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Isolation of 2-phenylethanol biosynthesis related gene and developmental patterns of emission of scent compounds in Persian musk rose (*Rosa moschata* Herrm.)

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ABSTRACT

The molecular properties of 2-phenylethanol were investigated in the single petals of Persian Musk rose at four floral developmental stages. The chemical compositions of the floral scent emissions were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). In this plant, the relative percentage of 2-phenylethanol increased as flowers developed. In the early stage of flower development, the patterns of emission of 2-phenylethanol were very low and then progressively increased reaching its highest value at full bloom stage. The partial CDS of phenyl acetaldehyde reductase (*PAR*) were isolated in the petals of Persian musk rose. Moreover, the expression pattern of *RmPAR* showed a significant difference during flower developmental stages and floral organ parts of *Rosa moschata*. The expression level of *RmPAR* constantly decreased from the budding stage (stage 1) to the full bloom stage (stage 3); It is reached at the highest level in the budding stage and rapidly decreases in the full bloom stage, and then increases again in the senescence stage (stage 4). In addition, the highest expression level of *RmPAR* was in the stamen and slightly higher in the petal, and the expression amount in the pistil was about half that in the stamen. Nevertheless, in the calyxes, *RmPAR* was lowly expressed.

1. Introduction

Rosa has sixteen species in Iran of which *R. moschata* Herrm. with the communal names of Persian Musk rose, Rose Anbar and Nastrane Shiraz are one of the most strongly scented rose species with characteristic floral scent molecules such as terpenoids, phenylpropanoids/benzenoids, and fatty acid derivatives (Jandoust and Karami, 2015). The distribution of Persian Musk rose is limited to many local regions of Iran including Fars and Kerman provinces; its wild origins are uncertain but are suspected to lie in the western Himalayas (Honarvar et al., 2011). As Persian Musk rose has not been confirmed clearly in history, but the supposition is that it is a parent of Damask rose (Jandoust and Karami, 2015). The water rose of Persian Musk rose has been used to strengthen heart muscles, stomach, liver, spleen, nerves, and gums and to strengthen intelligence in traditional medicine (Honarvar et al., 2011; Jandoust and Karami, 2015).

One of the prominent scent compounds produced by several fruits such as tomato, grape, strawberry and some roses as well as in the Persian musk rose is 2-phenylethanol (Jandoust and Karami, 2015; Karami et al., 2015; Chen et al., 2015; Sakai et al., 2007). In general, 2-Phenylethanol is used in the perfumery, cosmetics, and food industries (Hua and Xu, 2011). More than 10000 tons of 2-phenylethanol is used annually over the world (Scognamiglio et al., 2012; Hua and Xu, 2011). Moreover, 2-phenylethanol is used as a substrate to synthesize other flavors or therapeutic compounds including phenyl ethyl acetate ester (Scognamiglio et al., 2012). In the planta, the biosynthesis of 2-phenylethanol is achieved from L-phenylalanine (L-Phe) over pyridoxal-5′-phosphate (PLP)-dependent aromatic amino acid decarboxylases (AADC) and PAR (Chen et al., 2011, 2015; Sakai et al., 2007). AADC converted phenylalanine to phenyl acetaldehyde (PAld). Moreover, the preparation of PAld was derived from plant PAld synthase (PAAS), several families of the AADC, in *Petunia hybrida* (Kaminaga et al., 2006) and using AADC in *Arabidopsis* (Gutensohn et al., 2011) and *Solanum lycopersicum* (Tieman et al., 2006, 2007). The PAR action causes conversion of PAld into 2-phenylethanol (Chen et al., 2011; Tieman et al., 2006, 2007). Recent studies confirmed the intermediate compound of

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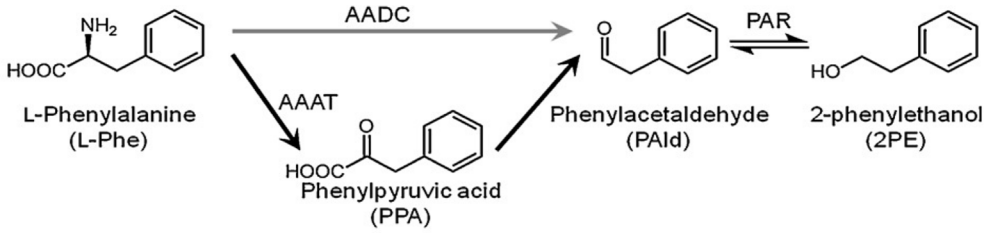


Fig. 1. The recommended biosynthesis pathways of 2-phenylethanol in the different type of Roses (Hirata et al., 2016).

