Floriculture and Ornamental Biotechnology ©2009 Global Science Books

Rosa damascena Mill., the Oil-bearing Damask Rose: Genetic Resources, Diversity and Perspectives for Molecular Breeding

Krasimir Rusanov[1] • Natasha Kovacheva[2] • Atanas Atanassov[1] • Ivan Atanassov[1*]

[1] AgroBioInstitute, 8 Dragan Tsankov, Sofia 1164, Bulgaria
[2] Institute of Rose and Aromatic Plants, 49 Osvobozhdenie, Kazanlak 6100, Bulgaria

Corresponding author: * **ivan_atanassov@abv.bg**

ABSTRACT

Modern rose oil production is nearly all based on cultivation of *Rosa damascena* 'Trigintipetala', the oil-bearing damask rose. Although cultivated for centuries, the genepool of damask rose cultivars used for industrial cultivation has been poorly characterized. The development of new breeding lines was based exclusively on clonal selection from vegetatively propagated industrial populations. Utilization of crosses within the species and with other rose species was generally avoided due to the industry's apprehension to changes in rose oil composition. This review summarizes the recent reports on DNA marker characterization of the genetic resources of industrially cultivated and naturally occurring damask roses. The needs and conditions for implementation of cross-breeding programmes in reference to the importance of this rose species for the perfume, cosmetic, pharmaceutical and food industries are presented. The opportunity for marker-assisted selection to assess the potential of the complex genome of *R. damascena* based on available molecular markers and the development of allele-/gene-specific markers is discussed.

Keywords: genetic diversity, rose oil, segmental tetraploid, SSR
Abbreviations: **AFLP**, Amplified Fragment Length Polymorphism; **RAPD**, Random Amplification of Polymorphic DNA; **RFPL**, Restriction Fragment Length Polymorphism; **SSR**, Simple Sequence Repeat

CONTENTS

INTRODUCTION

The genus *Rosa*, belonging to the *Rosaceae* family, includes over 200 species (Gudin 2000). Among them *R. damascena* Mill. is a unique species; some cultivars are used as garden roses, while others are the subject of large-scale industrial cultivation for rose oil production. These cultivars belong to the damask commercial or horticultural group of roses, well known for their strong fragrance (Widrlechner 1981). It is considered that the damask roses originated geographically and historically from ancient Persia (present-day Iran) and later spread throughout Europe and Northern Africa. By the 14th century damask roses were already cultivated as garden roses in Western Europe (Beales *et al.* 1998). Later, some of the old damask cultivars have been extensively used for European rose improvement.

The mass cultivation of *R. damascena* in Europe for production of rose oil was initiated during the 16th century in Bulgaria and Turkey, after the expansion of the Ottoman Empire (Topalov 1978). It is believed that the crude distillation of roses for rose oil began in Persia in the late 7th century AD and later spread into the provinces of the Ottoman Empire in the 14th century. The most common aroma concentrates derived from rose petals are rose oil and rose water, produced after steam distillation and rose concrete and rose absolute obtained after solvent extraction. Presently, the largest producers of rose oil from *R. damascena* are Bulgaria, Turkey and Iran, but growing volumes are also produced in India, China and other countries. For example, according to information from Biolandes Ltd. (Le Sen, France) the estimated volumes of the world production/market in 2006 consisted of approximately 3000 kg rose oil, 5000 kg absolute and dozens of tons of rose water (booklet 'Biolandes, La rose de Bulgarie', 2007). For the same year Bulgaria alone produced approximately 1900 kg rose oil, and during the last decade had consistently high annual production volumes varying between 1100 and 1900 kg.

The constitution of rose oil is very complex and involves more than 275 minor constituents and a small number of major compounds, including citronellol, geraniol, nerol, phenethyl alcohol, linalool, farnesol, eugenol and eu-

Received: 1 July, 2008. Accepted: 18 September, 2008.

Invited Mini-Review

genol methyl ether. A significant part of the rose oil odor is derived from two minor constituents, β-damascenone and β-ionone, (Kovats 1987; Ohloff 1994). The composition and quality of the extracted rose oil can vary significantly depending from the genotype of the cultivated damask rose (Younis *et al*. 2007); the environmental and climate conditions of cultivation; and the way of flower harvest, processing and rose oil distillation. Often the composition of the rose oil produced from the same plantation can vary from year to year, depending on the particular climatic conditions and when rose flowers are collected. Similarly, the cultivation of the same vegetatively propagated rose clone at different locations in the same geographic region also frequently results in variability in the quality and quantity of extracted rose oil (Nikolov *et al*. 1977; Nikolov *et al*. 1978; Topalov 1978).

One of the well known regions for cultivation of damask roses for production of high quality rose oil is the Rose Valley (the region around town Kazanlak) in Bulgaria and the Isparta region in Turkey. The quality of the produced rose oil is controlled through implementation of international standards for rose oil composition and characteristics (ISO 9842:2003, www.iso.org). Preservation of the composition and quality of the produced rose oil is the main prerequisite considered before the introduction of new rose oil-producing cultivars and changes in cultivation, flower processing or oil distillation practices.

Rose oil is an expensive product. The price of Bulgarian rose oil reached 5000 EUR/kg in 2007. It is used as an indispensable ingredient in virtually all fine perfume products. Rose oil and other rose products are also extensively used as fragrance components in a wide range of creams, soaps and pharmaceutical preparations. Rose products are also included in a number of food products like sweets, gelatines, diary desserts, etc., mostly in minute quantities. Dry petals from damask roses are included in various preparations of traditional herbal medicine and homeopathic products. The rose oil and other flower extracts have been the subject of numerous recent studies related to their antibacterial (Aridogan *et al.* 2002; Basim and Basim 2003), antioxidant (Achuthan *et al.* 2003; Ozkan *et al*. 2004), antiinfective and anti-inflammatory properties (Biswas *et al.* 2001), relaxant effects on tracheal chains (Boskabady *et al.* 2006) and even anti-HIV activity (Mahmood *et al.* 1996). In spite of the well documented positive effects observed from most of the studies, the identification of the respective active compounds requires further study.

Within this manuscript we present an overview on the recent progress on characterization of the genetic resources and diversity of *R. damascena* and discuss the opportunities for improvement of the oil-bearing damask rose.

