

Biotechnology of Scented Roses: A Review

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Abstract

Scented rose species are very important in the genus *Rosa* due to their use in highly-prized essential oil production and as garden roses. The fragrance of the rose flower (attar) captured by extraction is one of the most valuable flavor and fragrance products. A comprehensive study of the papers published in the field of in vitro culture and biotechnology of these species, particularly Damask rose, was performed. Then, in separate sections, the articles related to media preparation for callus culture, shoot regeneration and multiplication, rooting, and cell suspension culture were reviewed. In this paper, the stages of the in vitro culture of scented roses, including establishment, multiplication, in vitro rooting, acclimatization, and genetic variability are also considered. Subsequently, investigations on methods of in vitro culture of scented roses by callus, pollen, anther, cell suspension, and protoplast culture are described. Finally, technological improvements for micropropagation, biotechnology, pharmaceutical, and other properties of the Damask rose and other species of scented roses are discussed. Prospects of in vitro culture and biotechnological techniques of scented rose species are also discussed. Among scented roses, the focus of this paper is on four species, namely *Rosa damascena* Mill., *R. gallica* Linn., *R. centifolia* Linn., and *R. moschata* Herrm., which are highly prized for oil production.

Keywords: Chemical composition, in vitro culture, micropropagation, pharmaceutical properties.

Abbreviations: **ABA**, Abscisic acid; **AFLP**, amplified fragment length polymorphism; **BA**, N6-benzyladenine; **BAP**, 6-benzylaminopurine; **cv**, cultivar(s); **d**, day(s); **GA₃**, gibberellic acid; **GC**, gas chromatography; **GC/MS**, gas chromatography-mass spectrometry; **hr**, hour(s); **IAA**, Indole-3-acetic acid; **IBA**, Indole-3-butyric acid; **Kin**, kinetin; **min**, minute(s); **MS**, Murashige and Skoog medium (1962); **NAA**, α -naphthaleneacetic acid; **PCR**, polymerase chain reaction; **PG**, phloroglucinol; **PGR**, plant growth regulator(s); **RAPD**, randomly amplified polymorphic DNA; **SSR**, simple sequence repeats; **TDZ**, thidiazuron; **UV**, ultraviolet; **wk**, week(s); **yr**, year(s); **ZT**, zeatin.

Introduction

The genus *Rosa* which belongs to the family Rosaceae and the subfamily Rosoideae, comprises hundreds of species and thousands of cultivars. Roses are, undoubtedly, one of the most economically important and favorite ornamental and aromatic plants in the floriculture industry. Millions of rose bushes are planted in gardens or pots and billions of rose cut

flowers are sold annually around the world (Khosh-Khui and Teixeira da Silva, 2007). Only a few species among hundreds in the genus *Rosa* are scented (Gudin, 2000), which include *Rosa damascena* Mill., *R. gallica* Linn., *R. centifolia* Linn., *R. moschata* Herrm., *R. bourboniana* Desportes., *R. chinensis* Jacq., and *R. alba* Linn. (Kaur *et al.*, 2007). Among these, only the first four species are highly prized for oil production (Tucker and Maciarelo, 1988). The fragrance of the rose flower captured by extraction is one of the most valuable flavor

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and fragrance products used. The oil, flavor, and fragrance products of these species, like rose oil, rose water, rose concrete, and rose absolute, are used in foods, cosmetics, perfumery, beverages, soft drinks, ice-creams, and as aroma in ointments and lotions (Douglas, 2001; Pati, *et al.*, 2001; Douglas, 1993; Rusanov *et al.*, 2005a). The world production of these products was estimated to be 15-20 tons in 1986, with Bulgaria, Turkey, Morocco, France, and Italy being the largest producers (Douglas, 2001). Presently, Damask roses are grown in several European and Asiatic countries, including Iran, for the commercial production of rose oil and water.

R. damascena, an important species among the scented roses, yields a highly fragrant, commercially-important essential oil. Damask rose oil is primarily used as a fragrance component in pharmaceutical preparations (e.g., ointments and lotions), perfumes, creams, and soaps. Rose oil and absolute are also used extensively as ingredients for fruit-type flavors. Food products in which they are used include beverages, frozen dairy desserts, sweets, baked goods, gelatins, and puddings. Reported levels used are generally below 2 ppm and are thus in minute quantities (Douglas, 2001). It is notable that some scented roses are in the gene pool of modern cultivated garden roses, including *R. odorata* Sweet, *R. moschata* Herrm., and *R. damascena* Mill. (Ma, 1997). Modern cut-rose cultivars are not notable for their scent (Zuker *et al.*, 1998).

Botany, origin and genetics of scented roses

The Damask rose, also known as the Rose of Castile or the Bulgarian rose, originated in Asia Minor. It is a rose hybrid derived from *R. gallica* and *R. moschata* (Huxley, 1992). The original *R. damascena* is a deciduous shrub growing up to 2.2 m tall. Flowers are borne in large fragrant clusters, semidouble, and varying in color from

blush-white to deep pink (Fig. 1A), and the pedicels are long and covered with glandular bristles and small prickles. The leaves are pinnate with five (rarely seven) leaflets. The Damask rose is considered an important type of Old Rose and is also important for its prominent place in the pedigree of many other types. The leaves are usually a soft grey-green color above and downy beneath; the leaflets are oval and simply toothed and usually five per leaf. The branches are very prickly with hooked spines as well as prickly bristles, and the hips are pear-shaped and bristly (Gault and Synge, 1971).

R. moschata (Musk rose) is a species of rose that has long been in cultivation. Its wild origins are uncertain but are suspected to lie in the western Himalayas. *R. moschata* is a shrub that grows up to 3 m in height with single white 5cm flowers in a loose cyme or corymb blooming on new growth. The sepals are 2cm long with slender points. The flowers have a characteristic 'musky' scent which emanates from the stamens which is also found in some of its descendants. The prickles on the stems are straight or slightly curved and have a broad base. The light- or greyish-green leaves have 5 to 7 ovate leaflets with small teeth, the veins are sometimes pubescent, and the rachis possesses prickles. The stipules are narrow with spreading, free tips. Small, ovate fruits called hips are borne and turn orange-red in autumn. *Rosa moschata* J. Herrm. var. *nastarana* Christ in Boiss. (Persian Musk rose) (Fig. 1B) is hardier and grows as a shrub or climbs almost vigorously (Wymb, 1977).