PAld in biosynthesizing 2-phenylethanol in *Rosa hybrida* 'Hoh-Jun', *R. hybrida* cv. 'Yves Piaget', *R. damascena* and *R. rugose* (Hirata et al., 2016; Chen et al., 2015, Feng et al., 2015, Karami et al., 2015, Hirata et al., 2016; Chen et al., 2011; Sakai et al., 2007). In the diverse kind of roses, three enzymes comprising AADC, aromatic amino acid aminotransferase (AAAT) and PAR, were observed to be contributed in biosynthesizing 2-phenylethanol (Fig. 1) (Hirata et al., 2016).

In the present study, the *RmPAR* genes were isolated from *R. moschata*, which also be in the 2-phenylethanol biosynthesis pathway of *R. hybrid*; *R. damascena* and *R. rugosa*. Eventually, the spatial and temporal expression patterns of this gene were analyzed in floral organ parts and different developmental stages of *R. moschata*. Also, the relationship between biosynthesis and 2-phenylethanol accumulation was recognized.

2. Materials and methods

2.1. Plant materials and methods

Rosa moschata was used for extracting floral scent. In relation to the degree of blooming, the petals of flowers were picked up on the 26th of May 2016 during four stages, viz., the budding stage (S1), half bud opening stage (S2), full bloom stage (S3), and senescence stage (S4) (Fig. 2). The flowers in full bloom stage were soon softly detached into organs such as calyx, petal, stamen, and pistil. One part of the fresh petals was instantly used to perform headspace volatile analysis, and other remaining petals and detached flower organs were quickly frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.2. The headspace volatiles screening

The headspace volatiles screening was used as described by Karami et al. (2015). Briefly, three to five branches which have the most flowers were harvested in the early morning from the rose garden in Bajgah, Shiraz, Iran. The branches before transporting the samples to the laboratory were immediately placed in water in a vase. In the each of the floral development stages, petals of five flowers were randomly selected and mixed. About 3 g of each sample was immediately placed in a 20 ml headspace vial and sealed with a silicone rubber septum and an aluminium cap and then moved to the headspace tray. The headspace sampling was done using a Combi PAL System with auto-sampler, heater and, agitator. Though being agitated, the vial content was heated to a temperature of 45 °C, which was retained for 20 min. The temperature of the transmission line and sampling needle was 85 °C.

2.3. GC-MS analysis of the volatile compounds

The headspace was analyzed using a gas chromatograph with Agilent technologies technology with a capillary column HP-5 (5% phenyl methyl siloxane; 30 m × 0.32 mm i.d. × 0.25 μm). The temperature of the injector was 280 °C and the ratio of 1: 50 was used. The oven temperature was primarily ramped from 60 °C to 210 °C at 3 °C min^{-1} and held 8.5 min formerly amassed to 240 °C at 20 °C min^{-1}. Nitrogen was used as carrier gas at a flow rate of 1 ml min^{-1}. The detector temperature was kept at 290 °C. An Agilent 7890 GC-MS, equipped with a HP-5 MS column (30 mm × 0.25 mm × 25 μm), was used to confirm the identity of the compound. Helium was used as carrier gas and the sample was subjected to a ratio of 1:50. The isolated compounds were ionized using an ionization potential of 70 eV. The retention indices were determined using the retention times of series *n*-alkane and under similar chromatography conditions, the sample was examined.

2.4. Quantitative and qualitative analyses

Compounds were identified by comparing retention indices (RI, HP-5) with those reported in the literature and by comparing their mass spectrum with the Wiley GC-MS library, Adams Library, mass spectrometry data was published by Mass Finder 2.1 library, (Adams, 2007; McLafferty and Stauffer, 1989).

2.5. RNA extraction and purification and cDNA synthesis

Rose petals from each stage and flower organ in full bloom stage were instantly separate and frozen in liquid nitrogen and then frozen in −80 °C. According to the manufacturers protocol, high-quality total RNA was effectively isolated from 100 mg of each sample using RNeasy Plant Mini kit (Qiagen, Valencia, CA, USA). Total purified RNA was

Fig. 2. *R. moschata* flowers in full bloom stage.