GENETIC RESOURCES AND DIVERSITY

The genetic resources of oil-bearing *R. damascena* used for commercial production of rose oil in Bulgaria and Turkey, as well as the genetic diversity of damask roses growing in various regions of Iran were characterized in several recent studies. Two separate studies by Agaoglu *et al.* (2000) and Baydar *et al.* (2004) did not find polymorphism among *R. damascena* plants collected from various plantations in Turkey using RAPD (Random Amplification of Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism) and SSR (Simple Sequence Repeat) molecular markers. Similarly, Rusanov *et al*. (2005a) did not find differences in the SSR profiles of cultivars and lines subject to industrial cultivation in Bulgaria during the 20th century. The microsatellite analysis from the last study also demonstrates that the SSR profiles of the Bulgarian accessions were identical to those of several other oil-bearing rose accessions originating from Iran, India and Turkey, which possessed similar characteristics of distilled rose oil. Moreover, no differences were detected in the same study between the analyzed oil-bearing damask roses and the old damask roses *R. damascena versicolor* (a.k.a. 'York and Lancaster') and 'Quatre Saisons Continue' (a.k.a. 'Quatre Saisons' and 'Autumn Damask') cultivated as garden roses in West European countries. The results from these studies suggest that the oil-bearing damask rose used for industrial cultivation and the studied old garden damask rose genotypes originated from an initial *R. damascena* 'Trigintipetala' ortet.

It is considered that the damask roses were introduced into different parts of Europe from the Middle East during the 14th–16th centuries, thus their origin and centre of diversity could be located in this region. The results from three recent studies on genetic diversity among *R. damascena* plants collected from different locations in Iran provide strong evidence for the origin and centre of diversity of the damask roses. First, Pirseyedi *et al.* (2005) reported a high level of diversity between 12 accessions collected from separate commercial gardens located in different regions of Iran on the basis of AFLP analysis. Later, Babaei *et al.* (2007) reported significant diversity between 40 accessions collected from 28 provinces in Iran on the basis of analysis at 9 SSR loci. Cluster analysis of genetic similarities shows that the studied accessions belong to nine different genotypes. The main group of accessions (27 out of 40 analyzed) possessed a SSR profile identical to those of the *R. damascena* 'Trigintipetala' plants used for commercial production of rose oil in Bulgaria. Most of the accessions from the main group were collected from commercial production fields in the Isfahan province, the main rose production area of Iran. The rest of the genotypes involved a small number of accessions all of which possessed a considerable number of SSR alleles which were not present in 'Trigintipetala'. This suggests that the accessions from the small groups are not derived from self-pollination of the plants from the widely dispersed 'Trigintipetala'. In the most recent study using RAPD analysis of *R. damascena* accessions from different regions of Iran, Kiani *et al.* (2008) reported the distribution of 41 accessions into 10 different groups. Again, a large part of the accessions were grouped into a main cluster involving 'Trigintipetala' used for rose oil production in Bulgaria.

Together these results suggest that modern commercial rose oil production in Bulgaria, Turkey and predominantly in Iran is based on the cultivation of a single or very few closely-related genotypes originating from a common old *R. damascena* 'Trigintipetala' ortet spread into Europe from the Middle East. Some of the well known old damask rose cultivars (e.g. 'Kazanlik', 'York and Lancaster' and 'Quatre Saisons') cultivated in Western Europe as garden roses also originated from 'Trigintipetala'. The highlighted studies which genotype a number of landraces of *R. damascena* collected from various locations of Iran suggest that the region of Iran represents the centre of diversity for damask roses. Although some of the accessions possessing genotypes different from that of 'Trigintipetala' are used for commercial rose cultivation in Iran, their capacity for production of high quality rose oil remains to be determined. The recently discovered genetic diversity in *R. damascena* provides an important background for addressing the origin of this species and initiation of intraspecific cross-breeding programmes for improvement of oil-bearing damask roses.

PARENTAL ORIGIN AND GENETICS

The parental origin of the damask rose has been the subject of several speculations. Hurst (1941) suggests that damask roses possessing different flowering periods have different parental origins. According to this author the summer damask roses originated from the ancestral crossing of *R. gallica* L. and *R. phoenicia* Boiss., while the autumn damask roses (showing a second bloom in autumn) are derived from a cross between *R. gallica* and *R. moschata* Mill. Our recent study shows that 'York and Lancaster' and 'Kasanlik', which are summer damasks, possess an identical SSR profile with the autumn damask 'Quatre Saisons' (Rusanov *et al.* 2005a). Similarly, the study by Iwata *et al.* (2000) demonstrates identical RAPD profiles for these three cultivars.

Both results rule out the proposed different parental combinations of the summer and autumn damask roses. The only well documented study of the parental origin of the damask roses using DNA markers was reported by Iwata *et al*. (2000). Based on analysis of the internal transcribed spacer sequences of the nuclear ribosomal RNA and analysis of the *psbA-trnH* gene spacer sequence of the chloroplasts, the authors suggest a triparental origin of the old damask roses. The proposed model includes two successive crosses (*R. moschata* x *R. gallica*) x *R. fedschenkoana* Regel where *R. moschata* was pointed out as the maternal ancestor (the chloroplasts were derived from this species). From another perspective, our study on genetic similarity between the oil-bearing roses, which do not directly belong to the damask group, pointed out that the SSR profile of *R. damascena* differs in all alleles at several SSR loci from the profiles of the analyzed accessions of *R. moschata* and *R. gallica* (Rusanov *et al*. 2005b). This suggests that the precise analysis of the parental origin of damask roses requires probing of a number of genotypes closely related to *R. damascena* and its putative ancestors with molecular markers to provide information on allele configurations (e.g., SSR markers).

The recently reported centre of diversity of damask roses in Iran provides an exciting opportunity to address the origin of damask roses. The SSR analysis of *R. damascena* plants from this region reported by Babaei *et al.* (2007) pointed out that the studied accessions belong to 9 different genotypes. Furthermore, the genotype of the most abundant group was identical to the genotype of *R. damascena* 'Trigintipetala'. The other eight genotypes determined in the same study possess only a portion of the SSR alleles of 'Trigintipetala' while the rest of the SSR alleles were either unique or shared between the new genotypes. Such allele configurations exclude the possibility that the newly found genotypes are derived from self-pollination of 'Trigintipe-

Table 1 SSR markers used for characterization of *Rosa damascena* genetic recourses and diversity.

