata et al. (2000) suggested a triparental origin of the old Damask roses. Two recent studies with RAPD, AFLP, and SSR markers (Ağaoğlu et al. 2000; Baydar et al. 2004) did not detect polymorphism among the *R. damascena* plants grown in Isparta, Turkey. The latter is not surprising considering the relatively recent initiation of oil rose cultivation in Turkey based on material from Bulgaria (Baydar et al. 2004). Until now, there have been no results reported on the biodiversity of oil roses grown in Bulgaria and their genetic relations to oil-bearing *R. damascena* varieties cultivated in Asia as to garden Damask roses grown in Europe. Here, we report microsatellite analyses of a collection of oil roses grown in Bulgaria, oil-bearing *R. damascena* Mill. genotypes cultivated in India and Iran and garden Damask roses grown in Europe. The genetic relationships of the analyzed genotypes, the implications for damask rose collections, and the somatic stability of the microsatellite loci are discussed.

Materials and methods

Plant material

The rose plants examined in this study are listed in Table 1, including 26 oil-bearing *R. damascena* Mill.

Table 1 Rose genotypes investigated in this study

No.	Accession name	Specific characteristics[a]	Collection[b]	Region of cultivation
1	101	–	IRAP	Bulgaria
2	1071	–	IRAP	Bulgaria
3	1151	–	IRAP	Bulgaria
4	148-75	HY	IRAP	Bulgaria
5	51	–	IRAP	Bulgaria
6	6/79	HY	IRAP	Bulgaria
7	601	CT, RT, HOC	IRAP	Bulgaria
8	612	CT, HOC	IRAP	Bulgaria
9	615	CT	IRAP	Bulgaria
10	7/79	HY, HOC	IRAP	Bulgaria
11	70/74	–	IRAP	Bulgaria
12	809	–	IRAP	Bulgaria
13	831	RT, BST	IRAP	Bulgaria
14	9/77	Small flowers	IRAP	Bulgaria
15	Aleksandrovo	HY, HOC	IRAP	Bulgaria
16	Elejna	HY, RT, BST, long spines, specific leaf shape	IRAP	Bulgaria
17	Iskra	HY, BST	IRAP	Bulgaria
18	Janina	CT, RT, BST	IRAP	Bulgaria
19	L-49	RT	IRAP	Bulgaria
20	Svezhen	HY	IRAP	Bulgaria
21	Svezhen 188	Big flowers, BST	IRAP	Bulgaria
22	Svezhen 74	Big flowers, RT, BST	IRAP	Bulgaria
23	Svezhen-72	RT, BST, specific spine shape	IRAP	Bulgaria
24	K-IV	Specific leaf shape	IRAP	Bulgaria
25	Zaran	–	IRAP	Iran
26	Aligar	–	IRAP	India
27	Kazanlik (before1689)	–	RVM	Bulgaria
28	*Rosa damascena* (RVM)	–	RVM	–
29	Botzaris (1856)	–	RVM	Europe
30	Dom Pedro (1833)	–	RVM	Europe
31	Lamitie (1850)	–	RVM	Europe
32	*R. gallica* var. Red Damask (1800)	–	RVM	Europe
33	*R. damascena* (PSL)	–	PSL	Turkey
34	Ispahan (before 1832)	–	PSL	Europe
35	La Ville de Bruxelles (1849)	–	PSL	Europe
36	Madame Hardy (1832)	–	PSL	Europe
37	Rose de Rescht	–	PSL	Europe
38	Ville Bruxelles × Domes	–	PSL	Europe
39	York& Lancaster (before 1629)	–	DAR	Europe
40	Quatre Saisons (before 1633)	–	DAR	Europe

Accession names are given according to the collection from where the plant material was obtained. The approximate period since when the respective variety is known is given in *parentheses*. The region and country where accessions are originally cultivated are provided

[a] *CT* Cold tolerance, *HY* high yield, *HOC* high rose oil content, *RT* rust tolerance, *BST* black spot tolerance