Rosa gallica (Gallic Rose, French Rose, or Rose of Provins) is a species of rose native to southern and central Europe eastwards to Turkey and the Caucasus. It is a deciduous shrub forming large patches of shrubbery, the stems of which have prickles and glandular bristles. The leaves are pinnate, with three to seven bluish-green leaflets. The flowers are clustered one to four together, single with five petals, and are

fragrant and deep pink in color (Fig. 1C). The hips are globose to ovoid, measure 10-

13 mm in diameter, and are orange to brownish in color (Beales *et al.*, 1998).



Fig. 1. A. *Rosa damascena* Mill., B. *Rosa moschata* J. Herrm. var. *nastarana* Christ in Boiss., C. *R. gallica* Linn., D. *R. centifolia* Linn

Rosa ×*centifolia* (syn. *R. gallica* var. *centifolia* (L.) Regel) (the province rose, the cabbage rose, or Rose de Mai) is a hybrid rose developed by Dutch rose breeders in the period between the 1600s and the 1800s or possibly earlier. It is a complex hybrid bred from *Rosa gallica*, *Rosa moschata*, *Rosa canina*, and *Rosa damascena* (Huxley 1992); its exact hereditary history is not well-documented. Individual plants are shrubby in appearance, growing to 1.5-2 m tall, with long drooping canes and grayish green pinnate leaves with 5-7 leaflets. The flowers are round and globular, with numerous thin overlapping petals that are highly scented; they are usually pink (Fig. 1D), less often white to dark red-purple (Beales *et al.*, 1998). Five diploid species (including *Rosa moschata*), one triploid, and one tetraploid ancestral species of modern roses were studied for physical mapping of ribosomal DNA (Fernández-Romero *et al.*, 2001). *In situ* hybridization with the 18/25S rDNA probe revealed signals on one chromosome pair of all

diploid species, corresponding to a single nuclear organizer region per genome in these species. The intensity of the signals was higher in these two diploid species, including *Rosa moschata*, than in the other diploid species.

A molecular study showed that accessions of *R. damascena* from Bulgaria, Iran, and India and old European Damask rose cultivars (cvs.) have identical microsatellite profiles, suggesting a common origin (Rusanov *et al.*, 2005c). Recent studies on the molecular analysis of genetic diversity of *R. damascena* Mill. with randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) markers (Agaoglu *et al.*, 2000; Baydar *et al.*, 2004) did not show any polymorphism among *R. damascena* plants from various plantations in Turkey and Bulgaria. *R. damascena* can now be found in the wild in Morocco, Andalusia, the Middle East, and the Caucasus. As Damask roses were originally introduced from the Middle East into Western Europe, it

is thought that the origin and center of diversity of Damask roses can be found in this region. In Iran, the cultivation and consumption of Damask roses has a long history. Crude distillation of roses was probably developed in Persia in the late 7th century A.D. (Chevallier *et al.*, 1996; Beales *et al.*, 1998; Babaei, *et al.*, 2007).

While in the genus *Rosa* the chromosome number varies from $2n=2x=14$ to $2n=8x=56$ (Short and Roberts, 1991; Gudin, 2000), *R. damascena* Mill. is a tetraploid species (Horn, 1992). Various explanations for the evolution of *R. damascena* have been suggested. Widrlechner (1981) presented evidence to support the theory that the species originated in the eastern Mediterranean as a hybrid between *R. gallica* and *R. phoenicia*. It is also reported that the Damask rose is a tetraploid hybrid species derived from crosses between *R. moschata* and *R. gallica* (Horn, 1992). Another suggestion is that the Damask rose is a tetraploid species consisting of two groups, one being the Summer Damask, which flowers once in the summer of each year, and the second group being the Autumn Damask which has a second flowering in the autumn of each year. The Summer Damask is a hybrid between *R. gallica* and *R. phoenicia* (natives of the eastern Mediterranean), which looks like *R. multiflora* with hairy leaves, and the Autumn Damask which is a hybrid between *R. moschata* and *R. gallica* (Anonymous, 2006). A molecular approach using inter-simple sequence repeat (ISSR) markers was applied to seven species of *Rosa* including *R. damascena* and *R. moschata*. The optimal conditions for ISSR system were determined to be: MgCl₂ concentration of 2 mM, a 1 U quantity of Taq DNA polymerase, 30 ng template DNA, and a 1 μM concentration of primer. The reaction program was as follows: initial denaturation for 5 min at 94°C, 35 cycles of denaturation for 30 s at 94°C, annealing for 45 s at the annealing temperature specific for each primer, extension for 2 min at 72°C, and a final 10 min extension at 72°C

(Jabbarzadeh *et al.*, 2010a). Jabbarzadeh *et al.* (2010b) used inter-simple sequence repeat (ISSR) markers to study phylogenetic relationships among 7 rose species, including *R. damascena* and *R. moschata*. The dendrogram was constructed using the Jaccard coefficient and the UPGMA algorithm. The 7 rose species were classified into 3 major groups with within-group similarity values of >0.58. Group 1 included *R. banksiae*; group 2, *R. canina*, *R. chinensis*, *R. damascena*, *R. moschata*, and *R. hybrida*; and group 3, *R. foetida*. Using GC and GC/MS analyses, Karami *et al.* (2012) examined the chemical compositions of essential oil from nine distinct genotypes of Iranian *Rosa damascena* and concluded that the major hydrocarbons identified in all genotypes were nonadecane (10.7 to 51.2%), heneicosane (3.7 to 18%), eicosane (0.8 to 6.2%), and tricosane (0.5 to 2.4%).

Uses, chemistry, and chemical compositions in floral scents

Guterman *et al.* (2002) used genomic approaches to identify novel scent-related genes and created an annotated petal EST database of 2100 unique genes from two cultivars. They prepared and used DNA chips to perform expression analyses of selected clones. They also performed detailed chemical analyses of volatile compositions, identified secondary metabolism-related genes whose expression coincides with scent production, and discovered several novel flower scent-related candidate genes.