Locus	Origin	Linkage group*	Reference
RhE2B	*R. hybrida*	6	Rusanov *et al*. 2005a; Babaei *et al.* 2007
RhE3	*R. hybrida*	ND	Rusanov *et al*. 2005a
RhEO506	*R. hybrida*	2	Rusanov *et al*. 2005a; Babaei *et al.* 2007
RhAB26	*R. hybrida*	ND	Rusanov *et al.* 2005a
RhAB22	*R. hybrida*	6	Rusanov *et al.* 2005a
RhAB13	*R. hybrida*	4	Rusanov *et al.* 2005a
RhP519	*R. hybrida*	ND	Rusanov *et al*. 2005a; Babaei *et al.* 2007
RhAB1	*R. hybrida*	ND	Rusanov *et al.* 2005a
RhP507	*R. hybrida*	ND	Rusanov *et al*. 2005a
RhI402	*R. hybrida*	3	Rusanov *et al.* 2005a
RhD201	*R. hybrida*	ND	Rusanov *et al.* 2005a
RhBK4	*R. hybrida*	ND	Rusanov *et al.* 2005a
RhAB40	*R. hybrida*	4	Rusanov *et al.* 2005a
RhD221	*R. hybrida*	4	Rusanov *et al*. 2005a; Babaei *et al.* 2007
RhB303	*R. hybrida*	ND	Rusanov *et al*. 2005a; Babaei *et al.* 2007
RhO517	*R. hybrida*	1	Rusanov *et al.* 2005a
RhP524	*R. hybrida*	ND	Rusanov *et al.* 2005a
RhD206	*R. hybrida*	ND	Rusanov *et al.* 2005a
RhB19	*R. hybrida*	ND	Rusanov *et al.* 2005a
RhP518	*R. hybrida*	5	Rusanov *et al.* 2005a
RhAB15	*R. hybrida*	2	Rusanov *et al.* 2005a
RhJ404	*R. hybrida*	ND	Rusanov *et al.* 2005a
RhP50	*R. hybrida*	ND	Babaei *et al.* 2007
Rw10J19	*R. wichurana Crépin*	6	Rusanov *et al.* 2005a
Rw10M24	*R. wichurana*	5	Rusanov *et al.* 2005a
Rw3K19	*R. wichurana*	ND	Rusanov *et al.* 2005a
Rw5D11	*R. wichurana*	ND	Rusanov *et al.* 2005a
Rw14H21	*R. wichurana*	6	Rusanov *et al.* 2005a
Rw17I7	*R. wichurana*	ND	Rusanov *et al.* 2005a
Rw18N19	*R. wichurana*	ND	Rusanov *et al.* 2005a
Rw22B6	*R. wichurana*	5	Rusanov *et al.* 2005a
Rw32D19	*R. wichurana*	ND	Rusanov *et al.* 2005a
Rw55C6	*R. wichurana*	3	Rusanov *et al.* 2005a
Rw55D22	*R. wichurana*	7	Rusanov *et al.* 2005a
RMS023	*R. hybrida*	ND	Baydar *et al.* 2004
RMS027	*R. hybrida*	ND	Baydar *et al.* 2004
RMS029	*R. hybrida*	5	Baydar *et al.* 2004
RMS037	*R. hybrida*	2	Baydar *et al.* 2004
RMS057	*R. hybrida*	ND	Baydar *et al.* 2004
RMS070	*R. hybrida*	1	Baydar *et al.* 2004
RMS088	*R. hybrida*	6	Baydar *et al.* 2004
RMS089	*R. hybrida*	ND	Baydar *et al.* 2004
RMS146	*R. hybrida*	7	Baydar *et al.* 2004

* Linkage groups are given as published by Oyant *et al*. 2008 and Yan *et al*. 2005. ND = not determined

tala'. Thus the new *R. damascena* genotypes could be direct descendants of 'Trigintipetala' crossed with other roses growing nearby or they might share one or more common ancestral parents with 'Trigintipetala'. The reconstruction of the relationship between the new *R. damascena* genotypes and other closely related rose species endemic to this geographic region will provide important insight into the parental origin and genome structure of *R. damascena.*

The high stability of the SSR alleles in vegetatively propagated damask roses (Rusanov *et al.* 2005a) and the possibility to determine the allele configuration through MAC-PR analysis (Babaei *et al*. 2007) makes SSR analysis a very efficient tool for such studies. The MAC-PR (microsatellite DNA allele counting-peak ratios) method was recently developed for the analysis of SSR profiles of polyploid plants and provides information on SSR allele configuration based on quantification of microsatellite allele peak areas (Esselink *et al.* 2004). Another option for probing the origin of damask roses is the use of AFLP markers. In a recent study Koopman *et al*. (2008) applied AFLP markers to try to reconstruct the (species) relationships in *Rosa*. *Rosa damascena* 'Ispahan' was demonstrated to be closely related to *R. gallica* 'Boule de Nanteuil' and *R. centifolia* L. 'Blanche Moreau', but the authors did not include in the study *R. damascena* cultivars used for rose oil production. Extending the MAC-PR and AFLP analyses to the newly discovered *R. damascena* genotypes from Iran and closely related endemic *Rosa* species from this region should provide a better understanding of the origin of the overall group of damask roses.

The commercially cultivated *R. damascena* 'Trigintipetala' and the old garden damask roses are tetraploid plants (Saakov and Rieksta 1973; Topalov 1978), although diploid and hexaploid *R. damascena* samples were recently described in Iran (Tabaei-Aghdaei *et al.* 2007). In spite of the economic importance of oil-bearing *R. damascena*, the genome structure and genetics of this species is very poorly understood. For a long time the initiation of genetic studies in *R. damascena* was largely discouraged due to the expected negative impact of cross-breeding on the composition and quality of rose oil. Genetic studies were also substantially hampered by the lack of suitable marker systems and the complex tetraploid and possibly triparental nature of this species. The formation of a low number of seeds from cross or self-pollination combined with low seed germination complicate the establishment of segregating populations for genetic studies.

