ROSA DAMASCENA – GENETIC RESOURCES AND CAPACITY BUILDING FOR MOLECULAR BREEDING

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ABSTRACT

Rose genetics and genomics research has tremendously advanced during the last 10 years. This mini-review focuses on the current status of knowledge related to the worldwide genetic resources characterization of Rosa damascena, a species which belongs to the Damask group of roses and which has economic importance in several countries related to its cultivation for production of rose oil and rose water. We discuss the needs and options for R. damascena improvement and we present the research activities of the AgroBioInstitute related to R. damascena improvement in frame of the NSF funded DO02-105 "Centre for sustainable development of plant and animal genomics" project.

Keywords: *Rosa damascena*, oil rose, genetic resources, molecular breeding, AgroBioInstitute

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Introduction

The genus Rosa consists of more than 200 species (21). Among these *Rosa damascena Mill* (a.k.a. "the oil rose") belongs to the Damask group of roses which are known for their strong fragrance (53). *R. damascena* has been cultivated as garden rose in some west European collections but it is mainly grown for production of rose oil and rose water obtained after steam distillation of the rose flowers. It is considered that the oil rose originates from ancient Persia (today Iran) and has been later spread to Europe and Northern Africa. By the 14th century the Damask roses were already grown in West European rose collections as garden roses (10). Rose oil is mainly used in the perfumery and cosmetics industry as a base component of many of the modern perfumes but it also finds application in the food industry as a flavour additive. The main producers of rose oil are Bulgaria, Turkey, Iran and India. Smaller amounts of rose oil and mainly rose water are produced in the countries of Northern Africa. The price of the rose oil has been growing during the last years reaching 5000 EUR/kg in 2007. The rose oil and rose flower extracts have been studied for a number of health-beneficial effects including antibacterial activity (4, 8), antioxidant activity (2, 41), antiinfective and anti-inflammatory properties (11), relaxant effects on tracheal chains (13) and anti-HIV activity (35). Positive health-beneficial effects have been reported in most of the studies, however the identification of the respective active compounds requires further investigation. Rose oil (rose otto) has a complex composition consisting of 275 minor constituents and several major compounds including citronellol, geraniol, nerol, phenethyl alcohol, linalool, farnesol, eugenol and eugenol methyl ether. Two of the minor constituents β-damascenone and β-ionone play a significant role for the odour of the rose oil (31, 39). Currently, there is an acting ISO standard (ISO 9842:2003, International Standards for Business, Government and Society, available online: www.iso.org) applying for the rose oil composition.

Rosa damascena – cultivation for rose oil production, current status of the available genetic resources

It has recently been reported by several authors that the worldwide production of rose oil is based on a single or very few genotypes of *R. damascena* Mill (3, 6, 9, 29, 43, 46, 47). First, Agaoglu et al. (3) and Baydar et al. (9) analyzed the oil rose genetic resources in Turkey and could not find difference between the local accessions using RAPD, AFLP and SSR markers. Rusanov et al. (47) demonstrated the same for the available genetic resources in Bulgaria using SSR markers. In the same study the authors analyzed also accessions used for rose oil production from Iran, India and Turkey and revealed that they have the same genotype as the oil rose accessions from Bulgaria. The authors also found no difference between the old garden damask roses "York and Lancaster" (a.k.a. *R. damascena versicolor*) and "Quatre Saisons" (a.k.a. "Quatre Saisons Continue" and "Autumn Damask") grown in west European collections and the analyzed *R. damascena* accessions used for rose oil production. As it was already mentioned the Damask roses originated from ancient Persia where today Iran is situated. It could be expected that if any diversity in *R. damascena* exists it would be discovered in this region. Indeed, Pirseyedi et al. (43) reported high level of diversity among 12 accessions collected from different regions in Iran using AFLP markers. Later in 2007 Babaei et al (6) analysed the diversity among 40 accessions from 28 provinces in Iran using 9 SSR loci. The clustering analysis revealed 9 different groups of which the largest one consisting of 27 accessions possesses the same genotype as the *R. damascena* accessions in Bulgaria and Turkey. Kiani et al. (29) revealed again significant diversity among 41 accessions from different regions in Iran using

RAPD markers. The clustering analysis revealed 10 different groups as again the biggest group consisted of accessions with the same genotype as the ones used for rose oil production in Bulgaria and Turkey.

Bulgaria has been long time recognized as the main producer of highest quality rose oil. The cultivation of *R. damascena* in the country was initiated during the 16th century (52). The main rose plantations in the country are located in the famous "Rose valley" located near the town of Kazanlak. The "Rose valley" is located between two mountains (Stara planina and Sredna gora) which creates unique and beneficial climatic conditions (mild winter and not too hot summer) for the oil rose cultivation in this area. The local climate is especially suitable for the cultivation of the oil rose. Similarly, the oil rose cultivation areas in Turkey and Iran are located respectively in the Isparta region and the Isfahan province. The main producer of planting material in Bulgaria is the Institute for Roses and Aromatic Plants situated in Kazanlak in the heart of the Rose valley. Although the cultivation of *Rosa damascena* in Bulgaria is a tradition with a history of more than three hundred years, the production of rose oil in Bulgaria is based on the cultivation of a single genotype of the thirty-petaled *R. damascena* Mill f.Trigintipetala (47) which has been vegetatively propagated for centuries. As mentioned before the same genotype has been reported by several authors as the main genotype used in Turkey and Iran for production of rose oil. The amount and composition of the rose oil distilled from the rose petals is strongly affected by the genotype of the cultivated Damask rose, the climatic conditions, the time of rose petals harvesting, and the technology used for processing and distillation (37, 38, 52, 57).

