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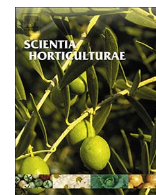

Physiological, phytochemicals and molecular analysis of color and scent of different landrace of *Rosa damascena* during flower development stages

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ABSTRACT

Rosa damascena is one of important flowers for the production of aromatic compounds mainly used in perfume industry. In this research, complete sequences of gene coding geranylgeranyl diphosphate synthesis (*GGPPS*) involved in monoterpenes biosynthesis pathway was isolated from *Rosa damascena* "Kamoor", and the relative expression of genes *GGPPS*, *DXR*, and *CCD1* was characterized during flower developmental stages (A, B and C) in petals of four landraces of damask roses (Ghalhar, Lavasanat, Kamoor and Minab) by real-time quantitative fluorescence PCR. The overall expression of *GGPPS* gene was lower as compared with those of gene *CCD1* and *DXR*. To further study, comprehensive analysis were done among physiological, molecular and phytochemical results to evaluate the relationship between main volatile compounds and colors. The output data revealed that damask roses with light colored flowers showed carotenoid accumulation and accommodation of higher carotenoid content and low anthocyanin content. Main volatile including citronellol, geraniol, neral, lianlyl acetate and dihydro citronellol acetate in flower development increase in the early stage, peaking in stage B and decreasing thereafter. These results suggest that the gene *GGPPS* may play role in the biosynthesis of volatile monoterpenes in *R. damascena* flowers, and this gene could be an important candidate genes for the regulation of secondary metabolism for Damask rose aromatic components.

1. Introduction

Rosa damascena Mill (Damask rose), the hybrid between *Rosa gallica* and *Rosa phoeinica*, belongs to the family Rosaceae (Khosh-Khui, 2014). Called as King of flowers originated in Iran and was introduced to Europe. Now, Damask rose are primarily grown in Iran, Bulgaria and Turkey (Baydar et al., 2004). *R. damascena* is one of the most important species in the genus *Rosa* genus, due to its prominent role in the perfume industry. Highly scented Damask rose are mainly cultivated for rose water, attar and other essential oils for the perfume industry as well as for use in the flavor and fragrance industry, (Mirzaei et al., 2016; Watanabe et al., 2009). Valuable products of *R. damascena* such as scent and color are mainly synthesized from Shikimic acid and methylerythritol 4-phosphate (MEP) pathways.

The most common compounds emitted from the flower of Damask rose are terpenoids including monoterpenes and tetraterpenes. Monoterpenes which represent up to 70% of scent content of rose (Magnard et al., 2015) are derived from basic C_5 unit isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMAPP) synthesized by mevalonic acid (MVA) and MEP pathways which are both localized in the cytosol and plastids. Additionally, the second phase of terpenoids synthesis starts by the reaction, which produces geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP). Geranyl diphosphate synthesis (GPS) allows the formation of the GPP which is a precursor of C_{10} monoterpenoids (Tholl, 2015). GPS as catalyzing enzyme of monoterpenes biosynthesis involved in production of Geraniol, Citronellol, and Nerol.

In the mid- twentieth century, researchers began focusing on structure of molecular compounds, but with the development of equipment in studying plant genetic and genomics, most studies shifted toward identification, separation and purification of key enzyme genes involves in biosynthesis pathways (Mahmoud and Croteau, 2001; Muñoz-Bertomeu et al., 2006; Feng et al., 2014). Biotechnological approaches characterized the key genes and enzymes regulating the

Abbreviations: RT-PCR, reverse transcription polymerase chain reaction; MEP, methylerthiol 4-phosphate; IDP, isopentenyl diphosphate; DMADP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FDP, farnesyl diphosphate; *GGPPS*, geranylgeranyl diphosphate synthesis; *DXR*, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; *CCD1*, carotenoid cleavage dioxygenase; FW, fresh weight; EOs, Essential oils; RCBD, randomized complete block design

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quality and quantity of precursors of main compounds like monoterpenes. In the recent years, the molecular study focused on genetics of rose scent production, resulted in identification of several genes involved in floral scent biosynthesis. These genes include 1-deoxy-D-xylulose-5-phosphate reductoisomerase (*DXR*) and carotenoid cleavage dioxygenase (*CCD1*), which catalyze the production of 1-deoxy-D-xylulose 5-phosphate and C_{13} norisoprenoid such as β- ionone β −damascenone and β −damascone (Feng et al., 2014; Hirata et al., 2012; Huang et al., 2009).

Flower color is affected by water content, as water deficiency causes flowers to turn dark (Schmitzer et al., 2010). Floral scent and color are important characters of rose flowers used in ornamental breeding programs. Dubois et al. (2012) shown that some genes play important role in the production of essential oils and pigments. Here, we study physiological and phytochemical changes during flower development in four landraces of *R. damascena* with different scent and color. Isolation and characterization of *GGPPS* gene, hypothesized to implicate in scent and color production, are reported here. The expression of *GGPPS, DXR* and *CCD1* genes, which are involved in scent and pigment production, was studied at different stages of flower development.

