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Effects of 28-homobrassinolide on growth, photosynthesis and essential oil content of *Satureja khuzestanica*

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Brassinosteroids are a group of plant hormones that have significant biological effects on plant growth and development. The effects of 28-homobrassinolide (HBR) on growth, photosynthesis, chlorophyll content, carbohydrate fractions and essential oil content of savory herbs (*Satureja khuzestanica* Jamzad) were investigated. Foliar application of 28-homobrassinolide at 10^{-10}, 10^{-8} and 10^{-6} M concentrations substantially increased growth. 28-homobrassinolide at 10^{-6} M concentrations improved herbage yield as reflected in the increase of foliar biomass. Exogenous application of 28-homobrassinolide increased the rate of photosynthesis. Growth promotion was also associated with increased chlorophyll content and resulted in the accumulation of carbohydrate fractions. At 10^{-6} and 10^{-8} M concentrations, 28-homobrassinolide also incremented the total content of essential oils. The quantitative analysis of savory oil from the savory plant treated with 10^{-6} M concentration revealed an increase in carvacrol and para-cymene contents and a decrease in the γ-terpinene content. Collectively, the present study demonstrates a positive impact of the new group of phytohormones on the agronomic performance of savory plant, a highly valued aromatic plant.

Key words: *Satureja khuzestanica*, 28-homobrassinolide, growth, photosynthesis, essential oil, carbohydrates, chlorophylls, savory.

INTRODUCTION

Satureja khuzestanica is an endemic plant that is widely distributed in the west of the Lorestan Province, Iran. This plant has reported therapeutic value as analgesic, antiseptic (Vosovgh-Ghanbari et al., 2010), antibacterial (Sahin et al., 2003; Azaz et al., 2002), antiviral (Abad et al., 1999) and antifungal (Boyraz and Ozcan, 2005; Zarrin et al., 2010; Sadeghi-Nejad et al., 2010). More than 26 constituents are identified in this species, in which carvacrol, γ-terpinene and *p*-cymine are dominant (Sadeghi-Nejad et al., 2010). Carvacrol [2-methyl-5-(1-methylethyl)-phenol] is generally recognized as a safe food additive and is used as a flavoring agent in baked goods, sweets and beverages (Farsam et al., 2004). Savory oil is a highly valued essential oil, which is used in soaps, perfumery and cosmetic industries. The value of this oil is increasing in international markets, and attempts were made to promote its cultivation by employing various suitable agrotechnologies. The growth and essential oil production of aromatic plants can be altered by the application of plant growth regulators. For example, application of triacontanol was found to be beneficial in improving the herbage yield (Silva et al., 2005); and a

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Abbreviations: HBR, 28-Homobrassinolide; **EBL,** 24-epibrassinolide; **BRs,** brassinoesteroids.

significant increase in essential oil accumulation in Japanese mint (Mentha arvensis) by chlormequat chloride was reported (Farooqi and Sharma, 1988). Brassinosteroids, including 28-homobrassinolid (HBR), comprise a specific class of low abundance plant steroids now regarded as a new class of phytohormones with a significant growth-promoting activity (Rao et al., 2002).

The studies conducted with brassinosteroid biosynthetic mutants of pea (*Pisum sativum*) (Nomura et al., 1997), tomato (*Solanum lycopersicum*) (Koka et al., 2000), and fava bean (*Vicia faba*) (Fukuta et al., 2002) provided compelling evidence for the vital role of this group of growth regulators for normal growth and development. Inhibition studies employing brassinozole, a specific inhibitor of brassinolide synthesis in plants, further fortified the view that brassinosteroids are indispensable for plant growth and development (Asami et al., 2000). The ability of brassinosteroids to improve the performance of cereals, vegetable crops, oil seed plants, and fruit crops has been widely reported (Ramaj et al., 1997; Khripach et al., 1999; Vardhini et al., 2006). In the present study, the effects of HBR on the growth, photosynthesis, chlorophyll, carbohydrate content and essential oil production of savory were investigated.