Rosa species are among model plant species which have powerful floral scents and have been used to isolate and characterize enzymes and genes involved in the biosynthesis of floral volatiles. More than 1700 compounds have been identified as floral scents in flowers of 90 families, including Rosaceae. Floral scent production and the composition of flower scents are genetically controlled, but environmental factors such as water stress or temperature influence the produced scents produced. In

flowering plants, attracting and guiding pollinators are the primary functions of floral scents. However, other functions, like defense or protection against abiotic stresses, may be included. Phytochemical analyses of rose scent compositions started in the 1970s, and more than 400 different volatiles have been identified in rose petals (Flament *et al.*, 1993; Dudareva and Pichersky, 2006; Schnepf and Dudareva, 2006). Fragrance is determined by mixtures of volatiles that can be grouped into the following five major series: hydrocarbons (mostly sesquiterpenes), alcohols (mostly terpenes such as geraniol, nerol, and citronellol), esters (mostly acetates such as hexyl acetate or geranyl acetate), aromatic ethers (3,5-dimethoxytoluene, benzyl methyl ether, and methyl-eugenol), and others such as aldehydes, aliphatic chains, rose oxides, and norisoprenes such as β -ionone (Antonelli *et al.*, 1997; Caissard *et al.*, 2005; Vainstein *et al.*, 2006; Debener and Linde, 2009). Among the five rose volatile groups, *R. damascena* and *R. gallica* were in group 2 of alcohol types without orcinol dimethylether, which emit between 35% and 85% alcohol, such as phenylethyl alcohol, citronellol, geraniol, and nerol (Flament *et al.*, 1993).

Rose oil is a colorless or yellow liquid and has a characteristic odor and taste of roses. At 25°C it is a viscous liquid. Upon gradual cooling it changes to a translucent, crystalline mass which can be liquefied by warming (Douglas, 2001). Although rose oil is a very complicated mixture of more than 100 different components, the major component recovered from the blossom is phenyl-ethyl alcohol. The other main components are the rose alcohols geraniol, citronellol, and nerol. Many others are present only in trace amounts but are very important for the overall quality of the oil. An example is the compound damascenone, which is an important odor constituent and is only present in minute amounts. As noted, phenyl-ethyl alcohol is a major oil component, but because of its solubility in

water, it is usually lost in the distillation waters unless collected as rose water. This alcohol is, however, present in the absolute extracted by solvent (Douglas, 2001). Working with scented cultivars of *Rosa hybrida* L., Lavid *et al.* (2002) showed that one of the most prominent compounds in the floral volatiles of many rose cultivars was the methoxylated phenolic derivative 3, 5-dimethoxytoluene (orcinol dimethyl ether). They reported that the cv. 'Golden Gate', which produced relatively high levels of orcinol dimethyl ether showed the most activity when compared with cv. 'Fragrant Cloud', a scented cultivar which emits almost no orcinol dimethyl ether. Caissard *et al.* (2005) concluded that sepals of a moss rose of the *R. damascena* group have a specific volatile organic compound pattern different from that of the petals.

Moreira *et al.* (2005) examined the antimicrobial activities of essential oils from a number of plants, including *R. moschata*, against 4 strains of *E. coli* and suggested the potential use of essential oils as possible food antimicrobial preservatives, particularly those produced by organic methods (Ardogan *et al.*, 2002).

Since carotenoids, beyond their other functions, intermediate in the biosynthesis of apocarotenoid aroma volatiles and act as pigments, particularly in floral and fruit tissues, Huang *et al.* (2009) searched for the genes responsible for the cleavage of carotenoids and cloned carotenoid cleavage (di-)oxygenase (CCD) genes from *R. damascena* using a degenerate primer approach which yielded a full-length cDNA (RdCCD1). The protein of the RdCCD1 gene was able to cleave a variety of carotenoids. Expression of RdCCD1 was studied by real-time polymerase chain reaction (PCR) in different tissues of the Damask rose, and results showed that the RdCCD1 transcript was present predominantly in the Damask rose flower.

Mannschreck and von Angerer (2011) prepared rose oil by distilling blossoms with water and analyzed the oils using gas

chromatography-mass spectrometry. They reported that the most important odorous components of many rose species were 2-phenylethanol, [beta]-ionone, [beta]-damascone, [beta]-damascenone, citronellol, rose oxide, geraniol, and nerol.

Karami *et al.* (2013), working on two distinct genotypes of Iranian *Rosa damascena*, isolated flowers at six stages of flower development using headspace extraction. They showed that the main floral headspace components were phenyl ethyl alcohol, β -citronellol, α -pinene, benzyl alcohol, and geranyl acetate. The relative percentage of phenyl ethyl alcohol increased as flowers developed in both genotypes. The first flower development stage of both genotypes had the lowest amount of β -citronellol. They also showed that geranyl acetate, an important contributor to aroma, was highest in stage 4 (outer petal whorl opened, inner petal whorl closed) (11.6 %) and stage 3 (sepals fully retracted, outer petal whorl beginning to loosen, petal color lightened) (22.7 %) in the first and second genotypes, respectively. This compound was not found in the early stages of flower development of either genotypes.

Scented rose propagation

Traditionally, Damask rose cultivars are propagated by suckers and, albeit rarely, by seeds, cuttings, budding, or grafting. Propagation from seeds may not breed true-to-type. Vegetative propagation is also very slow and time-consuming. *In vitro* techniques provide one of the most exciting procedures of producing new cultivars adaptable to different environmental conditions, providing rapid multiplication of superior cultivars, producing disease-free plants, and speeding up breeding programs. Thus, the rapid propagation of these genotypes is desirable.

In vitro culture of various *Rosa* species has been previously reviewed (Canli and Kazax, 2009; Skirvin *et al.*, 1990; Short and Roberts, 1991; Horn, 1992; Rout *et al.*,

1999; Roberts *et al.*, 2003; Pati *et al.*, 2005; Khosh-Khui and Teixeira da Silva, 2006; Debener *et al.*, 2009), but not specifically scented species. Here, a comprehensive review regarding the *in vitro* culture and biotechnology of scented roses, in particular *R. damascena* is attempted.