[b] *PSL* Collection of botanical, ancient, and modern rose cultivars, Professional School of Lullier, Switzerland; *IRAP* Institute of Rose and Aromatic Plants, Kazanlak, Bulgaria; *DAR* David Austin Roses Ltd., UK; *RVM* Roseraie du Val de Marne, L'Hay-les-Roses, France

accessions obtained from the collection of the Institute of Rose and Aromatic Plants (IRAP), Kazanlak, Bulgaria; 13 Damask rose varieties, and one *R. gallica* variety obtained from the Collection of Botanical, Ancient and Modern Rose Cultivars, Professional School of Lullier (PSL), Switzerland, Roseraie du Val de Marne, L'Hay-les-Roses, France and David Austin Roses, United Kingdom. DNA was extracted from young leaves according to Lefort and Douglas (1999).

Microsatellite analysis

The PCR reactions were performed on an Eppendorf Mastercycler in a total volume of 25 µl containing 1X PCR buffer (MBI Fermentas), 20–50 ng genomic DNA, 20 pmol of each primer, 2 m *M* of each dNTP, 1.5 m *M* $MgCl_2$ and 1 U *Taq* polymerase (MBI Fermentas). Two sets of primers, from *R. wichuraiana* (Zhang et al. 2002) and *R. hybrida* (Esselink et al. 2003), were used. The PCR conditions for amplification of the loci were as follows: 94°C for 3 min; 30 cycles of 94°C for 30 s, 50°C for 30 s (46°C for Rw18N19 and 46.5°C for Rw32D19), 72°C for 60 s; and a final elongation step at 72°C for 7 min.

In all cases, the forward primer was labeled with Cy5. Microsatellite fragment analysis was performed using high-resolution gels on an ALF Express II sequencer (Amersham Biosciences). Alleles were sized with Allele Locator, version 1.03, software (Amersham Biosciences). Internal standards were produced by PCR amplification of DNA fragments from pUC19 with a Cy5-labeled forward primer and a set of reverse primers producing sizes 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, and 610 bp.

Phylogenetic analysis

Because of the tetraploid nature of the analyzed Damask roses, it is difficult to deduce if a certain locus is present in one, two, or three copies, unless markers are of very high quality (Esselink et al. 2003). Therefore, for this study we treated all as dominant markers. A distance matrix was calculated using the Jaccard index and the clusters module of the ADE-4 software (Thioulouse et al. 1997). A dendrogram was computed by using the unweighted pair group method using arithmetic averages (*UPGMA*) option of the dendrogram module of the ADE-4 software.

Results and discussion

Transferability of microsatellite markers

Microsatellite analysis is widely used for genotyping and variety identification of vegetative propagated crops and species (Hokansson et al. 1998; Hvarleva et al. 2004), including the cut roses and rootstocks (Esselink et al. 2003). No data on isolation of microsatellites from *R. damascena* or other Damask roses have been reported until now. To select SSR markers for the present study, two sets of primers amplifying microsatellite regions derived from *R. wichuraiana* and *R. hybrida* were tested. The performed PCR amplification of genomic DNA isolated from four *R. damascena* genotypes demonstrated that 11 out of 28 SSR primer couples from *R. wichuraiana* and 22 out of 23 primer pairs from *R. hybrida* are successful in amplifying *R. damascena* DNA (Table 2). The observed difference in the efficiency of both tested microsatellite sets could be due to the genetic distance between the species (Saakov and Rieksta 1973). Alternatively, the difference may also be a result of the pre-selection according to marker robustness of the *R. hybrida* set (Esselink et al. 2003).

Analysis of Damask rose varieties

The analysis of 40 rose accessions with 11 SSR markers from the *R. wichuraiana* set showed that the old

Table 2 Microsatellite profiles of the accessions from the cluster of *R. damascena Mill* plants used for rose oil production and old European Damask roses