Table 1
The sequence of the primer prepared for the isolation of PAR-gene in *R. moschata*.

Primer	Sequence	Amplicon length (bp)	Ta
PAR-F	ATGAGCAACAAGGTGGTCTG	963	61.9
PAR-R	GCTGACGATACTCTTTTCCTTCA		

measured with Nanodrop ND 1000 Spectrophotometer (Wilmington, USA). DNase treatment was performed using the Fermentas DNase Kit (Fermentas, Hanover, MD) according to the manufacturer's instructions.cDNA synthesized *via* M-MuLV reverse transcriptase based on the process designated by Karami et al. (2015).

2.6. Isolation of phenylacetaldehyde reductase (PAR)

To isolate *PAR* sequence, primer pairs were designed based on the PARs conserved protein sequences (Table 1) from *R. damascena* (AB426519.2), *R. rugosa* (KP768084.1), and *R. hybrida* (AB972812.1) available at NCBI (www.ncbi.nih.gov). Sequence comparison was performed using GenBank Blast Searches (http://www.ncbi.nih.gov). The *PAR* sequence obtained from *R. moschata* deposited in NCBI with the Genebank number (KY115203.1).

2.7. Sequence analysis

The partial cds sequences of *R. moschta* PAR were evaluated by online blast of NCBI. Homologous searches with the BLASTn nucleotide levels and on the amino acid level with BLASTp were performed online (http://www.ncbi.nlm.nih.gov/blast/).

2.8. Real-time PCR analyses

Real-time PCR was carried out on RNA resulted from three independent test as reported by Karami et al. (2015). The primer pairs in current research were planned with Primer 3 (http://primer3.ut.ee). The Rose elongation factor α (*Ef α*) gene (AB370119.1) was utilized as the reference gene for normalizing data (Table 2). Relative Real-time PCR was obtained in a total volume of 20 ml comprising cDNA (2 µl), 1 × Syber Green buffer and a mixture of target gene primers (2 µl). A Line Gene K thermal cycler (Bioer, China) was used to perform amplification reactions under the subsequent conditions: at 94 °C for 2 min, 40 cycles of 94 °C for 10 s, 15 s at Ta °C (annealing temperature) and 30 s at 72 °C. Following 40 cycles, the animating of amplification products was carried out to 95 °C for controlling their melting curves and approving their specificity. For the Quantitative Real-Time PCR data, determination of relative expression level of *RmPAR* was performed by the threshold cycle (CT) technique with the Line- Gene K software and the technique of Larionov et al. (2005). Consequently, the level of expression of target mRNAs over reference values were computed using the equation of $2^{-\Delta\Delta CT}$ comparative Ct technique (Livak and Schmittgen, 2001). All samplings were performed with three replicates.

Table 2
Sequences of primers used for real-time PCR amplification and the resulting product size of *R. moschata*.

Primer	Sequence	Amplicon length (bp)	Ta
PAR-F	GCCTCGAACTCCTGATGTAGTGG	111	62
PAR-R	GCAGCATCCTCCGCCAAAGT		
EF-α-1F	CTGGACTGACTACTGAAG	103	60
EF-α -1R	ATGATGATGACCTGTGAG		

2.9. Statistical analysis

All data were evaluated in a completely randomized design with three replications. Data were stated as means ± standard deviation (SD). The statistical significance of differences between treatments was determined by Duncan's new multiple range test and IBM SPSS Statistics software version 21 at $p \leq 0.05$.

3. Results

3.1. Gene isolation and sequence analysis of 2-phenylethanol biosynthesis

A homology-based strategy was used to isolate of phenylacetaldehyde reductase (*PAR*) from *R. moschata*. A partial sequence of *PAR* was isolated from *R. moschata*. Genbank's BLAST nucleotide searches showed that the isolated partial *PAR* from *R. moschata* [KY115203.1] had more than 94% sequence identity with other known *PAR* genes of the genus of *Rosa* plant such as *R. damascena* [AB426519.2], *R. hybrida* [AB972812.1], *R. rugosa* cultivar Fenghua [KP768084.1] and *R. rugosa* cultivar Pingyin [KF700273.1] (Fig. 3).