Culture Media Requirements

Basic salts

In cell suspension culture, approximately 15% of chlorate resistant strains of Damask rose did not grow on mediums containing nitrate as the sole nitrogen source. These strains lacked the ability to reduce chlorate to chlorite. This observation supports the current idea that chlorate toxicity depends on the activity of nitrate reductase. These strains lost catalase activity following chlorate treatment, indicating that they took up and reduced chlorate. They have a mechanism for tolerating chlorate and its reduction products rather than avoiding them (Murphy and Imbrie, 1981). Cells of *R. damascena* were used to investigate the efflux of K^+ and HCO_3^- ions to the medium (Murphy *et al.*, 1983; Murphy, 1984). Dodras and Brown (2005) reported that in B deficient cells of *R. damascena* cv. 'Gloide de Guilan', a small oxidative burst, was observed coincidental with first cell death and increasing there after. Jabbarzadeh and Khosh-Khui (2005) used full strength Murashige and Skoog medium (1962) (MS) for a tissue culture of the Damask rose. However, Khosh-Khui *et al.* (2009) evaluated full strength MS, $\frac{1}{2}$ MS, $\frac{1}{3}$ MS, and $\frac{1}{4}$ MS and concluded that $\frac{1}{2}$ MS medium increased the rate of proliferation of musk rose.

Gelling agents

Enhanced growth and better shoot proliferation were found in cultures of *R. damascena* grown on a medium solidified with phytigel rather than agar (Kumar *et al.*, 2003). However, rooting was much more pronounced when microshoots were grown on medium gelled with agar. The

number of roots per rooted shoot was also higher in agar gel medium than in media containing phytagel. In separate experiments, the concentration of 8 g.L⁻¹ agar were used for both *R. damascena* and *R. moschata* (Jabbarzadeh and Khosh-Khui, 2005; Khosh-Khui *et al.*, 2009).

Growth regulators

Pati *et al.* (2001) reported that 6-benzylaminopurine (BAP) in a concentration of 5 µM was optimum for shoot proliferation in *R. damascena* and *R. bourboniana*. They also reported (Pati *et al.*, 2004) that culturing *R. damascena* cv. 'Jwala' shoots initially in an induction medium (half strength MS +3% sucrose, + 6.8 µM thidiazuron (TDZ) + 0.27 µM α-naphthaleneacetic acid (NAA) + 17.7 µM AgNO₃) and transferring them after 21 d to a regeneration medium (MS + 2.25 µM N6-benzyladenine (BA) + 0.0.054 µM NAA) was also optimum. *R. damascena* culture initiated on medium supplemented with TDZ and/or cultured continuously on media containing TDZ for 32-48 wk exhibited considerably more shoot proliferation and growth during subsequent cultures on medium containing BA (Kumar *et al.*, 2001). The combination of 2.5-3 mg L⁻¹ BA and 0.1 mg L⁻¹ indole-3-butyric acid (IBA) was the most suitable treatment for proliferation of Damask rose (Jabbarzadeh and Khosh-Khui, 2005). Khosh-Khui *et al.* (2009) reported that in *R. moschata* the highest numbers of shoots per explant (3.14 shoots) were produced when ½ MS + 1.25 mg L⁻¹ TDZ + 0.1 mg L⁻¹ NAA was used, and the highest percentage of proliferation was observed with the concentration of 1.25 mg L⁻¹ TDZ. They also showed that callus formation was observable in this species on ½ MS + 4.0 mg L⁻¹ BAP + 0.1 mg L⁻¹ NAA.

Kumar *et al.* (2001) showed that microshoots from the TDZ-induced culture of *R. damascena* rooted easily on IBA supplemented medium, but Pati *et al.* (2004) rooted the microshoots of *R.*

damascena cv. 'Jwala' by placing them on a half-strength MS liquid medium with 10.0 µM IBA and 3% of sucrose for 1 wk in the dark and later transferring them to a hormone-free medium where they were kept in the light (Pati *et al.* 2004). Jabbarzadeh and Khosh-Khui (2005) reported that the best treatment for rooting the Damask rose microshoots was 2.5 mg L⁻¹ 2, 4-D for 2 wk in an MS medium and then transferring the explants to an MS medium with no plant growth regulators (PGR). The best treatment for rooting musk rose microcuttings was 0.1 mg L⁻¹ IBA + 0.1 mg L⁻¹ 2, 4-D (Khosh-Khui *et al.*, 2009). It has been reported that PG enhanced the multiplication rates of roses *in vitro* so that the number of new axillary shoots per explant increased 30% when PG was added to medium containing BAP and NAA (Salekjalali, 2012). To increase shoot length, shoots on a modified MS medium were supplemented with 2 mg L⁻¹ GA, which resulted in longer shoots and a greater number of shoots per explant (Mahmoudi Noodezh *et al.*, 2012).

Stages of Scented Rose *In vitro* Culture

Establishment

Explant. Leaf, nodal section, and petiole explants have been used for the micropropagation of *R. damascena* (Kumar *et al.*, 2001). In cv. 'Jwala', leaf explants were used for the direct induction of shoot buds. Elite plants were also raised from nodal explants. Petioles from fully-developed young leaves were found to be ideal for the regeneration of shoots (Pati *et al.*, 2004). Among different explants, single-node explants measuring 1.5-2 cm long were selected by Jabbarzadeh and Khosh-Khui (2005), and explants measuring 6-10 mm long were placed in culture vessels containing a medium. Inserting the explants horizontally, vertically, or obliquely had no significant effect on proliferation rate. The results of the investigation of Khosh-Khui *et al.*

(2009) on musk rose indicated that single node explants were superior to shoot tips. Recently, nodal sections were used for *in vitro* propagation of Damask rose (Mahmoudi Noodezh *et al.*, 2012).