Locus	Allele size in base pairs	Locus	Allele size in base pairs
RhE2B	166 169 175 178	*RhE3*	166 180
RhEO506	208 220 226 259	*RhAB26*	162 166 178 188
RhAB22	156 160	*RhAB13*	142 146 178
RhP519	233	*RhAB1*	122 140 174
RhP507	131 164 215	*RhI402*	199 205 211
RhD201	196 199	*RhBK4*	166 174 178 182
RhAB40	230 252	*Rw10M24*	266 276 280
RhD221	208 217 223 227	*Rw3K19*	426 441
RhB303	120 124 126	*Rw5D11*	224 234 254 266
RhO517	258 261 264	*Rw14H21*	122 126
RhP518	130 133 139	*Rw17I7*	202 211
RhP524	118 121 229	*Rw18N19*	194 214 222
RhD206	205 211 304 307	*Rw22B6*	129 135 141
RhB19	138	*Rw32D19*	498 507 519
RhAB15	104 110 134 150	*Rw55C6*	258 272
RhJ404	91 118 145	*Rw55D22*	197
Rw10J19	264 267 366 396		

European Damask rose varieties Quatre Saisons, York, and Lancaster, and *R. damascena* genotypes used for rose oil production form one large "oil rose cluster" (Fig. 1) and possess identical microsatellite profiles (see Table 2). The complete dataset of microsatellite allele sizes for all damask accessions analyzed can be found at http://bulgenom.abi.bg. As might be expected, the rest of the analyzed European damask roses showed diverse levels of homology with this group due to the direct involvement of the old Damask varieties in their pedigree or sharing part(s) of the genome(s) of common ancestor species (Iwata et al. 2000).

The accessions from the oil rose cluster were further characterized with 22 microsatellite markers from the *R. hybrida* set. The performed analysis demonstrated that again, all roses from this cluster possess identical microsatellite profiles at the applied SSR markers (Table 2). The obtained data reveals genetic similarity of a large group of 31 rose genotypes consisting of several distinguishing sub-groups:

1. Twenty-four accession of *R. damascena* var. Kazanlik (phenotype *trigintipetala*) from the IRAP collection, used for rose oil production. These plants were selected from different regions of the Rose Valley, Bulgaria, during the period 1924–1980, and part of them show differences in cold tolerance, flower yield, and content of rose oil.
2. Two rose accessions from the IRAP collection, originating from the region of Azaran, Iran, and the region of Aligar, India. Both genotypes were considered as *R. damascena* due to their morphology and the composition of the rose oil (Staikov and Kalaidjiev 1980).
3. Two old European Damask rose varieties, one of which, 'Quatre Saisons', plays an important role in old European rose improvement in the 19th century.
4. Three *R. damascena* accessions from the Roseraie du Val de Marne and PSL collections including the old accession of var. Kazanlik, which had been known since 1689, and a *R. damascena* accession from the PSL collection originating from Turkey.

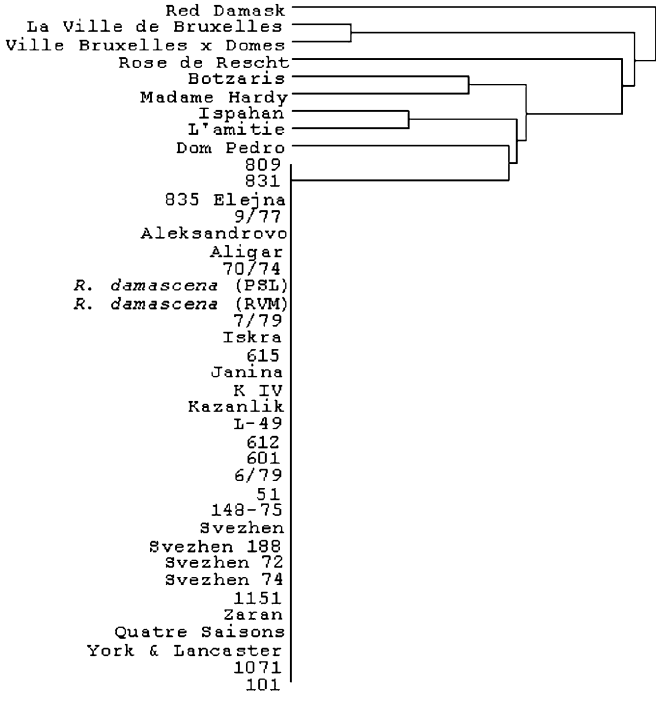

Fig. 1 Dendrogram of 39 Damask rose accessions and *Rosa gallica* var. Red Damask used as an outgroup. The dendrogram was constructed after analysis with a set of 11 microsatellite markers derived from *R. wichuraiana*