Improvement of the oil rose

Desired traits

The most desired traits related to oil rose cultivation are high flower yield, high rose oil content and resistance to diseases. The most common diseases which in some years could severely affect the cultivation of the oil rose are black spot (*Diplocarpon rosae Wolf*), rust (*Phragmidium mucronatum (Pers.) Schltdl.*), mildew (*Sphaerotheca pannosa (Wallr.; Fr) Lev.*) and botrytis blight (*Botrytis cinerea Pers.: Fr.*). Oil roses 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. The control over disease and pests is done through chemicals. Reduction of the costs for chemical spraying as well as the growing demand of organic rose oil production (22) makes the development of disease and pest tolerant oil roses a question of present interest.

As it was already mentioned the rose oil has a complex composition of over 275 compounds. It has been reported (1) that one of the rose oil compounds (methyleugenol) might cause tumors in rats. This makes the development of oil roses with lower amount of methyleugenol another question of present interest.

Perspectives for genetic engineering

The available gene transfer technologies present a unique opportunity for fast development of plants with improved characteristics. Development of disease tolerant transgenic hybrid roses was demonstrated by some authors (17, 33). However, presently several factors hamper the development of transgenic oil roses. Although at the AgroBioInstitute, Bulgaria we have successfully developed GM hybrid roses (12) using Agrobacterium mediated genetic transformation we were not able to develop GM oil roses (Borissova, personal communicaton). Plant regeneration for *R. damascena* has been reported by Ishioka and Tanimoto (25) and by Pati et al. (42) but it has to be revealed if these protocols are applicable for genetic transformation. Moreover, the currently active GMO law in Bulgaria (currently the biggest producer of rose oil in the world) prohibits the industrial cultivation of transgenic oil roses. Although the same law allows the laboratory experiments related to gene transfer in oil roses, currently it is not well justified to invest funds in the development of transgenic oil roses for industrial cultivation.

Perspectives for marker assisted breeding

During the past decades the improvement of the oil rose has been carried out only through intra-species clonal selection. Plants which show good characteristics in the field (vigour, high flower yield, high oil content) have been selected for introduction into genetic resources collection, further characterized and vegetatively propagated for industrial cultivation (5). Crossings between *R. damascena* and other rose species and especially *R. galica* have been attempted by rose breeders (51), however these hybrids have never made their way to industrial cultivation because they show changes in the rose oil composition.

The last 20 years have marked a tremendous progress in the application of DNA markers as a tool for marker assisted selection and breeding (7). During the last decade molecular markers have been successfully developed for roses and used in genetic diversity studies and identification of rose cultivars including *R. damascena* (9, 16, 19, 20, 29, 30, 47, 54) and development of molecular marker genetic maps (15, 18, 40, 44, 55). An integrated genetic linkage map was developed as an effort towards development of a reference genetic linkage map for rose (55). A number of rose genes and QTLs related to disease resistance, vigour, number of pickles, double corolla and recurrent blooming have been identified (15, 24, 34, 40, 56). In addition a large number of petal expressed cDNAs have been identified and stored in databases (14, 23, 27, 28). Genes involved in scent production were identified: sesquiterpene synthase (23), alcohol acetyl transferase (50), O-methyltransferases (32, 48, 49). Transciption factors controlling the rose floral development have also been identified and cloned (36).

All this puts a solid base for initiation of molecular marker breeding strategy for *R. damascena*. *R. damascena* has a complex tetraploid genome with a probable triparental origin

(26). Rusanov et al. (45) reported that *R. damascena* is most probably a segmental allotetraploid species where the type of inheritance (disomic or tetrasomic) depends on the locus position. Building a marker-assisted breeding strategy for *R. damascena* is not a straight-forward task because of the complex genome structure of this species. Currently, there is no widely available software for building genetic maps and finding QTLs for segmental allotetraploids like *R. damascena*. Probably the complex genetics of this species is the reason that *R. damascena* shows low affinity to self-pollination and cross-pollination. However, Rusanov et al. (45) reported that it is easy to create a segregating population of self-pollinated *Rosa damascena* through collection of seeds from industrial fields since the obtained seeds are the result of either self-pollination or pollination with neighbouring plants possessing the same genotype. This creates a good opportunity for start of genetics studies based on segregation of DNA markers and their association with the plant phenotypes.

Perspectives for genomics and metabolomics studies at the AgroBioInstitute (ABI)

Recently, ABI was recognized as a Centre of Excellence in plant and animal genomics by the National Science Fund in Bulgaria - project DO02-105 "Centre for Sustainable Development of Plant and Animal Genomics". The three year project includes both infrastructural upgrade of the ABI and the start of several key research subprojects. One of the research subprojects is dedicated to the oil rose improvement and is called "Oil rose cultivars with improved agronomic traits". This project will put the ground base for a MAS approach for the oil rose breeding. The foreseen work plan includes three interconnected directions: (1) genomics: characterization with molecular markers (SSR markers and SNP markers related to rose scent genes) of an enlarged segregating population of self-pollinated *R. damascena*; characterization with SSR and SNP markers of various hybrids between *Rosa damascena* and *Rosa galica*; (2) metabolomics: GC/MS metabolite profiling of the plants from the segregating population of self-pollinated *R. damascena* and of various hybrids between *Rosa damascena* and *Rosa galica*; (3) Inducible expression of transcription factors related to the expression of homeotic genes responsible for petal differentiation in *R. damascena* cell cultures. The ultimate goal of the genomics and metabolomics directions will be to obtain statistical association between the analyzed SSR and SNP markers with a desired metabolite profile of the analyzed rose plants. The Inducible expression of transcription factors related to the expression of homeotic genes responsible for petal differentiation in *R. damascena* cell cultures will aim to target the accumulation of rose petal specific metabolites in oil rose cell cultures.

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