MATERIALS AND METHODS

Plant material and treatment imposition

28-Homobrassinolide (HBR) was obtained from Sigma Aldrich (USA). Savory (*S. khuzestanica* Jamzad) Bourbon type plant cuttings were obtained commercially from the Khorraman Company in the industrial village of Khoramabad, Lorestan Province, Iran. Fresh and healthy plant cuttings of 14 cm height and having three top leaves (other leaves were carefully removed) were transplanted into nursery covers filled with garden soil and maintained at an adequate moisture level for 23 days to induce rooting. On the 23th day, uniform-size plants were sorted out, and one plant each was transplanted to earthen pots containing 10 kg of garden soil and compost in a 8:1 ratio. Plants were maintained in a greenhouse at 26/17°C (day/night) temperature under natural day length and watered three-times per week. 28-homobrassinolid was supplied to the plants at three concentrations namely, 10^{-10}, 10^{-8}, and 10^{-6} M as foliar spray on the 15, 40 and 80th days after planting. Distilled water spray was given to obtain plants to be used as control. On the 140th day, the height of the plants was measured and the rate of photosynthesis was recorded (as follows).

The pots were filled with water, the plants were gently removed without causing damage to the root system, and growth parameters were determined. Fresh leaf material was used for chlorophyll quantification and essential oil extraction. 5 g of leaf material was homogenized in 50 ml of 70% ethanol and the alcoholic homogenate was stored in deep freezer for carbohydrate analysis.

Growth parameters

The growth of the savory plants was recorded in terms of plant height, fresh and dry weight of shoots and roots, total leaf area, number of leaves per plant and leaf fresh weight. Leaf area meter model CI-203 (CID Inc., United States) was employed for recording the leaf area, by a destructive method. The readings were saved and exported to Microsoft Excel processing program, used to calculate total leaf areas.

Photosynthesis

The net photosynthesis was determined using a portable photosynthesis system (PAR 1500, block temperature 31°C, flow-500) using a LI-COR 6400 portable photosynthesis system on the upper most fully expanded leaf of intact plant.

Chlorophylls content

The chlorophylls were extracted in 80% (v/v) acetone and determined following the method of Arnon et al. (1949).

Analysis of carbohydrate fractions

The alcoholic homogenate was heated and centrifuged. The supernatant was used for the estimation of total sugars (Yoshida et al., 1976) and reducing sugars (Nelson et al., 1944). The precipitate was used for starch estimation (McCready et al., 1950). Nonreducing sugar content was calculated by adopting the formula described by Loomis and Shull (1937).

Essential oil extraction

50 g of freshly harvested leaf material was immediately transferred to a round bottom flask of the Clevenger apparatus. Water was added until the plant material was completely submerged and was then subjected to hydro-distillation for 3 h. The volume of the oil in the collecting tube of the apparatus was recorded. The percentage of oil content on fresh weight basis was calculated according to the following formula:

Oil content (%) in herb = Volume of the oil (ml) × 0.9*/weight of fresh herb (g) × 100

In which 0.9 is the specific gravity of savory oil. The oil was collected, 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 Hewlett-Packard GC/MS model 5971A equipped with flame ionization detector (FID), GP-100 printer-plotter and an electronic integrator using BP-1 [25 m × 0.5 mm (internal diameter) × 0.25 µm film thickness] capillary column coated with polydimethyl siloxane. Nitrogen was used as a carrier gas at 10 psi inlet pressure with a flow rate of 0.4 ml/min (linear velocity of 14 cm/s). Temperature was programmed from 60 to 220°C at the ramp rate of 5°C/min, 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 and 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 (Kovats et al., 1965). Retention indices were computed from gas chromatograms by logarithmic interpolation between n-alkanes. The homologous series of n-alkanes (C8-C22, Polyscience Inc., Niles, United States) were used as standard according to literature data (Davis, 1989), peak enrichment on co-injection with authentic compounds.

Quantitative data were obtained by electronic integration of peak areas without the use of response correction factors.

Statistical analysis

The experiments were repeated twice with 3 to 5 replicates of each

Table 1. Effects of HBR on growth of the savory plant.

