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Effect of brassinosteroids on growth and metabolite content of *Tinospora cordifolia* (Wild.) Miers ex Hook.F & Thomas

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ABSTRACT
The effect of brassinosteroids, a novel type of plant hormones on the growth of Tinospora cordifolia (Menispermaceae),an important medicinal plant was investigated. Foliar application of 24-epibrassinolide and 28-homobrassinolide, improved the vegetative growth of the T. cordifolia as reflected in terms of height of the plant, fresh and dry weight of shoot and root and foliage growth. BRs were also accounted in the enhancement of biochemical parameters like content of chlorophylls, soluble proteins and sugars. The studies demonstrated the useful impact of brassinosteroids in enhancing biomass production.

Keywords : *Tinospora cordifolia*, growth, chlorophylls, carbohydrates, proteins, Brassinosteroids

INTRODUCTION

Tinospora cordifolia(Wild.)Miers ex Hook.F & Thems (Menispermaceae) is a large deciduous climbing shrub found throughout India and also present in Srilanka, Bangladesh and China and is commonly known as Guduchi, Amruthavalli [1]. It is a traditional herbal medicine, used for several diseases like fever, diabetes, dyspepsia, jaundice, and skin diseases [2]. Almost all the parts of the plant are documented to be useful in ethnobotanical surveys conducted by ethnobotanists [3,4]. The isoquinoline alkaloid rich fraction from stem, including, palmatine, jatrorrhizine, and magnoflorine have been reported for insulin-mimicking and insulin-releasing effect and particularly magnoflorine has the potential of being developed as a new antidiabetic agent [5].

The growth of medicinal plants and production of secondary metabolites with of medicinal value can be altered by the application of plant growth regulators. Exogenous application of triacontanol was found beneficial in improving the herbage yield as well as artemisinin content in *Artemisia annua* [6]. Application of the cytokinin 6-benzylaminopurine (BAP) to *Mentha* plants resulted in increase in vegetative growth as well as essential oil production [7]. Foliar application of GA_3 and NAA enhanced the plant height, number of branches and leaf area of ajwain [8]. Increase in biomass production in *Andrographis paniculata* due to plant growth regulators was reported by *Gudhate et al*[9].

Brassinosteroids (BRs) are a new group of phytohormones with significant growth promoting nature [10,11]. BRs are considered as growth regulators with pleiotropic effects, as they influence diverse physiological processes like growth, germination of seeds, rhizogenesis, senescence and also confer resistance to plants against biotic and abiotic stresses [12]. The importance of the

brassinosteroids in improving the growth and yield of crop plants is well documented [13]. In the present study the effect of exogenous application of brassinosteroids on growth and metabolite content of *Tinospora cordifolia* is being investigated.

MATERIALS AND METHODS

Pot Culture Studies

Plant material: *Tinospora cordifolia* stem cuttings were collected from Botanical garden of Osmania University, Hyderabad, India.

Chemicals: Two bioactive brassinosteroids i.e. 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) procured from CID tech research Inc, Mississauga, Ontario, Canada were employed for the studies.

Propagation studies

15 cm nodal stem cuttings with two leaves were excised from the plants. These were planted in nursery covers filled with 3:1 mixture of garden soil and cocopit (obtained from local market). The nursery covers were watered immediately and transferred to Botanical garden. These were irrigated daily during initial 20 days and subsequently once in two days. The leafy cuttings were given brassinosteroid treatment as foliar spray on 30^{th} and 45^{th} day. Each compound was tested at 3 concentrations levels *viz*. 0.5, 1.0 and 2.0µM levels. The growing plants were given stick support. The plants were allowed to grow for 90 days. On 90^{th} day growth of the plant was recorded.

On 90^{th} day, the height of the plant was recorded and then plants were uprooted carefully from pots and dipped in bucket full of water to remove the adhering soil particles, ensuring safety of roots and the plants were blotted and employed for recording other growth parameters. The growth attributes recorded were fresh weight, dry weight (by keeping the plant material in oven at 110^{o}C for 24 hours), root parameters (employing WhinRhizo Root Scanner (XLRHIZO 2012a, Regent Instruments Inc, and Canada) and leaf area (by Leaf Area Meter (CI-203, CID Inc. Vancouver, Washington-USA).

Fresh leaf material was thoroughly homogenized in 70% (v/v) ethyl alcohol and employed for the estimation of soluble proteins and carbohydrate fractions.

Extraction and estimation of chlorophyll pigments

Chlorophyll pigments were extracted from fresh leaf material in 80% (v/v) acetone and estimated according to the method of Arnon [14].