Disinfection. Damask rose explants were pre-washed in a commercial dishwashing solution (about 0.1%) for 15 min and then placed under running tap water until used. They were surface-sterilized by immersion in 10% Chlorox (5.25% NaOCl) for 15 min and then rinsed 3 times in autoclaved distilled water (Jabbarzadeh and Khosh-Khui, 2005). To disinfect *R. moschata*, the explants were first washed under tap water for 30 min and then disinfected with different concentrations of Chlorox (5.25% NaOCl) and about 0.1% of a commercial dishwashing solution (Rika). The explants were kept in the disinfectant solution for 5 to 30 min and then rinsed 4 times with sterile distilled water. With this treatment the fungi contamination was controlled, but there was still some bacterial contamination; therefore, gentamicin, cefotaxim and ampicillin were tested. Lastly, the best treatment was 70% alcohol for 30 min and 20% Chlorox for 10 or 15 min plus 50 or 100 mg L⁻¹ of gentamicin (Khosh-Khui *et al.*, 2009). Endogenous contamination was eliminated with the antibiotic cefotaxim (Allahverdi Mamaghani *et al.*, 2010).

Cultural conditions. Anthers of *R. damascena* produced more callus in the dark than in the light. Generally, light reduced the number of anthers producing calli on different media (Tabaezadeh and Khosh-Khui, 1981). The effects of cool fluorescent light and photosynthetically active radiation (PAR) on the micropropagation of *R. damascena* were studied by Kumar *et al.* (2003). The plantlets raised under PAR light source showed higher survival and better *ex vitro* growth than those cultured under cool fluorescent light. When cell aggregates of *R. damascena* were exposed to ultraviolet (UV) light (254 or 360 nanometers) in the presence of 4'-methoxymethyltrioxsalen,

the proportion of cells resistant to NaClO₃ increased. However, the amount of increase was low (three times) and required very specific doses of UV light. The UV treatments did not select for chlorate-resistant cells over chlorate-sensitive cells (Murphy and Imbrie, 1981). Culture conditions were a photoperiod of 16 h with 1500 lux light intensity at 25±5°C for *R. damascena* and a 16 h photoperiod with 29 μmol m⁻² sec⁻¹ light intensity at 25±2°C for *R. moschata* (Jabbarzadeh and Khosh-Khui, 2005; Khosh-Khui *et al.*, 2009). Surface sterilization was carried out with 0.1% HgCl₂ for 5 min. The appropriate seasons for collecting explants were summer and autumn.

Multiplication

Proliferation. Comparing the micropropagation of 3 rose species including *R. damascena*, Khosh-Khui and Sink (1982) observed that all species exhibited shoot-tip proliferation on a medium containing MS basic salts plus supplements. The effects of TDZ and BA on the *in vitro* shoot proliferation of *R. damascena* were investigated by Kumar *et al.* (2001). Cultures initiated on medium supplemented with TDZ and/or cultured continuously on media containing TDZ for 32-48 wk proliferated more shoots during subsequent culture on a medium containing BA. Pati *et al.* (2004) concluded that the highest shoot regeneration of *R. damascena* cv. 'Jwala' was obtained when shoots were kept in the induction medium for 21 d and later transferred to the regeneration medium (Pati *et al.*, 2004). Jabbarzadeh and Khosh-Khui (2005) and Khosh-Khui *et al.* (2009) reported the micropropagation of Damask rose and musk rose, respectively. It was shown that the full-strength MS culture medium containing BAP (2 mg.L⁻¹) and NAA (0.1 mg.L⁻¹) supplemented with PG (100 mg.L⁻¹) produced the best results for shoot proliferation of *Rosa damascena* (Fig.2).



Fig. 2. Proliferation of *Rosa damascena*.

The highest level of shoot multiplication rate (5.9) was recorded at a combination of 5 mg.L^{-1} BAP and 0.1 mg.L^{-1} TDZ. Type and concentration of auxins did not significantly affect shoot multiplication or shoot length. Among various cytokinins, BAP was more effective than kinetin on shoot multiplication. There was no consistent response by both shoot multiplication rate and genotype to different concentrations of growth regulators (Allahverdi Mamaghani *et al.*, 2010). In a recent experiment, for optimal proliferation, a modified MS medium with higher levels of nitrates, calcium, and iron and supplemented with 4 mg.L^{-1} BAP and 25 mg.L^{-1} IAA was used (Mahmoudi Noodezh *et al.*, 2012).

Subculture. Shoots of *R. damascena* induced on a medium containing TDZ and then subcultured 8-12 times on a medium containing BA attained the capacity to grow and proliferate on a medium free from PGR (Kumar *et al.*, 2001). Jabbarzadeh and Khosh-Khui (2005) subcultured established shoots of Damask rose every 4 wk on fresh MS medium containing $2.5\text{-}3.0 \text{ mg L}^{-1}$ BA and 0.1 mg L^{-1} IBA. Among the multiplication stages, the first subculture resulted in the highest proliferation rate; differences between the second and fourth subcultures were negligible (Fig. 3). In *R. moschata* the highest proliferation was obtained in the first subculture, but in the second subculture there were fewer shoots (Khosh-Khui *et al.*, 2009).

In vitro rooting. Rooting in old world roses (including *R. damascena*) was lower compared to modern roses (Khosh-Khui and Sink, 1982). Microshoots of *R. damascena* from the TDZ-induced culture could be easily rooted on an IBA supplemented medium (Kumar *et al.*, 2001). Rooting was much more pronounced in microshoots of *R. damascena* when grown on a medium gelled with agar and kept under PAR light, and the number of roots per rooted shoot was also higher in agar gel medium as compared to medium containing phytigel (Kumar *et al.*, 2001).



Fig. 3. Five different subcultures of *R. damascena* (from left to right)

Pati *et al.* (2004) compared the rooting and growth performance of cuttings raised from *in vitro* and *in vivo* grown plants of *R. damascena*. They treated cuttings with different auxins and, after transferring the cuttings to the soil, recorded their growth performance. The auxin treated cuttings of *in vitro* raised plants responded better than the cuttings of *in vivo* raised plants. Raised plants from these cuttings showed a significantly better response for percentage of rooting, root number, root length, and bottom buds in control treatments. Jabbarzadeh and Khosh-Khui (2005) examined the effects of different treatments including using basal salt concentrations, applications of different auxins in the medium, using a quick-dip method, or using various concentrations of PGR, sucrose and agar. These treatments did not produce any roots in *R. damascena* microshoots. The best treatment for the rooting of shoots was obtained in MS medium with PGR and then transferring to a medium without PGR (Fig. 4). Since the

musk rose is a hard-to-root plant (Khosh-Khui *et al.*, 2009), after experimenting with different concentrations of media and auxins, the roots finally formed on $\frac{1}{2}$ MS medium containing 0.1 mg L^{-1} IBA + 0.1 mg L^{-1} 2,4-D. Quick-dip of microcuttings in 0 to 1500 mg L^{-1} IBA, NAA, and 2,4-D was not successful for rooting this species (Khosh-Khui *et al.*, 2010). Microshoots were rooted with $\frac{1}{2}$ strength of MS medium containing IBA at the concentration of 2 mg L^{-1} with up to 80% rooting (Salekjalali, 2012).