2. Materials and methods

2.1. Plant materials and methods

The landraces of Damask rose, cultivated in a randomized block design with tree replications were used in this study, in experimental Rosarium of Barij Essance Pharmaceutical Company, located in Kashan, Iran. Petal samples were carefully collected from four landraces of *Rosa damascena* including Ghalhar (white), Lavasanat (light pink) Minab (pink) and Kamoor (dark pink) and analyzed at three developmental stages including A (partially open flower), B (fully open flower) and C (senescent flower), according to Schmitzer et al. (2010).

Immediately after harvesting, the fresh weight of petals was measured and transported on ice to the laboratory. For molecular studies, petal samples were immediately frozen in liquid nitrogen, and stored at −80 °C for further analysis.

2.2. Evaluation of display physiological characteristics

Relative fresh weight (RFW), dry weight and water content were measured according to Celikel et al. (2011) by using three replicates of all petals from a single flower at individual test.

For measuring carotenoids, each 25 mg petals sample was dipped in 1 mL of carotenoid extraction buffer [acetone: methanol, 7:3] with 200 mg/mL $CaCO_3$, vortexed and left at room temperature for 1 h. Total extraction was partitioned with diethyl. An aliquot of total extraction was then saponified by mixing with 10% potassium hydroxide: methanol (w/v). After incubating the mixture at 4 °C overnight, the absorbance of the solution was read against a blank at 480, 680 and 666 nm using a spectrophotometer (BIO-RAD, USA). Total carotenoids were measured as described by Zhou (2012).

The color of petals was measured using HunterLab color measurement system (D 25-900, USA) equipment. The colorimeter was calibrated with a white tile plate before use, by placing the device placed vertically in the middle of each petals. Three dimensional measurement of brightness from 0 to 100 (0 = black, 100 = white) were evaluated in pure white (L^*), red-green scale (a^*) and yellow −blue scale (b^*) as described by Robertson (1977). Each individual test evaluated on five flowers per developmental stage according to Schmitzer et al. (2010).

2.3. Essential oils extraction

Rose oil from each landrace was extracted by hydro-distillation of 250 g fresh petal with 750 mL water for 1.5 h, using a 4 L Clevenger type apparatus (Mirzaei et al., 2016). The extracted essential oil was stored in the closed glass vial at 4 °C.

2.4. Gas chromatography (GC)

EOs sample was injected into port of a Thermo–UFM (Ultra-Fast model, Italy) gas chromatograph equipped with a P5 (non-polar) capillary column (10 m × 0.1 mm), which employed helium 0.5 mL/min as a carrier gas to split injection with ratio split 1:100. Oven temperature was set at 60 °C for 30 min, FID detector temperature was programmed at 285 °C at the rate of 80 °C/min and injector temperature was 280 °C. The percentage of the volatile compounds was computed from the peak area using normalization method.

2.5. Gas chromatography −mass spectrometry (GC/MS)

All samples were analyzed using a Varian 3400 gas chromatograph coupled with mass spectrometer model Saturn II, the ion trap system, ionized energy of 70 eV with a semi-polar DB-5 fused silica capillary column (30 m × 0.25 mm) and film thickness 0.25 mm: gas pressure 35 pound per square inch, column temperature 60–250 °C at rate of 3 °C/min, the temperature of injection chamber and the transfer line were set at 260 and 270 °C, respectively. Helium, as the carrier gas, was employed with a linear velocity of 31.5 cm/s, split ratio 1:60, scan time 1 s, mass range 40–300 a.m.u. Spectra identification was done with injection of *n*-tetradecan under the same condition and confirmed according to Wiley 275-L library and literature (Adams, 2012; Shibamoto, 1987; McLafferty and Stauffer, 1989). The Quantitative analysis mass fraction was calculated as following formula:

Content of each component (μg g^{-1}) = [Peak area of each component/Peak area of internal standard × concentration of internal standard (μg μL^{-1}) × volume of internal standard]/sample weight (g).

2.6. Reads assembly and identification of GGPPS gene

A total of 410 ESTs of *GGPPS* were downloaded from *R. hybrida* RNAseq projects (http://www.ncbi.nlm.nih.gov/sra/). Then, high similar EST was selected via Offline BLAST software v.2.7.0 (Altschul et al., 1997). After assembling EST sequences via align-then −assemble a consensus sequence was created using the Codon Code Aligner v. 5.0.1. Program (Fig. 1). Next, an ORF was found for consensus sequences via ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Finally, to validate results, specific primer (Table 1) were designed for PCR amplification of *GGPPS* gene using the Oligo Analyzer v.3.1 (http://eu.idtdna.com/calc/analyzer).

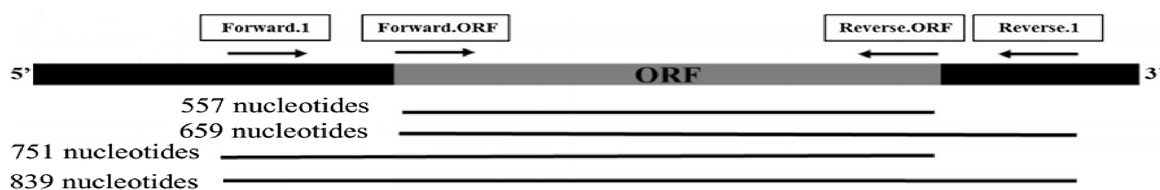

Fig. 1. Nested-PCR primers position and the length of *GGPPS* gene PCR products. Arrow and line show primers and PCR products, respectively. The figure is built by using EndNote software.

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