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Influence of 28-Homobrassinolide on Growth, Photosynthesis Metabolite and Essential Oil Content of Geranium [*Pelargonium graveolens* (L.) Herit]

K.N. Swamy and S. Seeta Ram Rao
Plant Physiology Laboratory, Department of Botany,
Osmania University, Hyderabad 500 007, India

Abstract: In this study, effect of 28-homobrassinolide on growth, metabolite content and essential oil production of geranium [*Pelargonium graveolens* (L.) Herit] was studied. 28-homobrassinolide increased plant growth by the amplification of photosynthetic rate and by the enzyme stimulation growth was increased. The growth promotion was associated with elevated levels of chlorophyll pigments, nucleic acids, soluble proteins, reducing sugars, non-reducing sugars and starch. 28-homobrassinolide at 3 µM concentration caused significant increase in essential oil content. The results suggest the potentiality of 28-homobrassinolide in enhancing plant growth.

Key words: 28-homobrassinolide, geranium, growth, metabolite content, essential oil

INTRODUCTION

Geranium is an important aromatic plant, yielding an essential oil which is highly priced for its very profound and strong rose-like odour (Farooqi and Sreeramu, 2001). The pure geranium oil is almost a perfume by itself and blends well with all other perfumes. It is widely used in scenting soaps and cosmetic industries. The oil has pesticidal, antibacterial and pharmacological activities (Balchin *et al*., 1998). The cost of oil increasing in International market. Therefore, of utmost necessary to improve in terms of its quantitative and qualitative values of this oil.

Brassinosteroids are a new group of plant hormones with significant growth promoting activity (Mandava, 1988; Clouse and Sasse, 1998). The ability of certain pollen extracts to promote growth lead to the discovery of this group of substances in plants. Collective efforts initiated by the scientists at various agricultural research stations of USDA resulted in the isolation of an active factor from the pollen grains of rape plant (*Brassica napus*) which was named as brassinolide (Grove *et al*., 1979). Till now, 65 brassinosteroids and 5 sugar fatty acid conjugates have been detected in the plant kingdom (Bajguz and Tretyn, 2003). Brassinosteroids are considered as plant hormones with pleiotropic effects as they regulate wide array of developmental processes such as growth, seed germination, rhizogenesis, flowering, senescence, abscission and maturation. Brassinosteroids also confer resistance to plants against various abiotic stresses (Sasse, 1999). In the present study, the effect of brassinosteroids on the growth and essential oil content of geranium is being investigated. In addition, the impact of foliar application of brassinosteroids on the content of various metabolites which have significant bearing on the growth was also evaluated.

MATERIALS AND METHODS

28-homobrassinolide was obtained from M/s CID tech Research Inc., Mississauga, Ontario, Canada. Geranium [*Pelargonium graveolens* (L.) Herit] Bourbon type plant cuttings were obtained from Central Institute of Medicinal and Aromatic Plants (CIMAP), Resource Centre, Hyderabad, India. This research project was conducted from 2003-2006.

Corresponding Author: S. Seeta Ram Rao, Plant Physiology Laboratory, Department of Botany, Osmania University, Hyderabad 500 007, India

Fresh and healthy plant cuttings measuring a length of 14 cm and having three terminal leaves (all the other leaves were excised) were transplanted in to nursery covers filled with garden soil and maintained under adequate moisture level for 20 days for rooting. On 20th day uniform size plants were sorted out and one plant was transferred to each earthen pot containing 10 kg of garden soil. Plants were grown in a glass house at 25/18°C day/night temperature under natural day length and watered alternate day. 28-homobrassinolide was supplied to the plants at three concentrations viz 0.5, 1 and 3 μM as foliar spray on 10th, 40 and 70th day after planting the rooted cuttings. Distilled water spray was given to control plants. On 120th day the height of the plants was measured. The pots were flooded with water and the plants were gently removed without causing any damage to the root system and other growth parameters were recorded. Fresh leaf material was used for chlorophyll estimation and essential oil extraction. Five gram of leaf material was homogenized in 50 mL of 70% (v/v) ethanol and the alcoholic homogenate was stored in deep freezer for further biochemical analysis.