3.2. Expression analysis of PAR in different developmental stages of R. moschata flowers

As shown in Fig. 4, the expression levels of *RmPAR* are significantly different during the development of *R. moschata* from budding stage to senescence stage. The expression level of *RmPAR* constantly decreases at the flower developmental stages; it was very low and scarcely to detect in the full bloom stage (stage 3). The expression level of *RmPAR* constantly decreased from the budding stage (stage 1) to the full bloom stage (stage 3); It is reached at the highest level in the budding stage and rapidly decreases in the full bloom stage, and then increases again in the senescence stage (stage 4).

3.3. Expression analysis of PAR in different flower parts of R. moschata

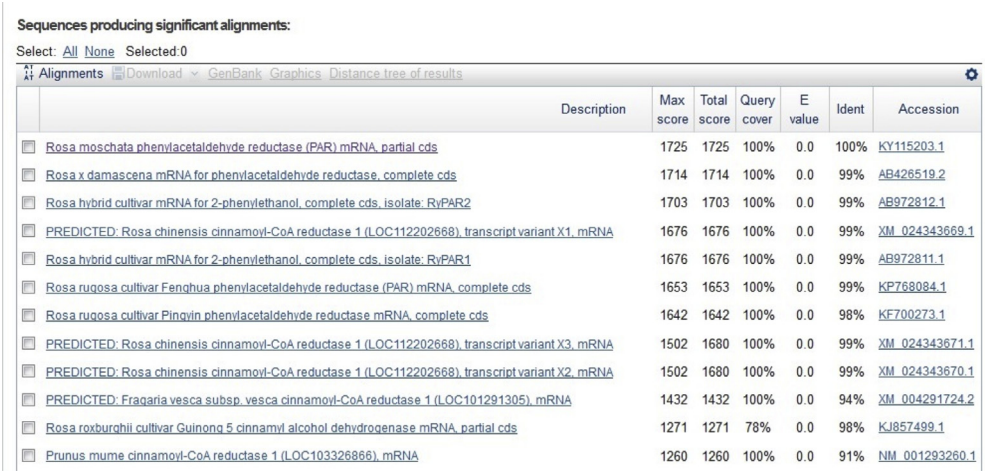
In all parts of *R. moschata* floral organs, *RmPAR* gene was expressed, however, their expression levels are significantly different (Fig. 5). The highest expression level of *RmPAR* was in the stamen and slightly higher in the petal, and the expression amount in the pistil was about half that in the stamen. Nevertheless, in the calyxes *RmPAR* was lowly expressed.

3.4. Headspace analysis of 2-phenylethanol in different flower developmental stages in R. moschata

Changes in the amount of 2-phenylethanol in different developmental stages of *R. moschata* were also measured to further examine the relationship between *RmPAR* expression and synthesis and accumulation of 2-phenylethanol. The 2-phenylethanol content progressively increased with the degree of flower opening. The lower 2-phenylethanol was detected at the beginning stages including budding stage (S1) and half bud opening stage (S2). However, the highest content of 2-phenylethanol (82.421%) was detected at the withering or senescence stage (S4); this value was approximately 2.48 times that at the budding stage (Fig. 6).

4. Discussion

Volatiles have been widely studied in open air (headspace) near the upper parts of the plants. In compared to solvent extractions of volatiles from plant tissues, the headspace analysis gives a more accurate picture of the volatile profile emitted by plants, (Tholl et al., 2006). The volatile analysis by headspace in this study shown that the relative percentage of 2-phenylethanol increased significantly at the floral developmental stages of *R. moschata*. Similar progresses of 2-phenylethanol were stated



