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The Phytochemical Changes of Violet Flowers (*Viola cornuta*) Response to Exogenous Salicylic Acid Hormone

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ABSTRACT: Violet is one of the ornamental plants with a good value in landscaping and herbal medicine. Salicylic acid is a signaling agent involving in secondary metabolite production. The aim of this study was to evaluate the physiological responses of violet flowers to exogenous salicylic acid. This experiment was conducted in the greenhouse, as a completely randomized design. Salicylic acid was sprayed on violet plants in four levels as 0, 0.1, 0.7, 1.5 mM and three replications. Flower diameter, flower stem length, fresh weight and dry matter percentage of violet flower were measured as morphological parametes. In laboratory parameters like antioxidant activity and anthocyanin variation were recorded using spectrophotometery method. The quercetin and rutin values were determined by HPLC. Results showed that salicylic acid significantly affected on flower diameter, total antioxidant capacity, rutin and quercetin contents. Therefore data analysis provides that high levels of salicylic acid increased morphological parameters and improved chemical substance involving to secondary metabolism promotion. Furthermore, using different concentrations of the hormone is required, to achieve the best quality and quantity of plant biomass and it is also necessary to achieve the best traits of ornamental and medicinal value

KEYWORDS: Anthocyanin, Quercetin, Rutin, Salicylic acid, Violet (*Viola cornuta*).

INTRODUCTION

Flowers are the beautiful signs of God's creation and the best gifts for human due to their mental and nutritional value. Medicinal plants have a particular value in the provision of community health and many drugs have emerged with plant origin in different parts of the world in recent years [18,6].Violet (*Viola cornuta*) is a plant of the Violaceae family flowering at the end of winter and early in spring [9]. Flowers, leaves, roots, seeds and even whole parts of this flower are used for medicinal purposes [7, 28]. The violet flowers contain volatile oil, rutin, syanyn, bright pigment, methyl salicylate glycosides and anthocyanins [42].

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Flavonoids are the largest group of natural phenolic that quercetin is one of them. Confronting with cancer cells and viruses, extensive anti oxidant activity, potent immune enhancing medicinal properties of this material is the most important attributes of them. Thus recently the study of the situation and availability of these compounds is more focused [27, 8, 37, 16, and 39]. Rutin is a glycoside of quercetin flavonoid that both of them were used in drugs inhibiting vein blood structure and were calculated as a component of multivitamins [22].

Salicylic acid (SA) is a phenolic compound that is widely found in plants and is now considered as a hormone-like substasnce which can play an important role in plant growth [31]. It has been showed that external salicylic acid application even in low concentration increase plant ability to enhance the induction of defense compounds such as phenolic compounds [20]. Salicylic acid is involved in the physiological processes that can stimulate flowering [14], increasing the synthesis of secondary metabolites [34]. Khandakr and colleagues [19] showed that the concentration of 10^{-5}mM increased the plant height, root length, leaf number and dry weight of red Cocks comb. Salicylic acid in cucumber [23], petunia [2], calendula [11], water garden cress [30] and borage [10] improves attributes such as shoot dry weight, chlorophylls and levels of carotenoid.

The effect of salicylic acid as a growth regulator of the cell as well as its direct and indirect influence on the production of secondary metabolites has been proven in some plants. Therefore, the main objective of this research was to study the variation of some ornamental parameters and secondary metabolites of violets under different levels of salicylic acid application.

MATERIALS AND METHODS

Used Apparatuses and Materials

Violet flower F_1 seeds were purchased from Tehran Farid Company and then surface was sterilized with sodium hypochlorite 20% for planting in greenhouse. The obtained seedlings were transferred to the 2.5 liter pots containing garden soil, cocopeat, manure and sand in 1:1:1:1 ratio. Pots were orderd based on completely randomized design with 3 replications. Treatments involved 4 different concentrations of salicylic acid (control, 0.1, 0.7 & 1.5 mM). Foliar sprays of different salicylic acid concentration were performed in 3 stages.

Morphological traits measurement

Flower diameter was measured using a digital caliper and the number of flower was continuously recorded. Flower stem length was determined with ruler accurately. Flowers were picked up after reaching full size and then were weighed on digital scale with 0.001gr accuracy. After drying in oven at 40 °C for 48 hours, the dry matter was recorded.

Antioxidant capacity (free radical scavenging) determination

To measure the amount of free radical scavenging [5], 1 mm of was mixed with l ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) at a concentration of 0.1 mM. After 15 min incubation in dark, radical activity was measured using spectrophotometer at a wavelength of 517 nm. Then the free radical scavenging was computed as the following formula, whears as recorded as sample absorbance and AC as control was used.

$$\text{Free radical scavering} = \mathbf{(}(Ac-As) / Ac\mathbf{)} * 100$$

HPLC	Merck Hitachi7100
Spectrophotometer	Mapadauv-1800
Oven	Fan azmagostar BM120
Digital scale	A & D Company Limiled
ultrasonic bath	Euronda 4D
Shaker	Labinko L46
centrifuge	Cnturion K2042
Salicylic acid	Merck K41142931
DPPH	Sigma Aldrich 257621
Methanol	Merck106007
Rutin	Merck500064
Quercetin	Merck551600

Anthocyanin measurement

To obtain the concentration of anthocyanins[43], dried flowers were pulverized with acidified methanol (ratio 1:10) and extracted in the dark at 4°C. The absorbance of samples was recorded at 520nm. Anthocyanin content calculated according to the formula of A=εbc, whears ε is extinction coefficient ($3300 mMcm^{-1}$), A is absorbance, b = 1 cm (width cuvette) and anthocyanin content (c) is mol/g.