Growth Parameters

The growth of the plants was recorded in terms of plant height, fresh weight and dry weight of shoot and root, total leaf area (Model CI-203 CID Inc. Vancouver Washington-USA), number of leaves/plant and leaf fresh weight.

Chlorophylls

The chlorophyll pigments were extracted and estimated according to the procedure of Arnon (1949) by extracting of plant material in 80% (v/v) acetone.

Photosynthesis

Net photosynthetic rate (P_N) in fully expanded leaves of intact plants under natural light was measured using a portable photosynthesis system LI-COR 6400 (PAR 1500, block temperature 31°C, flow-500).

Carbohydrate Fractions

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of total sugars (Yoshida *et al*., 1976) and reducing sugars (Nelson, 1944). Non-reducing sugars were calculated by the formula given by Loomis and Shull (1937). The residue was used for the estimation of starch (McCready *et al*., 1950).

Soluble Proteins

Soluble proteins in the ethanol homogenate were precipitated by adding 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 1% (w/v) sodium hydroxide. Lowry *et al*. (1951) method was employed for quantitative estimation of proteins.

Nucleic Acids

DNA and RNA fractions in the ethanol homogenate were separated by the method of Ogur and Rosen (1950). While, DNA estimation was done with diphenylamine reagent (Burton, 1968), RNA was quantified with orcinol reagent (Schneider, 1957).

Essential Oil Content

Freshly harvested leaf material was immediately transferred to round bottom flask of the Clevenger apparatus. Water was added till the plant material was completely submerged and was subjected to hydrodistillation for 3 h. The volume of the oil collected in the collecting tube of the apparatus was recorded. The percentage of oil content on fresh weight basis was calculated adopting the following formulae:

$$\text{Oil content (\%) in herb} = \frac{\text{Volume of the oil (mL)} \times \text{specific gravity (0.9)}^{*}}{\text{Weight of fresh herb (g)}} \times 100$$

*Specific gravity of geranium oil.

The oil of the plants were collected and dried over anhydrous sodium sulfate and stored at 5°C in a refrigerator until analyzed.

Composition of Essential Oil

The oil was analyzed using a Perkins Elmer gas chromatograph (Model 8500, Italy) equipped with Flame Ionization Detector (FID), GP-100 printer-plotter and an electronic integrator using BP-1 (25 m × 0.5 mm id × 0.25 μM film thickness) capillary column coated with polydimethyl siloxane, nitrogen was used as carrier gas at 10 psi inlet pressure with a flow rate of 0.4 mL min^{-1} (Linear velocity 14 cm sec^{-1}). Temperature was programmed from 60-220°C at ramp rate of 5°C min^{-1} with a final hold time of 10 min. Injector and detector were maintained at 250 and 300°C, respectively. Samples (0.1 μL) were injected with a split ratio of 1:80. The compounds in the essential oil were identified by comparing the retention times of the chromatogram peaks with those of authentic compounds run under identical conditions, by comparison of relative indices (Kovats, 1965). Retention indices were computed from gas chromatograms by logarithmic interpolation between n-alkanes. The homologous series of n-alkanes 8-C22. Poly Science Inc., Niles, USA were used as standard with literature data (Davis, 1990), peak enrichment on co-injection with authentic compounds. Quantitative data obtained by electronic integration peak areas (FID) without the use of response correction factors.

Statistical Analysis

The experiments were repeated twice with adequate replicates to obtain sufficient plant material for analysis. However values of 5 replicates of each parameter recorded were subjected to statistical scrutiny. For qualitative analysis of geranium oil, mean of 3 replicates from 3 μM concentration (where significant difference in (%) of oil obtained) was calculated. The data was analyzed by One-Way ANOVA, followed by Post Hoc Test (Multiple Comparison). The differences were considered significant if p was at least ≤ 0.05. The mean values have been compared and lower case alphabets are used in the tables to highlight the significant differences between the treatments.