Carbohydrate Fraction

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of total sugars [15] and reducing sugars [16]. The residue was used for the estimation of starch [17] and non-reducing sugar content was calculated by adopting the formulae given by Loomis and Shull [18].

Soluble Proteins

Soluble proteins in alcoholic homogenate were precipitated by adding 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 1% (w/v) sodium hydroxide. The method of Lowry *et al.* [19] was used for the quantification of proteins.

RESULTS AND DISCUSSION

Foliar application of brassinosteroids (EBL and HBL) on *Tinospora cordifolia* increased plant height (Table: 1). Among all the treatments, homobrassinolide at 2µM concentration proved to be highly effective in enhancing the plant height (39% over control). Brassinosteroid treatment caused substainal increase in fresh and dry weight of shoot system. An impressive 42.5% increase in fresh

weight as well as dry weight due to homobrassinolide application at 2µM concentration was observed. Similar increase in growth and biomass production in *Chlorella vulgaris* was obtained in response to brassinosteroids application [20]. An increase in the leaf biomass and leaf area in *Cucumis sativus* by exogenous application of brassinosteroids was reported [21].

All the root parameters recorded such as root area, width and length were found enhanced due to brassinosteroid feeding (Table: 1). Both the brassinosteroids used caused appreciable increase in root growth. The impact of brassinosteroids was much more impressive on root area. 28-homobrassinolide accounted 114% increase in root area at 2µM concentration. Swamy and Rao [22] demonstrated the ability of brassinosteroids to improve the root formation and growth in *Coleus* plant. Similarly, brassinosteroids promoted root growth in *Arabidopsis* [23]. Exogenous application of HBR enhancedthe root growth rate and root length in barley [24]. Müssig *et al*., [23] obtained 50% increase in root growth in wild type of *Arabidopsis thaliana* and upto 150% enhancement in BR-deficient mutant *dwfl-6 and cbb-3* by the application of brassinosteroids.

Exogenous application of brassinosteroids supported good foliage growth as evidenced by number of leaves and leaf area (Table: 2). The increase in leaf number and leaf area was found to be dose dependent. At 2µM concentration, 32% and 56% increase leaf area was obtained by EBL and HBL respectively. Earlier Swamy and Rao [25] found good enhancement in leaf area in case of geranium plants due to brassinosteroid application. Similarly, 2.4 times increase in leaf area was observed in *Coleus* plants treated with 3µM concentration of 28-homobrassinolide [22]. Exaggerated growth of *Arabidopsis* by brassinolide application has been reported [26].
Exogenous application of brassinosteroids resulted in a significant increase in chlorophyll levels in *T. cordifolia* (Table: 3). Particularly at 2µM concentrations both the brassinoasteroids were found to be highly effective in increasing the contents of chlorophyll pigments. Similarly, the amount of chlorophyll a, b and total chlorophyll were increased under BR treatments in *Gossypium* and *Vigna mungo* [27]. The Photosynthetic pigment levels are increased in salt stress induced rice seedlings with supplementation of brassinosteroids [28]. A stimulatory effect on the photosynthetic pigments was reported due to 24-epibrassinolide in *Wolffia arrhiza* [29] and in rape seedlings [30].

The foliar application of the brassinosteroids increased the carbohydrate contents in *T. cordifolia* (Table: 4). Increase in leaf area coupled with elevated levels of photosynthetic pigments might have resulted in enhancement of CO_2 assimilation, which might have contributed to high levels of carbohydrate productions. BRs promoted photosynthesis and growth by positively regulating synthesis and activation of a variety of photosynthetic enzymes including Rubisco in Cucumber [31]. Impaired carbohydrate metabolism and reduced biomass was found in a brassinosteroid-deficient *Arabidopsis* mutant [32].
Soluble protein content in brassinosteroids treated plants was greater than untreated control plants (Table: 4). Highest levels of soluble protein in *T. cordifolia* plants were observed at 2µM concentrations of 24-epibrassinolide as well as 28-homobrassinolide.Brassinosteroids application also accouted for enhancement of seed protein content in pea and lupine [33]. Brassinosteroids increased the root protein content in radish [34] and in cultured cells of *Chlorella vulgaris* [35].

CONCLUSION

The present study clearly demonstrated the positive influence of brassinosteroids on the growth of *Tinospora cordifolia*. Brassinosteroids application caused substantial increase in chlorophylls, proteins and carbohydrate fractions which have crucial bearing on growth and development.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

Tables

Table 1. Effect of brassinosteroids on vegetative growth of *Tinospora cordifolia*.