The successful application of a number of SSR markers from other *Rosa* species to *R. damascena* (**Table 1**) allows initiation of genetic and mapping studies for oil-bearing damask roses. In a recent study we characterized a small population of plants derived from seeds collected from a small isolated plantation of oil bearing *R. damascena* 'Trigintipetala' plants using SSR markers (Russanov *et al.* 2005c). Since all plants in the plantation originated from vegetative propagation of one oil-bearing rose cultivar, they could be considered to be the same genotype, i.e. clones. The SSR analysis suggested that all tested plants from the seedling population resulted from self-pollination of the plants of this genotype within the plantation. This demonstrates that a segregating population from *R. damascena* 'Trigintipetala' could be efficiently generated based on seeds from a small number of closely-planted, open-pollinated plants, which are vegetatively propagated from a genotype of interest.

Further analysis of the segregation of the SSR alleles within the characterized seedling population shows that some of the alleles segregate in a manner consistent with autotetraploid (polysomic) inheritance, while the segregation of other alleles was consistent with allotetraploid (disomic) inheritance. Thus, *R. damascena* could be described as a segmental allotetraploid with its type of allele inheritance depending on the chromosomal location of the corresponding locus. Segmental polyploidy was described for other modern roses as a result of various meiotic abnormalities and suppression of some chromosomal pairing arrangements (Shahare and Shastry 1963; Ma *et al*. 2000). A more detailed study of the allelic inheritance in *R. damascena* could provide greater insight into its complex genome structure. Also, mixed inheritance patterns could significantly complicate the interpretation of segregation data and must be considered in the planning and conducting of gene/genome mapping studies, QTL analysis and marker assisted selection.

IMPROVEMENT OF OIL-BEARING DAMASK ROSES

Prerequisites and limitations

Rose oil production is a very old industry based on cultivation of damask rose cultivars originating from one superior genotype (*R. damascena* 'Trigintipetala'). The cultivation of other rose cultivars results in distillation of considerably lower amounts of rose oil possessing a different composition and odour. The century old traditions to produce the finest quality of rose oil, its high and constantly growing price and well established world market make rose growers, rose oil producers and consumers very conservative and reluctant to introduce and use new genotypes since they will likely result in rose oils with a different composition and odour. Thus, the preservation of the traditional odour, essential characteristics and composition of the extracted rose oil are the ultimate prerequisites for evaluation of new rose cultivars as well as any changes in flower collection and distillation practices. This is the main reason cross-breeding is traditionally avoided in the improvement of oil-bearing damask roses.

Desired traits

An increase in flower yield, oil content and more generally plant vigour was and continues to be the main objectives for rose oil breeding programmes. The main diseases with a large economical impact on production of oil-bearing damask roses are black spot (*Diplocarpon rosae* Wolf), rust (*Phragmidium mucronatum* (Pers.) Schltdl.), mildew (*Sphaerotheca pannosa* (Wallr.; Fr) Lev.) and, depending on the climate conditions, botrytis blight (*Botrytis cinerea* Pers.: Fr.). Rose plants, and especially the flowers and young leaves, are attacked by a number of pests, some of the most important being *Agrilus mokrzeckii* Obubr., *Rhynchites hungaricus* Herbst, and *Macrosiphum rosae* L., among others. Disease and pest control is performed by limited sprays of pesticides before the start of flowering (Nedkov *et al*. 2005). Reduction of the costs associated with spraying and growing demands for the production of organic rose oil (Gunes 2005; Organic products 2007) make the development of new rose cultivars which are less susceptible to diseases and pests of increasing importance for oil rose improvement. The cultivation of oil rose landraces and cultivars well adapted to the local climate conditions and tolerant to cold temperatures, generally solve the problem of winter freezing (Raev 1984; Astadjov 1988; Haghighi *et al*. 2008). On the other hand, the insufficient and irregular rainfall in rose plantation regions have made drought tolerance a desired trait for oil rose improvement over the last decade (Gunes 2005).

Current status of oil rose breeding

As discussed above, hybridization was generally not applied for the improvement of oil-bearing damask roses. Some hybrids of *R. damascena* with *R. gallica* and other rose species demonstrate greater tolerance to diseases and extreme environmental conditions. In spite of this, they have not been used for industrial cultivation since the characteristics of the obtained rose oil did not match rose oil standards (Staikov and Kalaijiev 1980). The main method for improving oil-bearing damask roses during the last cen-

tury has been intra-clonal selection. *Rosa damascena* 'Trigintipetala' rose plants growing in rose plantations were screened for their vigour, flower yield and oil content. Plants that performed well were selected, propagated and further characterized (Astadjov 1975). At present, all industrial oil rose plantations in Bulgaria are based on cultivation of highly productive populations of selected *R. damascena* 'Trigintipetala' intraclonal lines including: population No. 5, 'Iskra', 'Svejen', 'Eleina' and 'Janina'. Population No. 5 (Astadjov 1988), 'Iskra' (Astadjov 1978) and 'Svejen' (Staikov and Astadjov 1975) were established by selecting clones with higher flower yield, oil content and the typical *R. damascena* 'Trigintipetala' odor. 'Eleina' and 'Janina' were developed through application of radiation and chemical mutagenesis and possess elevated tolerance to freezing and rust (*Phragmidium mucronatum*) (Raev 1984). In Bulgaria the oil rose planting material is produced by the Institute of Rose and Aromatic Plants, Kazanlak. Similarly, in Turkey and Iran the oil rose industrial plantations are established with planting material produced by rose growers after vegetative propagation of vigorous and productive plants (Haghighi *et al*. 2008).

PERSPECTIVES FOR BIOTECHNOLOGY

Gene transfer could provide straightforward solutions for improvement of oil-bearing damask roses and allow the development of disease- and pest-tolerant rose lines without significant changes to the odour and composition of the rose oil. Obtaining disease-tolerant transgenic plants was already demonstrated for cut roses (Dohm *et al.* 2002; Li *et al.* 2003). However, the opportunities for the development and industrial utilization of transgenic oil-bearing roses in the near future are greatly restricted by two factors.