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Rosa moschata phenylacetaldehyde reductase (PAR) mRNA, partial cds	1725	1725	100%	0.0	100%	KY115203.1
Rosa x damascena mRNA for phenylacetaldehyde reductase, complete cds	1714	1714	100%	0.0	99%	AB426519.2
Rosa hybrid cultivar mRNA for 2-phenylethanol, complete cds, isolate: RyPAR2	1703	1703	100%	0.0	99%	AB972812.1
PREDICTED: Rosa chinensis cinnamoyl-CoA reductase 1 (LOC112202668), transcript variant X1, mRNA	1676	1676	100%	0.0	99%	XM_024343669.1
Rosa hybrid cultivar mRNA for 2-phenylethanol, complete cds, isolate: RyPAR1	1676	1676	100%	0.0	99%	AB972811.1
Rosa rugosa cultivar Fenghua phenylacetaldehyde reductase (PAR) mRNA, complete cds	1653	1653	100%	0.0	99%	KP768084.1
Rosa rugosa cultivar Pingyin phenylacetaldehyde reductase mRNA, complete cds	1642	1642	100%	0.0	98%	KF700273.1
PREDICTED: Rosa chinensis cinnamoyl-CoA reductase 1 (LOC112202668), transcript variant X3, mRNA	1502	1680	100%	0.0	99%	XM_024343671.1
PREDICTED: Rosa chinensis cinnamoyl-CoA reductase 1 (LOC112202668), transcript variant X2, mRNA	1502	1680	100%	0.0	99%	XM_024343670.1
PREDICTED: Fragaria vesca subsp. vesca cinnamoyl-CoA reductase 1 (LOC101291305), mRNA	1432	1432	100%	0.0	94%	XM_004291724.2
Rosa roxburghii cultivar Guinong 5 cinnamyl alcohol dehydrogenase mRNA, partial cds	1271	1271	78%	0.0	98%	KJ857499.1
Prunus mume cinnamoyl-CoA reductase 1 (LOC103326866), mRNA	1260	1260	100%	0.0	91%	NM_001293260.1

Fig. 3. Comparison of amino acid sequences of biosynthesis gene of 2-phenylalanol from *R. moschata* to other plants derived from BLAST *RmPAR* nucleotide results versus NCBI database.

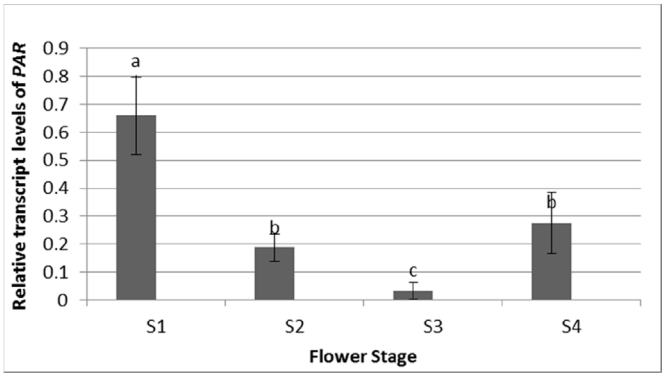


Fig. 4. The relative expression level of *RmPAR* during flower developing stages (S) of *Rosa moschata*. S1 (Stage 1) budding stage, S2 (Stage 2) half opening stage, S3 (Stage 3) full bloom stage, S4 (Stage 4) withering or senescence stage. Error bars are revealed the standard deviation of the mean.

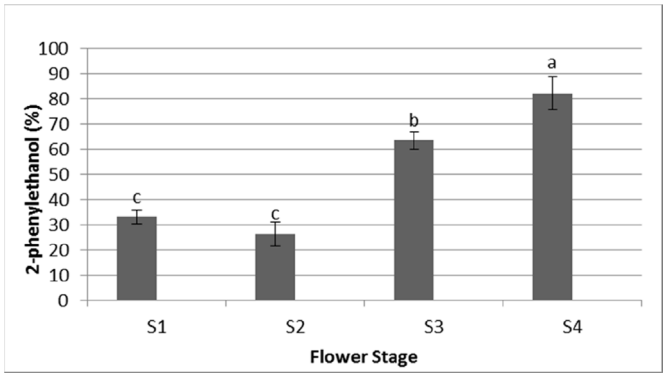


Fig. 6. The relative percentage of 2-phenylethanol in the volatile of *Rosa moschata* at four developmental stage. Error bars are revealed the standard deviation of the mean. S1 (Stage 1) budding stage, S2 (Stage 2) half opening stage, S3 (Stage 3) full bloom stage, S4 (Stage 4) withering stage.

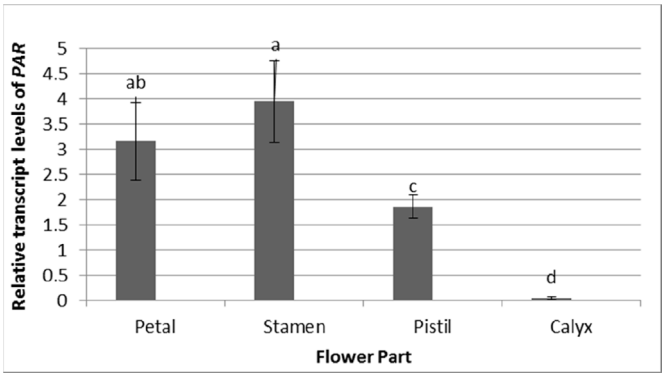


Fig. 5. The relative expression level of *RmPAR* in different floral organ parts of *Rosa moschata*. Error bars are revealed the standard deviation of the mean.