The identical microsatellite profiles of the group of oil and old European Damask rose genotypes strongly suggests that all of them originate from a single common ancestor. The obtained data suggests that clones of one genotype were spread in distant geographic regions of Europe and Asia and successfully vegetative propagated for centuries. The results of our study support the reported genetic uniformity of the *R. damascena* plants grown in Turkey (Ağaoğlu et al. 2000; Baydar et al. 2004) and the common origin of the old Damask rose varieties (Iwata et al. 2000). The reasons for the observed dominance of one ancestor Damask rose genotype are not clear. It could be result from its vigor and plasticity, allowing successful adaptation to diverse climate and soil conditions or from directed long-term propagation and cultivation of one superior variety because of the scent or the superior quality of rose oil and water. The claims that the roses from this genotype were mentioned in the old Greek literature (Hurst 1941) and cultivated by the Romans and found in Pompei (Krussmann 1962) support the second assumption. The obtained data from the present study also demonstrates that the entire rose oil industry in Bulgaria is based on a very narrow genepool consisting of a set of well performing clones. Similar results were reported earlier for the oil rose genetic resources in Turkey (Ağaoğlu et al. 2000; Baydar et al. 2004). This points out the need of well-targeted measures to extend the present genepool of oil rose. The results also indicate that for gene banks, it is worthwhile to characterize the material they contain by using DNA markers. This would immediately have pointed out the clonal nature of the collected material, as was demonstrated for poplar (Storme et al. 2004; Arens et al. 1998).

Somatic stability of rose microsatellites

The performed genotyping of long-term vegetative propagated oil rose genotype provide opportunity to assess the level of somatic stability of the studied microsatellite loci. A number of recent studies demonstrated that somatic mutation of microsatellites could take place in long-lived and/or vegetative propagated plants. The reported results show a large diversity of the rates of microsatellite mutations, depending on the plant species and locus studied. For examples:

- No somatic mutations were detected after analysis of rametes and megagametophytes of *Pinus strobes* L. at eight microsatellite loci (Cloutier et al. 2003).
- A single hyper-mutable locus, out of eight loci, was identified in rametes of *Robina pseudoacacia* (Lian et al. 2004)
- Variation in the alleles of two out of ten microsatellite loci was detected among the plantlets derived from the single donor tree of *Populus tremuloides* (Rahman and Rajora 2001)
- A high rate of intra-cultivar microsatellite variability was observed after analysis of 25 and 22 clones of the old grape cultivars Pinot noir and Chardonnay, where, respectively, 15 and 9 microsatellite loci out of the 100 analyzed possess mutated alleles in at least one clone (Riaz et al. 2002)

The modes of multiplication, cultivation, and selection of the oil rose very much resemble these of grape cultivars and clones, where plants of widely used cultivars are subject of intensive vegetative multiplication and cultivation, often followed by selection of the best performing plants and development of supposedly new clones from the same genotype. As mentioned previously, phenotypic differences are observed between the oil rose clones. Remarkably, the results from our study showed no mutated alleles in 33 microsatellite loci after analysis of 31 *R. damascena* accessions originating from common ancestor genotype, which have been vegetatively propagated for a long time (at least one to four centuries) in different geographic regions of Europe and Asia (Saakov and Rieksta 1973; Topalov 1978; Beales et al. 1998). This is in line with the observations of Vosman et al. (2004), who showed that mutants of hybrid tea rose varieties always showed the AFLP fingerprint of the initial variety. The observed high somatic stability of the analyzed microsatellites provides an exiting opportunity for their further application in studying the origin and evolution of Damask roses.

Conclusions

Microsatellite analysis of Damask roses demonstrates that: (1) the old European Damask roses and oil roses cultivated in different geographic regions have identical microsatellite profiles and originate from a common ancestor; (2) the oil rose cultivation is based on very narrow genepool; (3) gene banks maintain a large number of accessions that show identical genotypes; and (4) the long-term vegetative propagated Damask roses possess high somatic stability of the microsatellite loci.

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