HBR (M)	Plant height (cm)	Fresh weight (g)		Dry weight (g)		Number of leaves/plant	Total leaf area (cm^2/plant)	Leaf from weight (g/plant)
		Shoot	Root	Shoot	Root			
0	37.66[b]	64.75[b]	4.28[b]	9.01[c]	0.98[c]	77[d]	661.73[d]	33.9[d]
10^{-10}	39.03[b]	77.34[b]	4.58[b]	10.87[b]	1.02[b]	98[c]	718.55[c]	47.3[c]
10^{-8}	41.67[a]	81.99[a]	5.45[a]	11.26[b]	1.98[a]	119[b]	876.76[b]	62.0[b]
10^{-6}	43.60[a]	102.50[a]	6.01[a]	13.99[a]	2.11[a]	143[a]	1039.31[a]	75.2[a]

Note: The data presented are means and their standard errors (n = 5). Means followed by the same letters in column are not significantly different at P = 0.05.

Table 2. Effects of HBR on photosynthesis of the savory plant.

HBR (M)	Photosynthesis [μmol $CO_2/(m^2s^{-1})$]
0	11.21[c]
10^{-10}	14.63[b]
10^{-8}	18.30[a]
10^{-6}	18.60[a]

Note: The data presented are means and their standard errors (n = 5). Means followed by the same letters in column are not significantly different at P = 0.05.

Table 3. Effects of HBR on the chlorophyll content in savory leaves.

HBR (M)	Chl *a* (mg/g from weight)	Chl *b* (mg/g from weight)
0	0.715[c]	0.322[c]
10^{-10}	0.786[b]	0.357[b]
10^{-8}	0.813[b]	0.366[b]
10^{-6}	0.949[a]	0.413[a]

The data presented are means and their standard errors (n = 5). Means followed by the same letters in column are not significantly different at P = 0.05.

parameter recorded to obtain sufficient plant material for analysis and subjected to statistical scrutiny. For qualitative analysis of savory oil, mean of 3 replicates from 3 µM concentration (where significant differences in the percentage of oil were obtained) was calculated. The data was analyzed by one-way ANOVA. The differences were considered significant if P was at least ≥0.05.

RESULTS AND DISCUSSION

Exogenous application of 28- HBR substantially improved growth of savory plants as reflected in the increase of all growth parameters recorded (Table 1). The economic yield of the plant (herbage yield) as reflected in the leaf number, leaf weight and total leaf area was concomitantly increased due to HBR treatment (Table 1). Previous data indicates that exogenous BRs can act efficiently in plants as growth-promoters when applied at the appropriate dose and at the correct stage of plant development. In fact, increased growth of several species such as Arabidopsis (Arteca and Arteca, 2001), *Chlorella vulgaris* (Bajguz and Czerpak, 1998) or *Cucumis sativus* (Yu et al., 2004) by exogenous brassinolide applications has been reported. Likewise, the present study clearly demonstrates the favorable impact of HBR on growth of the aromatic plant, savory. Exogenous application of HBR resulted in a significant increase in the carbon dioxide fixation (Table 2). Savory plants treated with 10^{-6} M HBR exhibited the maximum rate of CO_2 fixation. In addition, HBR application was also associated with elevated level of chlorophylls (Table 3). Similarly, HBR application also resulted in the enhancement of carbohydrate profiles (reducing sugars, non-reducing sugars and starch) as compared to untreated control plants (Table 4). Higher chlorophyll levels coupled with an increase in photosynthesis might have contributed to the increased levels of carbohydrates observed. There are several reports of the increased chlorophyll levels in plants such as *C. vulgaris* after brassinosteroid application (Bajguz and Czerpak, 1998). Similarly, callus cultured on Murashige and Skoog medium supplemented with EBL formed many green buds and shoots in *Arabidopsis*

Table 4. Effects of HBR on the carbohydrate content in the leaves of the savory plant.

HBR (M)	Reducing sugars (mg/g from weight)	Nonreducing sugars (mg/g from weight)	Total sugars (mg/g from weight)	Starch (mg/g from weight)
0	1.80[c]	2.44[d]	4.22[d]	3.82[d]
10^{-10}	2.18[b]	2.87[c]	4.86[c]	4.02[c]
10^{-8}	2.33[b]	3.07[b]	5.29[b]	4.26[b]
10^{-6}	2.78[a]	3.45[a]	5.78[a]	4.67[a]

The data presented are means and their standard errors (n = 5). Means followed by the same letters in column are not significantly different at P = 0.05.

Table 5. Effects of HBR on geranial oil content of savory leaves.