First, there is a lack of an efficient genetic transformation procedure for *R. damascena* 'Trigintipetala'. Although successful plant regeneration from *R. damascena* 'Trigintipetala' and *R. damascena* grown in India (Ishioka and Tanimoto 1990; Pati *et al.* 2004) were reported, the applicability of these regeneration procedures for genetic transformation remains to be elucidated. At the AgroBioInstitute, Bulgaria we have successfully generated transgenic rose plants through somatic embryogenesis and *Agrobacterium* mediated genetic transformation of two *R. hybrida* cultivars (Borissova *et al.* 2005). However so far we have not been successful in genetic transformation of 'Trigintipetala' (A. Borissova pers. comm.).

Secondly, low acceptance towards cultivation and utilization of genetically modified plants has also substantial negative impact on the possibility for gene transfer application for oil rose improvement. For example, the current law on genetically modified organisms (GMOs) in Bulgaria prohibits the release of genetically modified plants into the environment of several crops including oil-bearing rose (Law on genetically modified organisms, 2005). Although the recent change in this law allows cultivation of genetically modified oil roses for research purposes (Amendment to the law on genetically modified organisms 2008), it is difficult to find public or industry funding to perform gene transfer research on oil-bearing roses.

Although earlier attempts for production of essential oil from *in vitro* cultures of *R. damascena* were not successful, cell suspension cultures of *R. damascena* were found to produce valuable individual compounds with biological activity or fragrance (Banthorpe and Barrow 1983; Resnikova and Rodeva 1985; Banthorpe *et al.* 1988; Pavlov *et al.* 2005). This suggests that large scale *in vitro* cultures of *R. damascena* could be efficiently used for production of valuable individual compounds through biosynthesis or biotransformation.

PERSPECTIVES FOR MOLECULAR BREEDING

The past decade has seen rapid progress in rose molecular genetics and rose flower scent research. Various types of molecular markers were successfully applied for characterization of rose genetic diversity and resources, as well for identification of rose cultivars (Debener *et al.* 2000; Esselink *et al.* 2003; Baydar *et al.* 2004; Esselink *et al.* 2004; Rusanov *et al*. 2005a; Yan *et al*. 2006; Kiani *et al*. 2008; Koopman *et al.* 2008). Several molecular genetic linkage maps were established for both diploid and tetraploid roses (Rajapakse *et al.* 2001; Crespel *et al.* 2002; Dugo *et al.* 2005; Yan *et al.* 2005; Oyant *et al.* 2008). An integrated map involving molecular and morphological markers was constructed towards the development of a reference map for rose (Yan *et al.* 2005). Genes and QTLs controlling the vigour, number of prickles, double corolla and recurrent blooming were localized (Crespel *et al.* 2002; Yan *et al.* 2007; Oyant *et al.* 2008). Genes related to disease resistance were identified and characterized (Linde *et al.* 2004; Hattendorf and Debener 2007). At the same time the application of a genomics approach for characterization of the transcriptome of rose petals led to the establishment of collections and databases of petal expressed cDNAs (Channelière *et al.* 2002; Guterman *et al.* 2002; Jung *et al*. 2004, 2008; http://www.bioinfo.wsu.edu/gdr/). A number of genes involved in scent production were identified: sesquiterpene synthase (Guterman *et al.* 2002), alcohol acetyl transferase (Shalit *et al.* 2003), *O*-methyltransferases (Lavid *et al.* 2002; Scalliet *et al.* 2002, 2006), etc.

The described recent progress in rose molecular genetics and biology outside of *R. damascena* provides a solid background and exciting opportunities for the application of molecular breeding in the improvement of the oil-bearing damask rose as well as for identification of new genes related to the biosynthesis of essential constituents of the rose oil. First, the established genetic linkage map developed from other rose species could serve as a starting point for more efficient application of the molecular markers into breeding programmes. The discovery that SSR markers developed for other rose species are transferable to *R. damascena* (Baydar *et al.* 2004; Russanov *et al.* 2005a; Babaei *et al.* 2007) makes them excellent anchor points for application of the established rose maps and alignment with the newly obtained data from gene mapping studies in oil-bearing rose. Moreover, the available sequence data from the identified petal-expressed genes involved in scent formation of other *Rosa* species could be converted to gene-/allele-specific molecular markers and directly used for molecular breeding for rose oil constituents.

In a recent pilot study we tested the possibility to develop allele-/gene-specific SNP (Single Nucleotide Polymorphism) markers for *R. damascena* based on the available sequence data of 15 petal expressed genes involved in scent formation in different *Rosa* species (unpublished data). The bidirectional sequencing of the PCR fragments amplified from genomic DNA of oil-bearing damask rose reveals that the sequences of six of the PCR fragments contain well detected SNPs sufficient for identification of the four alleles of the corresponding gene. No SNPs were detected in two other PCR fragments and the sequencing of the remainder of the PCR fragments produced overlapping, unreadable sequences possibly as a result of small deletions / insertions in some of the alleles of the studied gene or parallel amplification of a region of another member of the same gene family. Continuing analysis demonstrates the possibility for direct conversion of the sequence data into allele-/gene-specific SNP markers through sequencing of PCR-amplified gene regions. At the same time the results point out the necessity of using other types of markers (e.g. SCAR or PCR-RFLP) for solving 'problem' cases with overlapping sequences. The application of allele-/gene-specific markers offers a straightforward application of the gene sequence data into breeding programmes based on bulk segregant analysis and marker assisted selection. It allows characterization and identification of allele configuration related to a particular desired phenotype and selection of genotypes carrying the allele configuration.

