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Original Article

Correlation between Anthocyanin and Essential Oil Content of Damask Rose (*Rosa damascena* Mill.)

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Abstract

The essential oil of *Rosa damascena* Mill. is one of the most valuable and important base material in the flavor and fragrance industry. It has also some medicinal properties. The aim of this study was to determine the correlation between anthocyanin and essential oil content of Damask rose petals in 6 important Damask rose growing in some locations of Iran i.e. Meimand, Layzangan, Shiraz Eram Botanical Garden, Shiraz College of Agriculture, Kashan and Urumia. The results of this investigation indicated that the essential oil and anthocyanin contents were significantly different in the petals harvested at various locations. The highest oil content (0.155%) and anthocyanin content (2.368) was obtained from the Shiraz Eram Botanical Garden. A high positive correlation (r Sq Linear = 0.812) was obtained between oil and anthocyanin content of Damask rose.

Key words: *Rosa damascena* Mill, Anthocyanin, Essential oil, Positive correlation

Introduction

Rose is the most popular ornamental plant that has been systematically cultivated for its shape, fragrance, a wide variety of colors and essential oils [2]. The three species, *Rosa damascena* (pink damask rose), *R. centifolia* (light pink cottage rose), and *R. alba* (white cottage rose) are predominantly used in the production of rose oils and rose extracts, whereas the modern rose cultivars are exclusively cultivated for the floriculture and nursery industries [14]. In the perfume industry, *Rosa damascena* is the most important species for the production of attar of rose, made by distilling volatile oils from the flowers. It is also used widely in the manufacture of rose water, a flavoring agent [1, 4, 7]. At this time the provinces of Fars and Isfahan in Iran was the centre of global rose water production exporting to China and throughout the Islamic world [4]. Flower color, as well as fragrance, are essential for the attraction of pollinators and hence for the evolutionary success of plants. These two traits are equally important in terms of attracting consumers of ornamentals. Flower pigmentation has been intensively studied in the last several decades and today, the resultant deeper understanding of the underlying pathways has already been harnessed for crop improvement [17]. Flower color investigation of roses so far have shown that four anthocyanins, 3-glucosides and 3,5-diglucosides of cyanidin and peonidin , can be detected in flowers of wild Rose species, and also pelargonidin 3-glucoside and pelargonidin 3,5-diglucoside are detected in Rose cultivars [10]. A study by Nakamura *et al.* [11] indicated that a close correlation between the selection of flower color and essential oil component during daytime. Zvi *et al.* [17] indicated that by increasing in anthocyanin pigmentation, the production of volatile phenylpropanoid / benzenoid compounds increased up to ten fold in *Petunia*.

However, in recent years, a number of researchers have recognized that floral scent and floral color may occur in specific combinations for reasons other than concurrent selective pressures. In particular, the potential for shared biochemical pathways between pigment and volatile production has received significant attention, with the recognition of at least two independent sources of direct biosynthetic connections between these floral characters. First, the

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synthesis of anthocyanin pigment (blue, purple and reds in floral tissue) and the production of certain volatile benzenoid/phenylpropanoid compounds both represent branches of the shikimate pathway, through which plants produce a wealth of pigments, structural materials, phytohormones and defense compounds using phenylalanine as a common precursor [7-9, 16, 17]. Second, the plastid-localized 2-*C*-methyl-d-erythritol 4-phosphate (MEP) biosynthetic pathway in plants can lead to the production of carotenoid pigments (i.e., yellows, oranges and reds) and volatile homoterpenoid and apocarotenoid compounds [5-6, 13]. In either case, several researchers have hypothesized that pleiotropic interactions within biosynthetic pathways may pre-adapt plants to produce specific scent–color combinations, such that production of pigment determines the type and amount of volatile synthesis in floral tissue [6, 7, 13].
Several field studies have assessed this potential mechanism, with mixed results. Research with *Dactylorhiza romana* suggests that red and yellow color morphs differ in the relative amounts of benzaldehyde and linalool they emit, with yellow morphs emitting more benzaldehyde and less linalool while some red morphs released high amounts of linalool [13]. In contrast, assays in the *Orchis mascula* found no relationship between purple/white coloration and scent emission patterns [3], and no strong scent differences exist between red and white morphs of *Corydalis cava* [12]. Examination of floral scent and color in *Hesperis matronalis* has yielded conflicting patterns; a small-scale study revealed population specific differences between purple and white morph odors [9] while a larger study found no statistically consistent differences between color morphs in terms of scent [7]. Unfortunately, field studies such as these cannot control for differences in genetic background within a color morph. For instance, white flower morphs in a polymorphic population could arise from any of a number of null mutations within the anthocyanin pathway, with some reducing metabolic flux through the entire pathway, others increasing the accumulation of volatile precursors, and still others simply affecting the most proximate steps (e.g., a non-functional biosynthetic enzyme) in pigment biosynthesis. However, all of these mutants would be grouped together for analysis as “white” color morphs, obscuring the underlying mechanisms blocking the accumulation of pigment [7].
The aim of this investigation was to study the anthocyanin and essential oil contents of Damask rose petals in 6 important Damask rose growing in some locations of Iran (Meimand, Layzangan, Shiraz Eram Botanical Garden, Shiraz College of Agriculture, Kashan and Urumia).