HBR (M)	Essential oil content (%)
0	0.27[b]
10^{-10}	0.31[b]
10^{-8}	0.36[ab]
10^{-6}	0.38[a]

The data presented are means and their standard errors (n = 5). Means followed by the same letters in column are not significantly different at P = 0.05.

Table 6. Effects of HBR on essential oil composition of *Satureja khuzestanica* J.

HBR (M)	Content of aromatic substances (%)						
	Carvacrole	γ-terpinene	para-cymene	β-bisabolene	myrcene	α-thujene	α- terpinene
0 M HBL	81.17	3.47	3.18	1.12	1.01	0.77	0.71
10^{-6} M HBL	85.98	1.88	4.11	0.87	0.82	0.66	0.54

thaliana (Chen et al., 1996).

Yu et al. (2004) found significant increases in the initial activity of Rubisco and in the sucrose, soluble sugars, and starch contents, followed by substantial increases in sucrose phosphate synthase, sucrose synthase and acid invertase activities after EBR treatment. EBR increased the capacity of CO_2 assimilation in the Calvin cycle, as the result of the increase in the initial activity of Rubisco. Changes in photosynthetic rate due to EBR application were found to be associated with non-stomatal limitations, other than photochemical efficiency of PSII and photosynthetic pigments (Ali et al., 2008). Rice plants treated with brassinosteroid lead to restoration of chlorophyll levels and were associated with salt stress alleviation (Anuradha and Rao, 2003). Our results agree with previous literature. For instances, foliar spraying of mustard seedlings with HBR led to higher fresh and dry weights, enhanced carbonic anhydrase activity, net photosynthetic rate, pod number and seed yield (Hayat et al., 2000). Spraying sesame with HBR thrice at vegetative, flowering, and capsule formation stages increased growth, yield, sugars and soluble protein (Prakash et al., 2008). In *C*. *sativus*, EBL increased the activity of Rubisco and accounted for elevated levels of soluble sugars and starch (Yu et al., 2002). Impaired carbohydrate metabolism and reduced biomass was found in a brassinosteroid-deficient Arabidopsis mutant (Schluter et al., 2002). The chilling injury alleviation in cucumber seedlings by EBL was found to be associated with a sharp increase in the photosynthetic rate (Yu et al., 2002). Similarly, brassinolide caused an increase in sugar levels in *C*. *vulgaris*, and this was attributed to accelerated photosynthetic rates (Bajguz and Czerpak, 1998).

28-homobrassinolid employed in the study caused a significant increase in the essential oil content in savory plants when applied at 10^{-6} M concentration (Table 5). There was a slight increase in the content of carvacrol and para-cymene and a marginal decrease in γ-terpinene, β-bisabolene and myrcene contents (Table 6). The effect of HBR on essential oil yield might be mediated through the impact on growth and metabolism. HBR might trigger the intrinsic genetic potentiality of the plants to produce more essential oil. Higher levels of carbohydrates and their possible diversion to secondary metabolism might contribute to elevated levels of the essential oil in the savory plant. Further studies are needed to provide more insight on the role of brassinosteroids in the secondary metabolism. The increase in the essential oil content in lavender by EBL application was obtained (Youssef and Talaat, 1998). Application of indole-3-acetic acid resulted in a 4-fold increase in geraniol content *in vitro* developed plantlets and a 2.2-fold increase in 60-day-old plants of *M. officinalis* (Silva et al., 2005). A significant increase in essential oil accumulation in Japanese mint (*M. arvensis)* by chlormequat chloride was reported (Farooqi and Sharma 1988). The spirostane analogues of brassinosteroids were found to increase the production of leaves as well as to elevate the levels of essential oils in hydroponically grown mint (Maia et al., 2004). The ability of brassinosteroids to induce root formation by geranium stem cuttings was found (Swamy and Rao, 2006), thus expanding the practical utility of this new group of hormones to plant propagation. The results of the present study demonstrated the improvement of herb yield coupled with the elevated levels of aromatic substances in savory. A great role for brassinosteroids in the 21st century agriculture is being envisaged (Khripach et al., 2000) and the present findings add another dimension to the practical utility of HBR.

Summing up, HBR were considered to be promising compounds for application in agriculture because they positively impacted several aspects of regulatory activity on growth and development of savory plants and their economic value as a yield-promoting agent was confirmed by their positive effect on the resultant quality.

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