One critical question is what kind of segregating popu-

lation could best be used for molecular breeding of oil-bearing rose in order to avoid unwanted changes in the rose oil composition, odour and content? As discussed earlier, *R. damascena* is a segmental allotetraploid possessing a complex genome structure with low seed set and poor seed germination. The same is generally true for interspecific hybrids involving the oil-bearing damask rose which makes the application of multiple back-crossing a very difficult task. The recently discovered large genetic diversity among the *R. damascena* accessions collected from Iran (Pirseyedi *et al.* 2005; Babaei *et al.* 2007) could be used as a source for the transfer of desired traits through intraspecific crosses with *R. damascena* 'Trigintipetala' and is expected to result in less changes in rose oil characteristics. Although very promising, this requires better characterization of the available *R. damascena* accessions for the genetic nature of the desired traits and parameters of their rose oil. A second option is to utilize the heterogeneity and heterozygosity of the *R. damascena* 'Trigintipetala' genome. A preliminary observation shows that most of the plants from the seed progeny of self-pollinated oil-bearing damask rose (see above) have 'Trigintipetala' odour, but display segregation for morphological traits. This suggests the possibility for selection of improved clones from self-pollinated 'Trigintipetala' populations which possess improved agronomic traits, yet have similar odour and oil rose composition. The characterization of such a population will provide an opportunity to identify regions of the genome including desired traits and, at the same time, would be good starting material for initiation of well targeted molecular breeding programmes in oil-bearing damask rose.

CONCLUDING REMARKS

Cultivation of the oil-bearing damask rose and distillation of rose oil from its petals is a centuries-old industry based on very conservative traditions and production practices. It resulted in what we know today as modern rose oil, one of the finest essential oils derived from one superior form, *R. damascena* 'Trigintipetala'. Recent DNA marker studies show that the present industrial cultivation of *R. damascena* is based on one or very few genotypes undistinguishable from 'Trigintipetala'. The high genetic diversity discovered among the landraces of *R. damascena* growing in Iran points to this region as being the centre of diversity for this species. This latter finding offers a new opportunity for addressing the origin of *R. damascena* and provides new genetic resources for cultivar improvement.

The characterization of a small segregating population of self-pollinated seedlings of *R. damascena* 'Trigintipetala' suggests that 'Trigintipetala' is a segmental allotetraploid with a type of allele inheritance depending on the chromosomal location of the locus. The complex genome structure of *R. damascena* and the ultimate prerequisite for preservation of traditional odour, essential characteristics and composition of the distilled rose oil make the improvement of the oil-bearing damask rose a very challenging task. The recent progress in molecular genetics, genetic map construction and gene identification in other rose species, together with the demonstrated transferability of molecular markers, generate a critical mass of molecular genetics data and methods for initiation of molecular marker assisted breeding programmes for improvement of oil-bearing *R. damascena.* Such breeding programmes could involve intraspecific crosses with recently discovered genetically diverse genotypes of *R. damascena* or the utilization of the high heterozygosity of the *R. damascena* 'Trigintipetala' genome. Thus, the enlarging knowledge on molecular genetics of oil-bearing *R. damascena* 'Trigintipetala' makes its further improvement possible along with the preservation of its traditional oil quality and characteristics.

REFERENCES

Achuthan C, Babu B, Padikkala J (2003) Antioxidant and hepatoprotective effects of *Rosa damascena. International Journal of Pharmacognosy* **41**, 357-361

Agaoglu Y, Ergul A, Baydar N (2000) Molecular analyses of genetic diversity of oil rose (*Rosa damascena* Mill.) grown in Isparta (Turkey) region. *Biotech-nology and Biotechnological Equipment* **14**, 16-18

Amendment to the law on genetically modified organisms (2008) *State Official Gazette of the Republic Bulgaria* **43** (in Bulgarian)

Aridogan BC, Baydar H, Kaya S, Demirci M, Ozbasar D, Mumcu E (2002) Antimicrobial activity and chemical composition of some essential oils. *Archives of Pharmacal Research* **25**, 860-864

Astadjov N (1988) Improved populations of Kazanlushka oil bearing rose. *Rastenievudni Naouki* **6**, 21-26 (in Bulgarian)

Astadjov N (1978) Iskra - new cultivar of Kazanlushka oil bearing rose. *Rastenievudni Naouki* **8**, 59-63 (in Bulgarian)

Astadjov N (1975) Evaluation of accessions of Kazanlushka oil bearing rose. *Rastenievudni Naouki* **5**, 19-26 (in Bulgarian)

Babaei A, Tabaei-Aghdaei SR, Khosh-Khui M, Omidbaigi R, Naghavi MR, Esselink GD, Smulders MJ (2007) Microsatellite analysis of Damask rose (*Rosa damascena Mill.*) accessions from various regions in Iran reveals multiple genotypes. *BMC Plant Biology* **7**, 12-19

Banthorpe D, Branch S, Poots I, Fordham W (1988) Accumulation of 2-phenylethanol by callus derived from leaf-bud of *Rosa damascena*. *Phytochemistry* **27**, 795-801

Banthorpe D, Barrow S (1983) Monoterpene biosynthesis in extracts from cultures of *Rosa damascena*. *Phytochemistry* **22**, 2727-2728

Basim E, Basim H (2003) Antibacterial activity of *Rosa damascena* essential oil. *Fitoterapia* **74**, 394-396

Baydar N, Baydar H, Debener T (2004) Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. *Journal of Biotechnology* **111**, 263-267

Beales P, Duncan W, Fagan G, Grant W, Grapes K, Harkness P, Hughes K, Mattock J, Ruston D, Sutherland P, Williams T (1998) *Botanica's Roses. The Encyclopedia of Roses*, Random House, Australia, 704 pp

Biolandes, La rose de Bulgarie (2007) Booklet, Biolandes, 40420 Le Sen France. Available online: http://www.biolandes.com

Biswas N, Gupta S, Das G, Kumar N, Mongre P, Haldar D, Beri S (2001) Evaluation of Ophthacare eye drops – a herbal formulation in the management of various ophthalmic disorders. *Phytotherapy Research* **15**, 618-620

Borissova A, Hvarleva T, Bedzhov I, Kondakova V, Atanassov A, Atanassov I (2005) Ag*robacterium*-mediated transformation of secondary somatic embryos from *Rosa hybrida* L. and recovery of transgenic plants. *Biotechnology and Biotechnological Equipment* **19**, 70-74

Boskabady M, Kiani S, Rakhshandah H (2006) Relaxant effects of *Rosa damascena* on guinea pig tracheal chains and its possible mechanism(s). *Journal of Ethnopharmacology* **106**, 377-382