Materials and Methods

Plant material
The petals of *Rosa damascena* were collected during flowering period, which begins in May to mid-June, (depending on the amount of sunshine and the altitude of the growing region) from Meimand, Layzangan, Shiraz Eram Botanical Garden and Shiraz College of Agriculture (south region of Iran), Kashan (central region of Iran) and Urumia (north region of Iran).The flowers were harvested in the early morning, when starting to bloom.

Anthocyanin content
To determine the anthocyanin content, 100 mg of fresh flower tissue was extracted in 1 mL of methanol containing 1% (v/v) HCl, following overnight incubation in the dark at – 4 °C with shaking at 150 rpm. The extract was centrifuged at 10500 g for 10 min. The anthocyanin content in the supernatant was determined using the formula: $A_{530} – 0.25 A_{657}$ [8].

Essential oil isolation
The collected flowers of investigated plants were air dried at room temperature (less than 25°C) in a shady location for 10 days. The dried samples (50 g, four times for each location) were subjected to hydrodistillation for 4 h using an all glass Clevenger-type apparatus, to extract oils according to the method outlined by the European Pharmacopoeia and was measured gravimetrically (w/w%).

Results and Discussion

The results of this investigation indicated that the essential oil contents were significantly ($P \leq 0.05$) different in the petals harvested at various locations. The highest oil content (0.155%) was obtained from the Shiraz Eram Botanical garden which was significantly different compared to other locations (Table 1). Sefidkon *et al.* [15] reported the essential oil yield of four *Rosa damascena* samples (two samples from national botanical garden of Iran with source of Kashan and Oskou, one sample from Kashan and one sample from Chaloos road) and indicated that a significant difference were present between all tested samples.

Table 1. Essential oil and anthocyanin contents of Damask rose in different exprimental sites.

Exprimental site	Essential oil (%)	Anthocyanin
Meimand	0.055 c*	1.444 b
Layzangan	0.1040 b	2.085 a
Shiraz Eram Botanical Garden	0.1552 a	2.368 a
Shiraz College of Agriculture	0.03615 d	0.8045 c
Kashan	0.053 c	0.8608 c
Urumia	0.1107 b	1.748 b

* Means in each column with the similar letters are not significantly different at 5% level of probability using Lsd test.

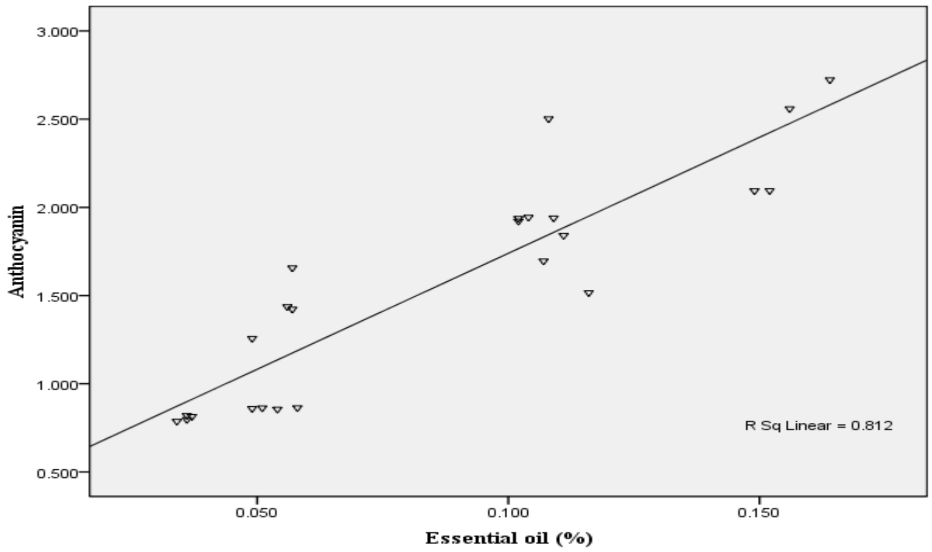

Fig 1. Correlation between essential oil and anthocyanin contents of Damask rose in exprimental sites.

The findings of the present study indicated that the anthocyanin contents were significantly ($P \leq 0.05$) different in the petals harvested at various locations. The highest anthocyanin content (2.368) was obtained from the Shiraz Eram Botanical Garden (Table 1). A high positive correlation (r Sq Linear = 0.812) was obtained between oil and anthocyanin content of Damask rose (Fig. 1). In the other hand, by increasing anthocyanin concentration in the petals the essential oil content increased in the experimental site. Zvi *et al.* [17] reported that an interlinking trait are present in co-engineering of scent and colour biosynthesis in *Petunia* flowers. It has also demonstrated that fragrance is affected by modulation of anthocyanin biosynthesis, reveals an intriguing link between the two secondary metabolic pathways in carnation (*Dianthus caryophyllus*) [16]. In most studies that explicitly address the connections between floral odor and pigmentation, mutations to the anthocyanin pathway lead directly to changes in emission of benzenoid molecules such as methyl benzoate and benzaldehyde [16, 17]. Other studies have hypothesized connections between anthocyanin and benzenoids due to the assumption of conserved biochemical pathways [7]; a similar link between benzenoids and anthocyanin may be expected here.

Over all, the results reported herein revealed that the high level of genetic variation among *R. damascena* landraces grown in Iran [1, 4] and different environmental conditions [15] affect the essential oil and anthocyanin contents in Damask rose. Moreover, there are considerable correlation between essential oil content and anthocyanin concentration which can be used as an essential oil quantity index in this plant.

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