Phytochemical characteristics determination

Extracts was prepared according to the proposed method of Samee &Vorarat[35], with slight modification. After of extract preparation, samples were treated in an ultrasonic bath for 10 min and then centrifuged in 3500 r.p.m. The samples were passed through micro injection filter (0.45µm) and injected to HPLC. Merck Hitachi apparatus equipped with Lacrom Pump Model 7100 – diode array detector- (285 nm) and C18 Column (length 25 cm and in diameter 4.6 mm) was used in present study. The standard calibration curve of rutin obtained was used from different concentrations of 0, 10, 50,200, and 300 ppm and for quercetin the concentrations of 0, 5, 10, 50 and 100 ppm. The correct amount of compounds was computed based on the obtained formula for each compound.

STATISTICAL ANALYSIS

Data analysis was performed using SAS software and mean comparison was performed with LSD test at the 5% probability.

RESULTS AND DISCUSSION

The results from the analysis of variance are presented in Table 1. Obtained data suggest that salicylic acid had significant effect on flower diameter, antioxidant capacity, rutin content and quercetin content.

Data showed that the highest flower diameter (5.16 cm) was observed in the plants which were treated with salicylic acid at its highest concentration (Figure 1)

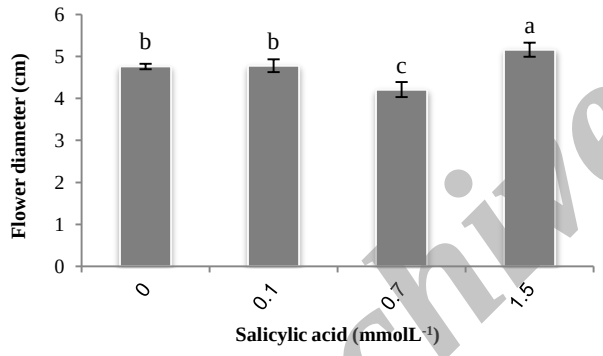
Fresh weight of flower was affected by hormones. With 0.1 $mmolL^{-1}$, the highest fresh weight was obtained. In medicinal plants industry, dried plant organs are used commonly and there is a direct relation between dry matter and plant material quality. By applying the 0.7 mM salicylic acid, highest percentage of dried flowers (14.46%) was observed.Contrary to that lowest flower yield was achieved at control level. Induce flowering,

growth, respiration and ethylene synthesis are important effects of salicylic acid hormone [38].

Table 1.Variance analysis of of salicylic acid treatment on violets (*Viola cornuta*).

Source Change	Degrees of freedom	Flower diameter	Flower stems length	Fresh flower weight	Dry matter of flowers	Flower Anti oxidant capacity	Flower Anthocyanin	Flower Rutin	Flower Quercetin
Hormon levels	3	0.45^{**}	$0.28^{n.s}$	$0.004^{n.s}$	$1.96^{n.s}$	57.17^{*}	$0.0008^{n.s}$	916582.78^{**}	8.42^{**}
Experimental error	8	0.02	0.37	0.004	1.92	12.82	0.0004	8172.02	0.002
Coefficient of Variation٪	-	3.1	15.3	14.9	10.3	6.5	14.8	9.1	0.08

* & ** significant at 5 and 1% probability level and n.s is not significant**.**



Figurer 1. Effect of Salicylic acid on flower diameter

This regulator increases protein production and by creation new isozymes a band that induces flower buds and increases their number [21]. Stimulatory effects of salicylic acid can grow through increased growth and cell division in meristematic regions and increase the effect of this hormone on other plant hormones [36]. In the same study, salicylic acid hormone on African violets [15], and Gompherna flower [17], found similar results with this study.

The length of flower stem in plants due to better feature of flowers appearance is the important parameter in ornamental plants. In this study, the length of flower stem increased as the salicylic acid increased from 0.1 to 1.5 $mmolL^{-1}$(4.2 cm). Expansion and cell division is regulated by salicylic acid hormone, indeed there is a balance between growth and aging by this hormone [32]. Salicylic acid together with auxin also affect elongation and cell division [13]. This hormone also played in specific proteins synthesis that called kinase protein; these proteins also play an important role in the regulation of division, differentiation and cell morphology formation [44]. Several reports of the effect of salicylic acid on shoot and root length of the plants available. In soybean and cucumber plants it has been showed that by increasing the level of this hormone an increase in nitrate reductase activity resulted to increase in measured traits [38, 12]. Results of Mehrabian and colleagues [25] showed that compared to other concentrations 0.1 and 0.2 mM SA, increased the shoot length, root length, fresh and dry weight of maize seedlings.