Channelière S, Rivière S, Scalliet G, Szecsi J, Jullien F, Dolle C, Vergne P, Dumas C, Bendahmane M, Hugueney P, Cock M (2002) Analysis of gene expression in rose petals using expressed sequence tags. *FEBS Letters* **515**, 35-38

Crespel L, Chirollet M, Durel CE, Zhang D, Meynet J, Gudin S (2002) Mapping of qualitative and quantitative phenotypic traits in *Rosa* using AFLP markers. *Theoretical and Applied Genetics* **105**, 1207-1214

Debener T, Janakiram T, Mattiesch L (2000) Sports and seedlings of rose varieties analysed with molecular markers. *Plant Breeding* **119**, 71-74

Dohm A, Ludwig C, Schilling D, Debener T (2002) Transformation of roses with genes for antifungal proteins to reduce their susceptibility to fungal diseases. In: *20th International Eucarpia Symposium - Section Ornamentals*, 3-6 July 2001, Melle, Belgium, pp 105-111

Dugo M, Satovic Z, Millan T, Cubero J, Rubiales D, Cabrera A, Torres A (2005) Genetic mapping of QTLs controlling horticultural traits in diploid roses. *Theoretical and Applied Genetics* **111**, 511-520

Esselink G, Smulders M, Vosman B (2003) Identification of cut rose (*Rosa hybrida*) and rootstock varieties using robust sequence tagged microsatellite site markers. *Theoretical and Applied Genetics* **106**, 277-286

Esselink G, Nybom H, Vosman B (2004) Assignment of allelic configuration in polyploids using the MAC-PR (microsatellite DNA allele counting-peak ratios) method. *Theoretical and Applied Genetics* **109**, 402-408

Gudin S (2000) Rose: Genetics and breeding. *Plant Breeding Reviews* **17**, 159-189

Gunes E (2005) Turkey rose oil production and marketing: a review on problem and opportunities. *Journal of Applied Sciences* **5**, 1871-1875

Guterman I, Shalit M, Menda N, Piestun D, Dafny-Yelin M, Shalev G, Bar E, Davydov O, Ovadis M, Emanuel M, Wang J, Adam Z, Pichersky E, Lewinsohn E, Zamir D, Vainstein A, Weiss D (2002) Rose scent: genomics approach to discovering novel floral fragrance–related genes. *The Plant Cell* **14**, 2325-2338

Haghighi M, Tehranifar A, Nikbakht A, Kafi M (2008) Research and current profile of Iranian production of damask rose (*Rosa damascenea* Mill.) *Acta Horticulturae (ISHS)* **769**, 449-455

Hattendorf A, Debener T (2007) Molecular characterization of NBS-LRR-RGAs in the rose genome. *Physiologia Plantarum* **129**, 775-786

Hurst C (1941) Notes on the origin and evolution of our garden roses. *Journal of the Royal Horticultural Society* **66**, 77-289

Ishioka N, Tanimoto S (1990) Plant-regeneration from Bulgarian rose callus. *Plant Cell, Tissue and Organ Culture* **22**, 197-199

ISO 9842:2003, International Standards for Business, Government and Society. Available online: www.iso.org

Iwata H, Kato T, Ohno S (2000) Triparental origin of Damask roses. *Gene* **259**, 53-59

Jung S, Jesudurai C, Staton M, Du Z, Ficklin S, Cho I, Abbott A, Tomkins J, Main D (2004) GDR (Genome Database for Rosaceae): integrated web resources for Rosaceae genomics and genetics research. *BMC Bioinformatics* **5**, 130

Jung S, Staton M, Lee T, Blenda A, Svancara R, Abbott A, Main D (2008) GDR (Genome Database for Rosaceae): integrated web-database for Rosaceae genomics and genetics data. *Nucleic Acids Research* **36 (Database issue)**, D1034-D1040

Kiani M, Zamania Z, Khalighia A, Fatahia R, Byrne D (2008) Wide genetic diversity of *Rosa damascena Mill*. germplasm in Iran as revealed by RAPD analysis. *Scientia Horticulturae* **115**, 386

Kovats ES (1987) Composition of essential oils. Bulgarian oil of rose (*Rosa damascena Mill*). *Journal of Chromatography* **406**, 185-222

Koopman W, Wissemann V, De Cock K, Van Huylenbroeck J, De Riek J, Sabatlno G, Visser D, Vosman B, Ritz C, Maes B, Werlemark G, Nybom H, Debener T, Linde M, Smulders M (2008) AFLP markers as a tool to reconstruct complex relationships: A case study in *Rosa* (*Rosaceae*). *American Journal of Botany* **95**, 353-366

Lavid N, Wang J, Shalit M, Guterman I, Bar E, Beuerle T, Menda N, Shafir S, Zamir D, Adam Z, Vainstein A, Weiss D, Pichersky E, Lewinsohn E (2002) *O*-Methyltransferases involved in the biosynthesis of volatile phenolic derivatives in rose petals. *Plant Physiology* **129**, 1899-1907

Law on genetically modified organisms (2005) *State Official Gazette of the Republic Bulgaria* **27** (in Bulgarian)

Li X, Gasic K, Cammue B, Broekaert W, Korban S (2003) Transgenic rose lines harboring an antimicrobial protein gene, Ace-AMP1, demonstrate enhanced resistance to powdery mildew (*Sphaerotheca pannosa*). *Planta* **218**, 226-232

Linde M, Mattiesch L, Debener T (2004) Rpp1, a dominant gene providing race-specific resistance to rose powdery mildew (*Podosphaera pannosa*): molecular mapping SCAR development and confirmation of disease resistance data. *Theoretical and Applied Genetics* **109**, 1261-1266

Ma Y, Crane C, Byrne D (2000) Meiotic behavior in a tetraploid rose and its hybrid progeny. *HortScience* **35**, 987-1184

Mahmood N (1996) The anti-HIV activity and mechanisms of action of pure compounds isolated from *Rosa damascena*. *Biochemical and Biophysical Research Communications* **229**, 73

Nedkov N, Kunev K, Kovacheva N, Stanev S, Djurmanski A (2005) *Manual for Cultivation of Essential Oil Plants*, IREMK, Kazanlak, Bulgaria, 160 pp (in Bulgarian)