RESULTS AND DISCUSSION

The treatment of geranium plants with 28-homobrassinolide resulted in the substantial increase in the vegetative growth of the plant as reflected in increase in all the vegetative parameters recorded in the study (Table 1). Among the three concentrations, 3 μM proved to be highly effecting in improving the growth of the plants. Exaggerated growth in *Arabidopsis* by brassinolide application was reported (Arteca and Arteca, 2001). Similar enhancement of root growth in *Arabidopsis* due to application of 24-epicastasterone and 24-epibrassinolide was reported by Mussig *et al*. (2003). The improvement of rooting and plant growth by brassinosteroid application was found by Swamy and Rao (2006). The economic yield of the plant as reflected in the leaf number, leaf fresh weight and total leaf area were also found increased due to 28-homobrassinolide treatment (Table 2). Herbage yield was found maximum incase of geranium plants treated with 3 μM of 28-homobrassinolide.

28-homobrassinolide enhanced the chlorophyll levels (Table 3). Exogenous application of 28-homobrassinolide resulted significant increase in carbon dioxide fixation. Similarly application of 28-homobrassinolide increased the total chlorophyll contents and its fractions in *Brassica juncea*

Table 1: Effect of 28-homobrassinolide on growth of geranium plant

HBL (μM)	Plant height (cm)	No. of branches per plant	Fresh weight (g)		Dry weight (g)	
			Shoot	Root	Shoot	Root
0.0	28.8±1.4c	4.4±0.7c	53.7±1.7d	3.11±0.3b	7.80±1.3c	0.75±0.01c
0.5	32.2±0.6b	6.4±0.5bc	69.0±0.9c	4.07±0.9b	9.32±2.6b	0.90±0.03b
1.0	33.1±0.7b	8.6±0.1b	75.3±1.1b	4.60±0.5b	10.01±1.8b	1.05±0.02a
3.0	35.4±0.9a	10.6±0.6a	92.4±1.3a	6.06±1.4a	12.09±0.9a	1.15±0.06a

The data presented above are Mean±SE (n = 5). Mean followed by the same alphabet in column is not significantly different at p = 0.05 level

Table 2: Effect of 28-homobrassinolide on geranium leaf

HBL (μM)	No. of leaves per plant	Fresh weight (g)	Total leaf area $(cm)^2$
0.0	56.6±2.0d	36.9±1.2d	593.6±15.76d
0.5	70.2±1.8c	50.6±1.0c	795.2±23.54c
1.0	80.4±1.7b	56.9±0.8b	979.4±23.27b
3.0	95.8±1.5a	69.6±0.6a	1225.5±20.09a

The data presented above are Mean±SE (n = 5). Mean followed by the same alphabet in column is not significantly different at p = 0.05 level

Table 3: Effect of 28-homobrassinolide on the chlorophyll content and photosynthetic rate of geranium leaves

HBL (μM)	Chlorophyll a (mg g^{-1} fr.wt.)	Chlorophyll b (mg g^{-1} fr.wt.)	Total chlorophyll (mg g^{-1} fr.wt.)	Photosynthesis (μM CO_2 m^2 sec^{-1})
0.0	0.751±0.14c	0.324±0.02c	1.075±0.16d	11.7c
0.5	0.829±0.05b	0.364±0.03b	1.193±0.08c	14.9b
1.0	0.908±0.13ab	0.375±0.12b	1.283±0.25b	16.6b
3.0	0.971±0.06a	0.436±0.05a	1.407±0.11a	19.5a

The data presented above are Mean±SE (n = 5). Mean followed by the same alphabet in column is not significantly different at p = 0.05 level

(Alam *et al*., 2007). Talaat and Youssef (1998) also reported that photosynthetic pigments in *Hibiscus sabdariffa* were increased by brassinosteroids. 28-homobrassinolide application also resulted in elevated carbondioxide fixation as compared to untreated control plants.

Foliar application of 28-homobrassinolide to geranium plants caused sharp rise in the levels of all the three carbohydrate fractions (Table 4). Higher chlorophyll levels coupled with increase in photosynthesis might have contributed to increased levels of carbohydrate fractions. In *Cucumis sativus*, 24-epibrassinolide increased the activity of RUBISCO as well as accounted for elevated levels of soluble sugars and starch (Yu *et al*., 2004). The growth promotion in tomato by brassinosteroid application was associated with elevated levels of carbohydrates (Vardhini and Rao, 2003).