One important factor in the medicinal plant is their antioxidant capacity. In this study, the effect of salicylic acid on this attribute was significant. In level of 0.7 mM treatments (Figure 2), antioxidant capacity, reached up to a maximum (54.9%). Under stress conditions plants

increase the activity of antioxidant defense enzymes to protect themselves against further damages [1]. It seems that treating by salicylic acid enhances the antioxidant

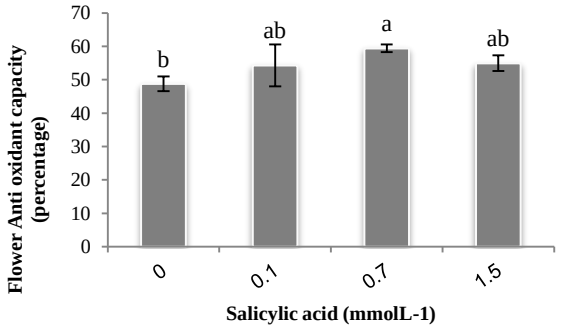


Figure 2.Effect of salicylic acid on anti oxidant capacity

Although the content of anthocyanins tend to decrease with increasing salicylic acid, but no significant difference was observed. It has been showed that salicylic acid affect phenolic accumulation by activation different enzymes like phenylalanine ammonia lyase(PAL) and chalcone synthase (CHS). Thus reduction of anthocyanin could be explained by the conversion of flavonoids to each others or more active substances [4].Another reason for this result can be attributed to inhibition of ethylene synthesis [33]. This result is similar to Bernard and colleagues [3] in tea plant.Both Rutin (Figure 3) and quercetin (figure 4), as important flavonoids compounds, were significantly varied in different levels of salicylic acid application (Figures 3 & 4).

protection of the cell membrane, photosynthetic pigments, and causes to improve the growth index and secondary metabolites, ultimately [29].

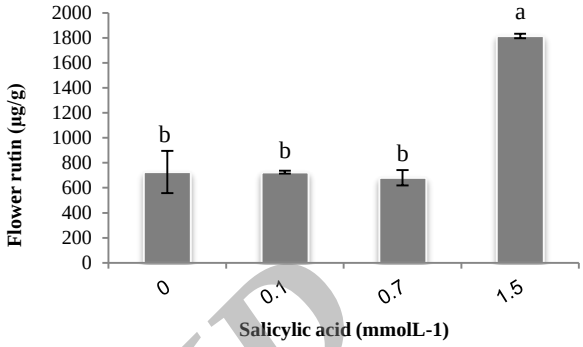


Figure3. Effect of Salicylic acid on rutin content

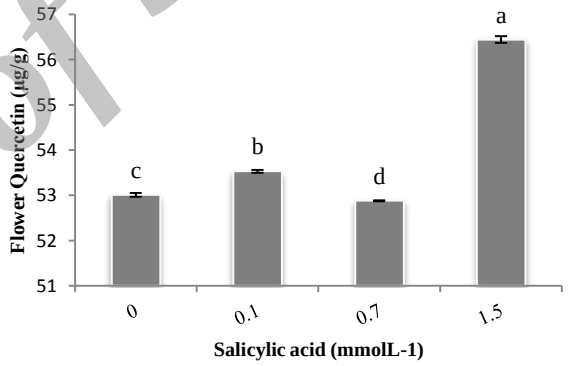


Figure 4. Effect of Salicylic acid on quercetin content

Salicylic acid induces enzyme activity to increase active ingredients and flavonoids. This hormone is known as a key message component in the specific activation plant response and these responses lead to biosynthesis and accumulation of plant secondary compounds [31]. Rutin was shown to consist of bioactive compounds that may help protect plants against ultraviolet radiation or pathogens and could be used to prevent the side effects of cancer treatments, diabetes, and hypercholesteremia [24, 26]. The results of Sun and colleagues [40], rutin content of leaves of buckwheat (*Fagopyrumtartaricum*) markedly increased after treated with salicylic acid, by

increasing the activity of enzymes are involved in rutin production. Based on the role of salicylic acid in plant defense system, it seems that external salicylic acid application induces pseudo stress conditions in plant enhances the antioxidant protection via secondary metabolite accumulation [29]. Rutin and Quercetin are valuable glycoside polyphenolic compounds of violet and many of other plants that are important in medicine. in most cases a direct relationship was observed between the amount of salicylic acid application and and defense compounds accumulation [41].

CONCLUSION

The results of this study showed that salicylic acid had effective influence on plant morphological characteristics, such as size and the number of flowers.

In addition, the effects of this hormone on secondary metabolites accumulation indicate that salicylic acid as a plant hormone and stress signaling compound induces pseudo stress conditions and encourages plant to start its tolerance system. If salicylic acid applied in suitable amount act as a good target in secondary metabolite production. It should be mentioned that high amount of this compound can limit the secondary product production. Based on the obtained results it can be concluded that salicylic acid in the range of 1.5mM could be appropriate for viola secondary metabolite production.

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