Nikolov N, Dragostinov P, Tsoutsoulova T, Apostolova B, Portarska F (1978) Essence de rose bulgare et autres produits aromatiques de la rose oleifere de Kazanlik. *Pharmachim* **205**, 35-40

Nikolov N, Dragostinov P, Tsousoulova A, Portarska F, Apostolova B, Nenov N (1977) Bulgarian Rose Oil and Its Quality. In: *7th International Congress of Essential Oils*, 7-11 October, 1977, Kyoto, Japan, pp 159-163

Ohloff G (1994) *Scent and Fragrances. The Fascination of Odors and their Chemical Perspectives*, Translated by Pickenhagen W, Lawrence B, Springer-Verlag, Berlin, Germany, 253 pp

Organic products (2007) Market news service, International Trade Center, *UNCTAD/WTO* Issue No **2**

Oyant L, Crespel L, Rajapakse S, Zhang L, Foucher F (2008) Genetic linkage maps of rose constructed with new microsatellite markers and locating QTL controlling flowering traits. *Tree Genetics and Genomes* **4**, 11-23

Ozkan G, Sagdic O, Baydar N (2004) Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Food Science and Technology International* **10**, 277-281

Pati P, Sharma M, Sood A, Ahuja P (2004) Direct shoot regeneration from leaf explants of *Rosa damascena Mill*. *In Vitro Cellular and Developmental Biology - Plant* **40**, 192-195

Pavlov A, Popov S, Kovacheva E, Georgiev M, Ilieva M (2005) Volatile and polar compounds in *Rosa damascena* Mill 1803 cell suspension. *Journal of Biotechnology* **118**, 89-97

Pirseyedi S, Mardi M, Davazdahemami S, Kermani M, Mohammadi S (2005) Analysis of the genetic diversity of 12 Iranian Damask rose (*Rosa damascena Mill.)* genotypes using amplified fragment length polymorphism markers. *Iranian Journal of Biotechnology* **3**, 225-230

Raev R (1984) Mutation breeding of Kazanlushka oil bearing rose (*Rosa damascena Mill*.). *Rastenievudni Naouki* **8**, 92-98 (in Bulgarian)

Rajapakse S, Byrne D, Zhang L, Anderson N, Arumuganathan K, Ballard R (2001) Two genetic linkage maps of tetraploid roses. *Theoretical and Applied Genetics* **103**, 575-583

Resnikova S, Rodeva B (1985) Synthesis and biotransformation of essential oil components in mint and rose cell cultures. In: Butenko R (Ed) *Plant Cell Cultures – Advances in Science and Technology in USSR*, MIR, Moscow, USSR, pp 108-124

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

Rusanov K, Kovacheva N, Atanassov A, Atanassov I (2005b) Microsatellite analysis of oil-bearing roses which do not belong to the species *Rosa damascena* Mill. *Bulgarian Journal of Agricultural Science* **11**, 1-9

Rusanov K, Kovacheva N, Atanassov A, Atanassov I (2005c) Lessons from the microsatellite characterization of a segregating population derived from seeds of open pollinated *Rosa damascena mill f. Trigintipetala* plants. *Biotechnology and Biotechnological Equipment* **19**, 72-79

Saakov S, Rieksta D (1973) *Roses*, Zinatne, Riga, USSR, 116 pp (in Russian)

Scalliet G, Lionnet C, Le Bechec M, Dutron L, Magnard J, Baudino S, Bergougnoux V, Jullien F, Chambrier P, Vergne P, Dumas C, Cock M, Hugueney P (2006) Role of petal-specific orcinol *O*-methyltransferases in the evolution of rose scent. *Plant Physiology* **140**, 18-29

Scalliet G, Journot N, Jullien F, Baudino S, Magnard J-L, Channelière S, Vergne P, Dumas C, Bendahmane M, Cock JM, Hugueney P (2002) Biosynthesis of the major scent components 3,5-dimethoxytoluene and 1,3,5-trimethoxybenzene by novel rose *O*-methyltransferases. *FEBS Letters* **523**, 113-118

Shahare M, Shastry S (1963) Meiosis in garden roses. *Chromosoma* **13**, 702-724

Shalit M, Guterman I, Volpin H, Bar E, Tamari T, Menda N, Adam Z, Zamir D, Vainstein A, Weiss D, Pichersky E, Lewinsohn E (2003) Volatile ester formation in roses. Identification of an acetyl-Coenzyme A geraniol/citronellol acetyltransferase in developing rose petals. *Plant Physiology* **131**, 1868-1876

Staikov V, Kalaijiev I (1980) Results from the hybridization of the oil bearing rose (*Rosa damascena Mill*.). *Rastenievudni Naouki* **5**, 21-33 (in Bulgarian)

Staikov V, Astadjov N (1975) New varieties of oil bearing rose. In: *Nauchna Sesia*, 15-16 May, Sofia, Bulgaria, pp 110-117 (in Bulgarian)

Tabaei-Aghdaei S, Babaei A, Khosh-Khui M, Jaimand K, Rezaee M, Assareh M, Naghavi M (2007) Morphological and oil content variations amongst Damask rose (*Rosa damascena* Mill.) landraces from different regions of Iran. *Scientia Horticulturae* **113**, 44-48

Topalov V (1978) *The Kazanlushka rose and the rose production in Bulgaria* (in Bulgarian). Christo Danov, Plovdiv, Bulgaria

Widrlechner MP (1981) History and Utilization of *Rosa damascena*. *Economic Botany* **35**, 42-58

Yan Z, Bai Y, Teixeira da Silva JA (2006) Molecular markers and their uses in genetic studies in rose. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol 1), Global Science Books, Isleworth, UK, pp 498-503

Yan Z, Denneboom C, Hattendorf A, Dolstra O, Debener T, Stam P, Visser PB (2005) Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. *Theoretical and Applied Genetics* **110**, 766-777

Yan Z, Visser PB, Hendriks T, Prins TW, Stam P, Dolstra O (2007) QTL analysis of variation for vigour in rose. *Euphytica* **154**, 53-62

Younis A, Khan MA, Khan AA, Riaz A, Pervez MA (2007) Effect of different extraction methods on yield and quality of essential oil from four *Rosa* species. *Floriculture and Ornamental Biotechnology* **1**, 73-76