28-homobrassinolide also influenced the levels of soluble proteins in geranium plants (Table 5). An increase in protein content was found due to 28-homobrassinolide application. In Chinese cabbage protoplasts, the cell division rate and amount of soluble protein increased by the addition of 24-epibrassinolide to culture media (Nakajima *et al*., 1996).

28-homobrassinolide application caused an increase in the contents of nucleic acids (Table 6). Key (1969) suggested that phytohormones regulate the growth of the plants by effecting nucleic acid metabolism. Bajguz (2000) reported the brassinosteroid induced changes in nucleic acid content in *Chlorella vulgaris*. In *Arachis hypogaea* higher contents of RNA were reported when treated with brassinosteroids (Prakash *et al*., 2003).

28-homobrassinolide employed plants at 3 μM concentration enhanced the aromatic oil yield when compared to untreated plants (Table 7). There was slight increase in the content of geraniol and marginal decrease in citronellol, linalool, isomenthone content due to 28-homobrassinolide 3 μM (Table 8). The effect of 28-homobrassinolide on essential oil yield might have mediated through the impact on growth and metabolism. 28-homobrassinolide might have trigged the intrinsic genetic potentiality of the plants to produce more essential oil. Higher levels of carbohydrates and their

Table 4: Effect of 28-homobrassinolide on the carbohydrate fractions in the leaves of geranium plant

HBL (μM)	Reducing sugars (mg g^{-1} fr. wt.)	Non-reducing sugars (mg g^{-1} fr. wt.)	Total sugars (mg g^{-1} fr. wt.)	Starch (mg g^{-1} fr. wt.)
0.0	1.81± 0.02c	2.40±0.05d	4.21±0.07d	3.81±0.08c
0.5	2.10±0.04b	2.75±0.08c	4.85±0.12c	4.14±0.11b
1.0	2.25±0.12b	3.01±0.10b	5.26±022b	4.23±0.15b
3.0	2.49±0.01a	3.22±0.05a	5.71±0.06a	4.78±0.09a

The data presented above are Mean±SE (n = 5). Mean followed by the same alphabet in column is not significantly different at p = 0.05 level

Table 5: Effect of 28-homobrassinolide on the soluble protein content of geranium leaves

HBL (μM)	Soluble proteins (mg g^{-1} fr. wt.)
0.0	4.79c
0.5	5.32c
1.0	6.59b
3.0	7.93a

The data presented above are Mean±SE (n = 5). Mean followed by the same alphabet in column is not significantly different at p = 0.05 level

Table 6: Effect of 28-homobrassinolide on the Nucleic acid content of geranium leaves

HBL (μM)	DNA content (μg g^{-1} fr. wt.)	RNA content (μg g^{-1} fr. wt.)
0.0	221±25c	506±13b
0.5	260±23b	530±11b
1.0	282±17b	555±14b
3.0	329±32a	618±08a

The data presented above are Mean±SE (n = 5). Mean followed by the same alphabet in column is not significantly different at p = 0.05 level

Table 7: Effect of 28-homobrassinolide on oil percentage and yield of geranium

HBL (μM)	Oil (%)
0.0	0.21± 0.05c
0.5	0.24±0.02b
1.0	0.26±0.01b
3.0	0.29±0.07a

The data presented above are Mean±SE (n = 5). Mean followed by the same alphabet in column is not significantly different at p = 0.05 level

Table 8: Effect of 28-homobrassinolide on essential oil quality of rose scented Geranium*

HBL	Aromatic substances (%)						
(μM)	Linalool	Isomenthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Geranyl tiglate
0.0	9.9	9.5	20.6	18.1	7.4	5.6	1.2
3.0	8.7	9.8	19.1	21.4	7.2	5.1	1.1

*Mean of three replicates

possible diversion to secondary metabolism might have contributed to elevated levels of essential oils in geranium plant. Similarly, the Spirostane Analogues of Brassinosteroids (SABS) were found increasing the production of leaves as well as improved the essential oils in hydroponically grown mint (Maia *et al*., 2004). The present findings demonstrate clearly the ability of 28-homobrassinolide to enhance the growth and metabolite content which further enhanced the essential oil content in the plant. Khripach (2000) envisaged a great role for brassinosteroids in 21st century agriculture. The results of the present study, present case for the use of brassinosteroids for improvement of aromatic plants.

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