during the development of the flower of *R. hybrida, R. canina* and some genotypes of *R. damascena*. This explanation shows that the main molecule in all oil samples is 2-phenylethanol and its content is steadily increasing during the development process of the flower (Roccia et al., 2019; Feng et al., 2015; Karami et al., 2015; Hosni et al., 2011; Rusanov et al., 2011; Shalit et al., 2004). The main scent compound released from flowers of some hybrid roses (Rosa 'Hoh-Jun' and Rosa 'Yves Piaget'), *R. damascena*, and *R. rugosa* is 2-phenylethanol. This compound is synthesized from L-Phe *via* the intermediary PAld by two key enzymes, AADC and PAR (Feng et al., 2015; Karami et al., 2015; Chen et al., 2011; Sakai et al., 2007). In the current study, we isolated a key 2-phenylethanol biosynthesis related gene, *RmPAR*, from the Persian musk rose. The results of the BLAST comparison of this gene with the same genes found in other plants indicated that the *RmPAR* of *R. moschata* is highly comparable to the *PAR* of *R. hybrid*, *R. damascena*, and *R. rugosa* (Fig. 2). Therefore, *RmPAR* may control the mutual precursor of 2-phenylethanol biosynthesis in *Rosa* plants.

The divergent trends of expression levels of *RmPAR* showed with the relative percent of 2-phenylethanol from the budding stage to the full bloom stage. Particularly, *RmPAR* expression progressively decreased from the budding stage to the full bloom stage (Fig. 4), while the relative percent of 2-phenylethanol gradually increased (Fig. 5). This result may be ascribed to the detail of the aromatic components biosynthesis process in plants including as synthesis, accumulation, and then slowly release procedure (Roccia et al., 2019; Feng et al., 2015). In general, the current research demonstrated that the first steps of flowering (S1 and S2) are different in perfume attributes and fully open flowers (S3 and S4). This can be because of the fact that the last step is under development and producing volatile compounds is associated with their potential activity in attraction and direction of pollinators or protection of plant parts from their adversaries (Dudareva et al., 2004). In this way, several reports have revealed that fragrances of flower are adjusted to last for a longer time for stabilizing pollination (Karami et al., 2015; Hosni et al., 2011; Dudareva et al., 2004).

In parallel to this study, *PAR* transcripts expression of petals for two

different genotypes (SSG and WSG) of damask rose at six steps of floral developmental were examined to prove the contribution of *PAR's* in the biosynthesis of 2-phenylethanol (Karami et al., 2015). The results of earlier research showed higher relative transcript levels corresponding to *PAR* in the petals of damask rose at steps 3 and 4 in SSG and WSG comparing with current research. Moreover, the *PAR* expression pattern exhibited a considerable difference between two genotypes throughout flower developmental steps (Karami et al., 2015). It has been shown that *PAR* can help produce aromatic molecules at the molecular level (Karami et al., 2015; Chen et al., 2011). The maximum transcripts have been found in step 4, which shows a relationship to the highest release of 2-phenylethanol in this stage of *R. damascena*, which has earlier reported (Karami et al., 2013, 2015; Chen et al., 2011). Other genes of rose flower presented the maximum transcription at the similar unfurling step, while the release of volatile compounds was more (Guterman et al., 2002; Lavid et al., 2002). In *R. damascena,* it has been revealed that the petals odor dominated with 2-phenylethanol, which is considered as bug attractants (Karami et al., 2015; Shalit et al., 2004; Dobson et al., 1999). In current research, the chemical analysis corresponding to *R. moschata* obviously presented that the release of higher amount of perfume compounds into the peak has reached throughout the final steps of flower growth. This is a very common property that has been found in a large number of species such as *A. majus* (Dudareva et al., 2004), *Clarkia breweri* (Picherky and Gershenzon, 2002), and diverse rose cultivars (Sun et al., 2016; Karami et al., 2015; Feng et al., 2015; Picone et al., 2004; Shalit et al., 2004; Guterman et al., 2002; Watanabe et al., 2002). In general, this relation between scent emission and flower opening represents the absorption capacity of pollinators for flower (Negre et al., 2003). In the *R.rugosa*, the high expression of *RrAADC* in the budding stage can result in the synthesis of phenylacetaldehydeand then *RrPAR* expression quickly increased as phenylacetaldehyde regularly accumulated, which caused permanent increase in 2-phenylethanol content, thereby increasing the degree of flow opening (Feng et al., 2015). In our research, in the budding stage the expression level of *RmPAR* was highest. The most synthesized phenylacethaldehyde may be converted to 2-phenylethanol under the expression of high *RmPAR* and the precursor substance that is being used continuously as a result, *RmPAR* expression declined rapidly to the lowest level in the full bloom stage. The conversion rate of phenylacetaldehyde is then reduced due to the reduction of *RmPAR* expression. In the other study in *R. rogusa*, it has been shown that despite the continuous decrease in *RrAADC* expression in the half-opening stage, the synthesis and accumulation of phenylethaldehyde is promoted. This portent faintly increased *RrPAR* expression again at the withering stage and marginally preeminent 2-phenylethanol content in the flowers (Feng et al., 2015). In contrast to our study, a positive correlation between the expression levels of *PAR* and the emission of 2-phenylethanol in the *R. hybrida* cv. 'Yves Piaget' was revealed (Chen et al., 2015). Petals are the main parts of flowers that release scent components. In *R. hybrida* cv. 'Yves Piaget' flowers, the petal was the main source of perfume although stamens prepared a significant involvement due to the high concentrations of volatiles in this organ (Chen et al., 2015).