Treatments	Plant height (cm)	Shoot fresh weight (gm)	Shoot dry weight (gm)	Root fresh weight (gm)	Root dry weight (gm)	Root length (cm)	Root width (cm)	Root area (cm^2)
Control	46.74±1.682	30.21±1.02	3.356±0.25	3.23±0.23	0.358±0.015	10.28±1.015	4.21±0.646	45.23±6.42
0.5µM EBL	49.14±1.721	33.56±2.23	3.728±0.56	3.52±0.02	0.391±0.036	12.03±0.817	4.86±0.269	56.56±10.86
1µM EBL	54.06±2.020	35.85±1.56	3.983±0.21	3.71±0.52	0.412±0.054	13.11±2.113	5.32±1.114	67.79±8.15
2µM EBL	59.16±2.243	38.05±1.04	4.22±0.58	4.05±0.41	0.450±0.112	15.29±2.074	5.20±0.890	76.86±5.87
0.5µM HBL	55.48±2.343	36.61±0.98	4.067±1.01	3.65±0.09	0.405±0.210	13.15±0.630	4.81±1.085	61.17±4.84
1µM HBL	60.46±1.553	39.14±2.04	4.348±0.87	3.96±0.36	0.441±0.063	15.02±1.035	4.92±0.979	70.06±4.09
2µM HBL	64.96±2.447	43.02±2.21	4.780±0.63	4.66±0.54	0.517±0.241	17.07±1.015	5.62±1.049	96.89±5.88

The data presented above are Mean ± S.E. (n=5).

EBL=24-epibrassinolide, HBL=28-homobrassinolide.

Table 2. Effect of brassinosteroids on foliage growth of *Tinospora cordifolia*.

Treatments	No. of Leaves	Leaf area (cm^2)
Control	15.0±1.414	33.02±0.665
0.5µM EBL	16.4±1.568	37.11±0.594
1µM EBL	18.2±1.280	39.73±0.484
2µM EBL	19.6±2.088	43.61±0.942
0.5µM HBL	18.3±2.130	46.35±0.489
1µM HBL	20.8±2.130	48.18±0.661
2µM HBL	22.8±2.922	51.59±1.014

The data presented above are Mean ± S.E. (n=5).

EBL=24-epibrassinolide, HBL=28-homobrassinolide.

Table 3. Effect of brassinosteroids on Photosynthetic pigments of *Tinospora cordifolia*.

Treatments	Chlorophyll-a (mg g^{-1} FW)	Chlorophyll-b (mg g^{-1} FW)	Total Chlorophylls(mg g^{-1} FW)
Control	1.23±0.009	0.440±0.0031	1.676±0.011
0.5µM EBL	1.24±0.010	0.444±0.0036	1.690±0.013
1µM EBL	1.26±0.008	0.457±0.0041	1.717±0.012
2µM EBL	1.29±0.007	0.479±0.0031	1.777±0.010
0.5µM HBL	1.29±0.021	0.452±0.0036	1.746±0.025
1µM HBL	1.33±0.010	0.473±0.0016	1.805±0.010
2µM HBL	1.42±0.018	0.489±0.0025	1.917±0.019

The data presented above are Mean ± S.E. (n=5).

EBL=24-epibrassinolide, HBL=28-homobrassinolide.

Table 4. Effect of brassinosteroids on the contents of carbohydrate fractions and soluble proteins.

Treatments	Reducing sugars (mg g^{-1} FW)	Non reducing sugars (mg g^{-1} FW)	Total sugars (mg g^{-1} FW)	Starch (mg g^{-1} FW)	Soluble proteins (mg g^{-1} FW)
Control	0.780±0.241	1.021±0.25	1.803±0.451	12.02±0.841	4.01±0.026
0.5µM EBL	0.968±0.532	1.043±0.14	2.015±0.521	12.85±1.021	4.52±0.191
1µM EBL	1.092±0.086	1.104±0.25	2.203±0.652	13.54±0.471	5.91±0.129
2µM EBL	1.096±0.065	1.137±0.13	2.234±0.235	15.26±0.751	6.18±0.134
0.5µM HBL	1.086±0.014	1.113±0.35	2.207±0.412	13.65±0.045	5.70±0.197
1µM HBL	1.142±0.471	1.165±0.05	2.312±0.054	14.97±0.652	6.29±0.148
2µM HBL	1.289±0.035	1.315±0.07	2.617±0.035	17.05±2.011	8.68±0.101

The data presented above are Mean ± S.E. (n=5).

EBL=24-epibrassinolide, HBL=28-homobrassinolide.

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