Acclimatization. It has been shown that a combination of photosynthetically active radiation light (PAR) and phytagel is a useful option for the micropropagation of Damask rose (Kumar *et al.*, 2003). Jabbarzadeh and Khosh-Khui (2005) acclimatized the plantlets of Damask rose in a soil mixture consisting of peat moss and sand 1:1 (v/v) and successfully transferred the plantlets to the greenhouse after 3 wk. Gradual acclimatization was recommended for this species (Fig. 4).



Fig. 4. Rooting (left), acclimatization (middle) and acclimatized plant (right) of *R. damascena*

After the successful root production of musk rose, the plantlets were acclimatized in a medium consisting of 1:1 (v/v) peat moss and perlite; the plantlets were successfully transferred to the greenhouse after 25 d (Khosh-Khui *et al.*, 2009). Damask rose plantlets were acclimatized in a soil mixture consisting of mineral perlite and peat moss (2: 1 v/v) and successfully transferred to the greenhouse after 4 wk (Salekjalali, 2012).

Genetic Variability

The variability between clones of *R. damascena* was studied by Stefanov (1975). Up to 10-day differences in flowering dates were shown. The clones also varied in number of buds (500-700/plant), number of petals/flower (31-40), number of stamens (63-111), average flower weight (2-2.5 g), and essential oil content (0.029-0.070%). Patra *et al.* (1987) studied 35 variable selections (on the basis of performance in

flower biomass production and oil yield) of *R. damascena* which had been taken from an original population of 576 plants. They found a non-significant correlation between oil content percentage and the characteristics of flower number, total fresh flower biomass, fresh weight of a single flower, and flowering period. They concluded that oil content is the only character useful for the improvement of essential oil yield and that the biosynthesis of essential oils is not limited by flower biomass yield. A strain of cultured cells of *R. damascena* resistant to shortwave radiation (254 nanometers) was reported by Murphy *et al.* (1979). Murphy and Imbrie (1981) concluded that it was UV light-induced mutations in cell suspension culture of Damask rose that were leading to chlorate resistance.

Microsatellite analysis of 26 oil-bearing *R. damascena* Mill. accessions and 13 garden Damask roses demonstrated that *R. damascena* Mill. accessions from Bulgaria, Iran, and India and old European Damask rose cvs. had identical microsatellite profiles, suggesting a common origin (Rusanov *et al.*, 2005c). In the same study, it was also shown that modern industrial oil rose cultivation is based on a very narrow gene pool and that oil rose collections contain many genetically identical accessions. A long-term vegetative propagation of the Damask roses also revealed high somatic stability for the microsatellite loci analyzed. The diversity of forty landraces of Damask rose collected from 28 provinces of Iran was evaluated by Tabaei-Aghdai *et al.* (2007). They showed positive correlations between the number of petals and flower weight and also number of stamens ($r = 0.630$), while the correlation between number of petals and peduncle length was negative. They also found a negative correlation between oil content and number of stamens. The dendrogram created from data of cluster analysis for morphological characters of eight landraces of Damask rose indicated no relationship of genetic

variation with their collection sites. Pearson's coefficients showed a positive and strong correlation between flower weight and other morphological characteristics (Farooq *et al.*, 2011).

***In vitro* Culture Methods of Scented Rose**

Callus culture

Pati *et al.* (2001) initiated friable callus from cultures of stem and leaf segments of *R. damascena* on MS medium supplemented with varying concentrations of PGR. Among the different concentrations of TDZ and BAP a friable green callus was obtained in musk rose with 2.5-3 mg L⁻¹ TDZ + 0.1mg L⁻¹ NAA or 4 mg L⁻¹ BAP + 0.1 mg L⁻¹ NAA on ½ MS medium using a single node explant (Khosh-Khui *et al.*, 2009).

Pollen and anther culture

Induction of callus from anther culture of *R. damascena* was first reported by Tabeezadeh and Khosh-Khui (1981). Among the media tested, MS medium with 2 mg L⁻¹ indol-3-acetic acid (IAA) and 0.4 mg L⁻¹ kinetin (Kin) was generally the best for anther culture of *R. damascena*. Culture of *R. damascena* when a few petals are visible on the flower bud was recommended.

Cell suspension culture

Murphy and Imbrie (1981) measured the sensitivity of *R. damascena* cultured cells to chlorate by plating samples of suspensions in agar containing NaClO₃. This sensitivity depended on the age of the cultures that were plated. Chlorate-resistant colonies isolated from 5- to 7-d cultures retained their resistance through many generations of growth in medium lacking NaClO₃; they also retained resistance when mixed with sensitive cells. Suspension cultures of *R. glauca* and *R. damascena* were established and used to study the structure of primary cell wall (Joseleau and Chambat, 1984). Callus and suspension cultures of *Rosa damascena* were kept under different

conditions to study secondary metabolites (Banthorpe *et al.*, 1986). Effects of a fungal elicitor and UV radiation on ion transport and hydrogen peroxide synthesis by Damask rose cells were studied by Arnott and Murphy (1991). The low molecular metabolites produced by *Rosa damascena* Mill. 1803 cell suspension culture were studied by Pavlov *et al.* (2005).

Protoplast culture

Protoplast culture of *R. damascena* was studied by Pati *et al.* (2001). The researchers initiated friable callus from stem and leaf segments and reported efficient protoplast culture procedures from cell suspension culture of *R. damascena*. However, the regeneration of plants posed a developmental block in both parental and hybrid calli. Pati *et al.* (2008) reported the fusion of protoplasts of *Rosa damascena* and *R. bourboniana* using polyethylene glycol as the fusogen and later immobilizing them in a thin layer of alginate. They tracked the fused protoplasts and the hybridity of heterokaryons, and the callus development was later confirmed by molecular characterization.