In the different organ of *R. rugosa*, the expression levels of both *RrAADC* and *RrPAR* varied in the different flower organs of the *R. rugosa*. However, the petal had the highest amount of 2-phenylethanol, but the level of expression of both genes in petals was much lower than stamens or pistils and even lower than in the receptacles and calyxes (Feng et al., 2015). In compared to stated study, the level of *RmPAR* expression was the highest in the stamen and faintly higher in the petal, and the quantity of expression in the pistil was about half that in the stamen. Moreover, the expression of *RmPAR* is dimmed in the calyxes. In our study, this phenomenon can be attributed to the fact that high levels of *RmPAR* expression in stamens carry the mobility of enzymes and substrates to petals and promote the synthesis of large amounts of 2-phenylalethanol in petals. However, further examination is required to clarify the basic mechanism.

5. Conclusion

According to the experiments carried out herein, it can be stated that the *RmPAR* gene expression is very different during the flowering development. The level of *RmPAR* expression incessantly decreased at the flower developmental stages; it was very low and hardly detected in the full bloom stage. Moreover, the expression level of *RmPAR* continuously decreased from the first stage to the full bloom stage; it stretched the highest level at the budding stage and quickly decreased at the full bloom stage, and then increased over again at the senescence stage. The content of 2-phenylethanol progressively improved with the stage of flower opening. The lower 2-phenylethanol was perceived at the beginning stages including budding stage and half bud opening stage. The highest 2-phenylethanol content was detected at the withering stage. Based on the idea that the gene expression follows a descending trend from the early stages till the stage before senescence but the scent emission keeps on an ascending trend during this period, the hypothesis proposed, as well, about *R. rugosa* appears to have become more robust that it is most likely the plant's policy to produce enzymes and precursors of scent production and accumulation and then gradually makes use of them to produce and emit scent (Feng et al., 2015).

According to the fact that the gene expression was also found high during withering and senescence stage and that the 2-phenylethanol release was in its peak, it can be concluded that the plant tries emitting 2-phenylethanol scent to attract pollinators in line with its survival practices before being completely withered and this mechanism is plant-specific in establishing communication with the environment in its periphery. Perhaps, it can also be hypothesized that this mechanism of action is well associated with the short life of the *R. moschata*. This plant finishes flower development stage rather quickly and it spends a short time from bud development to senescence and withering so it can be stated that the plants firstly begin gene expression to produce scent precursors and then start scent production and emission with the onset of perfect flower opening stage that lasts till the end of the development stage and senescence and the interval between these two stages is very short. In fact, the plant makes use of this strategy to survive and perform pollination through attracting pollinators to its scent. However, there is a need for further and more precise research, especially repetition of scent examination using other headspace methods so that the results can be more reliably evaluated and interpreted. Also, the investigation of PAR enzyme during various growth stages can assist us in making more accurate conclusions and expressing more realistic hypotheses.

Acknowledgements

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