Technological Improvements

Micropropagation

Khosh-Khui and Sink (1982a) micropropagated *R. damascena* using shoot-tip proliferation on MS medium with PGR. Both rooting and acclimatization to the planting medium were lower in *R. damascena* compared with *R. hybrida*. Micropropagation protocol was established in *R. damascena* (Kumar *et al.*, 2001) using leaf, nodal section, and petiole explants. Pati *et al.* (2004) reported a protocol for *in vitro* propagation of *R. damascena* cv. 'Jwala'. *In vitro* rooting of microshoots was accomplished, and plantlets transferred to the soil showed a 90% survival rate. Ishioka and Tanimoto (1990) reported that when callus tissues of Bulgarian rose were cultured on the medium with no ammonium nitrate and containing IAA and BA, buds

formed in the callus. The number of buds was significantly increased by the simultaneous addition of calcium ionophore. Jabbarzadeh and Khosh-Khui (2005) studied the factors affecting micropropagation of Damask rose and reported that plantlets were acclimatized using a soil mixture consisting of peat moss and sand 1:1(v/v) and were successfully transferred to the greenhouse after 3 wk. In another investigation, the successful acclimatization of musk rose micropropagated plants and their subsequent transfer to the field were crucial steps for the commercial exploitation of *in vitro* technology reported by Khosh-Khui *et al.* (2010). After successful root production, the plantlets were acclimatized in a medium consisting of 1:1 (v/v) peat moss and perlite, and plantlets were successfully transferred to the greenhouse after 25 d.

Molecular Characterization

The origin of cvs. 'Champneys' Pink Cluster', 'Blush Noisette' and 'Napoleon' roses was investigated using RAPD (Wagner *et al.*, 2002; Manners *et al.*, 2004). They reported that nearly all polymorphic DNA banding patterns in profile accessions of 'Champneys' Pink Cluster' were accounted for in *R. chinensis* Jacquin and *R. moschata*. They also concluded that these two species are closely related. In another investigation (Frederick *et al.*, 2002), *R. moschata* cultivars were compared by RAPD. The researchers concluded that all cultivars except 'Bremo' were similar, and despite morphological differences, small genetic mutations rather than major genetic alterations were responsible for variability among musk rose cultivars. Lavid *et al.* (2002) identified and characterized two closely related cDNAs from a rose petal library, each of which encoded a protein capable of methylating the penultimate and immediate precursors (orcinol and orcinol monomethyl ether, respectively) to give the final orcinol dimethyl ether product. Baydar *et al.* (2004) reported that even though *R.*

damascena plants grown in Turkey were from the same original populations, they showed some morphological differences. Baydar *et al.* studied the genetic relationships among Damask rose plants using microsatellite and AFLP markers. Twenty-three AFLP and nine microsatellite primer pairs were used. Baydar *et al.* detected no polymorphism among the plants and concluded that all differences among *R. damascena* plants originated from point mutations not detectable by molecular markers. Using RAPD-PCR, Lewis *et al.* (2004) reported that the DNA profile of 'Bremo Double Musk' did not match any of the other *R. moschata* cultivars, but 'Beremo' was a true musk. Characters of different rose cultivars and species by fingerprinting are reported (Zuker *et al.*, 1998).

Rusanov *et al.* (2005b) analyzed a population of *R. damascena* plants derived from seeds of open pollinated plants which were at 22 microsatellite loci and demonstrated that *R. damascena* is a segmental allotetraploid with a polysomic or disomic type of inheritance depending on the chromosomal location of the corresponding locus. Babaei *et al.* (2007) used nine polymorphic sequenced-tagged microsatellite sites (SSRs) and analyzed the biodiversity of 40 accessions of *R. damascena* collected from major and minor rose oil production areas in Iran. All microsatellite markers showed a high level of polymorphism (5-15 alleles per microsatellite marker, with an average of 9.11 alleles per locus). Cluster analysis of genetic similarities revealed that these SSRs identified a total of nine different genotypes. A genotype from Isfahan province, Iran was identical to the Bulgarian genotype. Because the identification of clones and cultivars and their genetic relationships are important for breeding programs, Kaur *et al.* (2007) studied the genetic diversity among six selected elites of *Rosa damascena* using 58 primers, and they found 368 fragments of which 43 were polymorphic. They

identified a decamer primer OPV4 (CCCCTCACGA) which could distinguish six oil-rich cultivars of *R. damascena*. Jabbarzadeh *et al.* (2009) optimized DNA extraction for ISSR studies in seven important rose species of Iran including *R. damascena*. DNA isolation from rose species is particularly difficult because of their large amounts of polysaccharides and polyphenols and other compounds. These substances decreased both the yield and quality of the DNA. Thus, the researchers designed a simple and efficient method for DNA isolation which could overcome this problem.

The biotechnology of roses was reviewed by Canli and Kazaz (2009). Kaul *et al.* (2009) crossed cultivars 'Jwala' and 'Himroz' of *R. damascena* with *R. bourboniana* attempting to combine the oil quality of *R. damascena* and the recurrent flowering habit of *R. bourboniana*. The F1 plants were evaluated using morphological, RAPD, and microsatellite (SSR) markers. They concluded that this approach was advantageous for its rapidity and simplicity in identifying hybrid plants at the juvenile stage. Recently, through work on a collection of Damask from Pakistan and Iran, their characterization was determined through microsatellite markers. SSR markers confirmed the high level of diversity of the *Rosa damascena* germplasm within Iran and showed that the Pakistani genotypes were similar to those from the Iranian provinces of Isfahan, Kerman, and Fars. (Farooq *et al.*, 2013).

Pharmaceutical and other Properties

Pharmaceutical compounds such as ascorbic acid and essential oils are extracted from callus cultures of scented roses (Banthorpe *et al.*, 1983). Arnott and Murphy (1991) studied the effects of a fungal elicitor and UV radiation on ion transport and hydrogen peroxide synthesis by Damask rose cells. The elicitor preparation induced an efflux of K⁺ and a

production of H₂O₂ by cell suspensions of *Rosa damascena*. The kinetics and amounts of K⁺ efflux and of H₂O₂ production and degradation were similar to those observed when the cells were treated with UV radiation (254 nm). It is concluded that cultured cells of *R. damascena* treated with an elicitor derived from *Phytophthora* spp. produced H₂O₂. It is also hypothesized that in Damask rose cells, H₂O₂ is produced by a plasma membrane NAD(P)H oxidase (superoxide synthase) (Bolwell *et al.*, 1998).

R. damascena is also cultivated for its medicinal properties, and this use is steadily increasing in the world. In recent years, the anti-HIV, antibacterial and antioxidant activities of *R. damascena* essential oil have been demonstrated (Mahmood *et al.*, 1996; Achuthan *et al.*, 2003; Basim and Basim, 2003; Özkan *et al.*, 2004). Achuthan *et al.* (2003) reported that the fresh juice of the Damask rose flower exhibited promising *in vitro* antioxidant potential. The partially purified acetone fraction from silica gel column chromatography was found to be active with antioxidant properties. The low molecular metabolites (volatiles and polar compounds) produced by *Rosa damascena* Mill. 1803 cell suspension cultured under different treatments were studied by Pavlov *et al.* (2005). They showed that the main groups of volatiles were hydrocarbons and free acids and their esters and only traces of terpenoids were found.

Antibacterial activity of the extract of *R. damascena* was determined using the agar diffusion method against 15 species of bacteria by Özkan *et al.* (2004). Both fresh and spent flower extracts were effective against all bacteria except *Escherichia coli* O157:H7. However, fresh flower extract was more effective than spent flower extract. Banthorpe and Barrow (1983) showed that callus from the stem of *R. damascena* did not accumulate monoterpenes. However, cell-free extracts of the cultures converted isopentenyl pyrophosphate (IPP) into geraniol and nerol

some 300 times more efficiently than did the optimum extracts from the parent plants. They concluded that the crucial enzymes for monoterpene synthesis were present in the cultures. In another investigation (Banthorpe *et al.*, 1986), callus and suspension cultures of *Rosa damascena* kept under different conditions accumulated negligible amounts of monoterpenes. However, enzymes that converted mevalonate and isopentenyl pyrophosphate into geraniol and nerol were extracted from the apparently inactive callus with activities up to 100 times greater than those from the parent plant. These activities were optimum in cultures that were slow growing or were in the stationary phase. Researchers concluded that both callus and suspension cultures rapidly metabolized exogenously supplied monoterpenes via oxidative pathways.

It was observed that most callus lines derived from explants of axillary buds of *R. damascena* on standard media did not produce essential oil. However, after exposure to a suitable diurnal variation of light and temperature, a small proportion (5 of the 36 lines assayed) accumulated 2-phenylethanol (the main component of the natural oil) and its β-D-glucoside (Banthorpe *et al.*, 1988). The UV absorption ability of various extracts of *R. damascena* was evaluated by Tabrizi *et al.* (2003). The sun protection factor (SPF) was determined, and it was concluded that the UV absorption ability of the prepared cream was due to the presence of flavonoid compounds within the extracts.

Conclusion and Future Prospects

Because of the low oil content in *Rosa* genus and the lack of natural and synthetic substitutes, rose oil is one of the most expensive essential oils in the world market (Baydar and Baydar, 2005). An oil yield of 0.01-0.02% (0.1-0.2 ml oil kg⁻¹ flowers) was achieved in trials. Around 5 tons of flowers would be needed to produce one liter of rose oil (Douglas, 2001).

Considering that Damask rose is a difficult-to-propagate plant (Hajian and Khosh-Khui, 2000), any efforts to commercialize the *in vitro* culture of Damask rose would be worthwhile. However, protocols of *in vitro* techniques in *R. damascena* are restricted to particular genotypes and must be adapted for specific purposes. Research is needed to understand more about the processes underlying and controlling adventitious regeneration and somatic embryogenesis as well as inherent plant factors in Damask rose and other scented rose species.

A high-density genetic map with a number of anchor markers has been created by Yan *et al.* (2005) to be used as a tool in dissecting genetic variation in roses. Linkage maps for 88 individuals were constructed using a total of 520 molecular markers including AFLP, SSR, PK, RGA, RFLP, SCAR, and morphological markers. The SSR markers together with RFLP markers provided good anchor points for future map alignment studies in roses and related species. Using biotechnological techniques to improve essential oil production and selecting new cultivars would advance the use of scented rose species not only for oil extraction but also for use as garden plants. These new techniques would also be fruitful in achieving faster rates of multiplication in tissue culture methods and may be of great commercial value in establishing plantations.

Chlorate-resistant colonies of *R. damascena* isolated from 5- to 7-d cultures retained their resistance through many generations of growth in medium lacking NaClO₃ (Banthorpe *et al.*, 1986). The continuation of such research will undoubtedly result in Damask roses and scented rose plants resistant to other adverse environmental factors. Condliffe *et al.* (2003) believed that sexual hybridization traditionally used for development of rose

new cvs. is time-consuming, and by introducing one useful trait another may be eliminated. For example, improving floral form and production over many decades has resulted in the loss of scent by many modern rose cvs. Therefore, the introduction of specific genes into scented rose cultivars could facilitate the generation of new cultivars with improved traits without the problems associated with conventional breeding. Plant phenotype, yield, and resistance to diseases and pests are characteristics that can be manipulated on an individual basis.

Rusanov *et al.* (2009) reviewed the worldwide genetic resources characterization of *R. damascena* and discussed the needs and options for improving this *Rosa* species. Similarly, Canli and Kazaz (2009) reviewed the biotechnology of roses and concluded that this technology has become an important part of rose breeding and propagation programs, since it can eliminate sterility problems through embryo rescue, shorten breeding cycles via *in vitro* germination, create variation by *in vitro* mutagenesis, and lead to cultivar development via somaclonal variation. Optimizations of the tissue culture protocols for scented roses are crucial to integrating this technology into commercial applications. Genetic transformation is also a promising tool as it eliminates the difficulties associated with sexual hybridization like lengthy breeding cycles, sterility, polyploidy, and high levels of heterozygosity which are available in almost all rose species including scented roses.

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