Effect of brassinosteroids on rooting and early vegetative growth of Coleus \[Plectranthus forskohlii\] (Willd.) Briq.] stem cuttings

K N Swamy and S Seeta Ram Rao*
Department of Botany, Osmania University, Hyderabad-500 007, India.

Received 23 October 2008; Accepted 22 June 2009

Brassinosteroids are new class of phytohormones and now considered as the sixth group of hormones in plants. They influence varied growth and developmental processes such as growth, germination of seeds, rhizogenesis, flowering, senescence and abscission and they are considered as plant hormones with pleiotropic effects. The present paper emphasizes the importance of 28-homobrassinolide and 24-epibrassinolide in the root formation and root growth in case of coleus \[Plectranthus forskohlii\] (Willd.) Briq. The study revealed the ability of brassinosteroids in increasing the root formation and root growth of treated cuttings over control.

Keywords: Brassinosteroids, \[Plectranthus forskohlii\], Coleus, Rooting, Forskolin, Phytohormones.

IPC code Int. cl.8—A61K 36/00, A61K 36/53, A01G 31/00

Introduction
Coleus \[Plectranthus forskohlii\] (Willd.) Briq. syn. \[Coleus forskohlii\] Briq.] is an important medicinal plant belonging to the family Lamiaceae. The tuberous roots are rich source of forskolin, a diterpenoid (Fig. 1). The forskolin is being developed as a drug for hypertension, glaucoma, asthma and congestive heart failures\(^1\). Coleus is being used in Ayurvedic medicine for heart disease, relieving spasmodic pain and reducing overweight. The complex herbal mixtures containing root extract of coleus is gaining popularity in US for their putative ‘fat burning’ properties\(^2\). Coleus can be propagated both by seeds and stem cuttings, the latter being the easy and common practice. Plant growth regulators are being increasingly employed to improve rooting and vegetative propagation of plants and helps in quick multiplication of such plants and increase roots availability\(^3\).Brassinosteroids are a new group of phytohormones with significant growth promoting activity and are essential for many processes in plant growth and development\(^4,5\). The ability of certain pollen extracts to promote growth led to the discovery of this group of substances in plants. Collective efforts initiated by the scientists at various Agricultural Research Stations (ARS) of USDA resulted in the isolation of an active factor from the pollen grains of rape plant \[(Brassica napus\] Linn.) which was named as brassinolide\(^6\). As the first steroidal plant growth regulator was isolated from \[Brassica napus\], a generic name ‘brassinosteroids’ has been given to this new group of phytohormones. Brassinosteroids are polyhydroxy steroids. They have a common-cholestane skeleton and their structural varieties come from the kind and the orientation in the A/B rings and side chain\(^7\). Brassinosteroids are hormones with pleiotropic effects as they regulate processes such as growth, seed germination, flowering, senescence and abscission\(^8\). Brassino-
steroids have been reported so far from 60 species which includes 51 angiosperms (12 monocots and 39 dicots), 6 gymnosperms, 1 pteridophyte (Equisetum ravens Linn.), 1 bryophyte (Marchantia polymorpha Linn.) and 1 alga (Hydrodictyon reticulatum (Linn.) Lagerh) and are probably ubiquitous in plant kingdom. The role of brassinosteroids in improving the productivity of various crop plants such as vegetables, fruits, oil seed plants are well documented. In the present study the effect of brassinosteroids on rooting of stem cuttings and early root growth of coleus has been investigated. In addition the impact of basal application of brassinosteroids to stem cuttings on the early vegetative growth was also studied.

Materials and Methods

28-homobrassinolide and 24-epibrassinolide (Fig. 2) were procured from M/s CID tech Research Inc, Mississauga, Ontario, Canada. Coleus [Plectranthus forskohlii (Willd) Briq.] plants were obtained from Central Institute of Medicinal and Aromatic Plants (CIMAP), Resource Centre, Boduppal, Hyderabad and maintained in Botanical Garden of the Department at Osmania University, Hyderabad.

Treatments

From 130 day old stock plants of coleus stem cuttings were obtained. 14 cm long, healthy plant cuttings with two pairs of terminal leaves (all the other leaves were excised) were sorted out for the experiment. Each of the brassinosteroid was tested at two concentration levels, viz. 50 µM and 100 µM. Basal part (2 cm) was dipped for 5 minutes in each concentrations of brassinosteroid solution. Distilled water dipped plant cuttings were used as controls. The treated plant cuttings were transplanted immediately to nursery covers which were filled with garden soil. Sufficient number of replicates was maintained for the experiment so as to terminate the experiments periodically for recording data. The nursery covers were placed in glass-house and the plants were watered weekly thrice.

Rooting

For recording the rooting, nursery covers were flooded with water and then the covers were cut with scissors and the plants were gently separated from the soil without causing any damage to the root system. The number of roots formed per cutting was recorded on 15th and 30th day after the treatment. Further counts could not be possible due to web like growth of root system.

Root growth

Fresh weight and dry weights of the roots per plant was recorded on 30th and 60th day after the treatment. The roots were dried separately in an oven at 70°C for 48 h. and then the dry weight was recorded.

Shoot growth

The shoot growth of the plant was recorded on 60th day in terms of fresh and dry weights; foliage growth was measured by recording leaf area [employing Leaf Area Meter (Model CI-203) CID Inc. Vancouver Washington-USA], number of leaves, fresh weight and dry weight of leaves per plant. The dry weights were recorded after 48h and kept in an oven at 70°C.

Fig. 2—Chemical structure of Brassinosteroids
Statistical analysis
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 \( \leq 0.05 \).

Results and Discussion
Application of brassinosteroids to coleus stem cuttings substantially increased rhizogenesis as reflected in number of roots formed compared to control plant cuttings (Table 1). Among all the treatments 28-homobrassinolide at 100 µM concentration was found highly effective in inducing adventitious roots on stem cuttings of coleus. An increase of 85% and 76%, on 15\(^{th}\) and 30\(^{th}\) day, respectively in the number of roots formed was observed due to the application of 28-homobrassinolide (100 µM). The application of brassinosteroids might have triggered the innate genetic potentiality of the plants for rhizogenesis there by improved the root formation. The process of root initiation and root formation on stem cuttings is controlled by plant growth regulators\(^{13}\). The ability of indole butyric acid in rooting of plant cuttings is well known. IBA enhanced rooting on Himalayan yew (\textit{Taxus baccata} Linn.) stem cuttings\(^{14}\). Increase in rooting of stem cuttings of aromatic plants such as Rosemary (\textit{Rosmarinus officinalis} Linn.), Thyme (\textit{Thymus vulgaris} Linn.) and Peppermint (\textit{Mentha piperita} Linn.) by the application of synthetic auxins was observed. The ability of polyamines in the induction of adventitious root formation was demonstrated\(^{15-16}\). The results of the present study clearly revealed the ability of brassinosteroids to stimulate root formation on coleus, an important medicinal plant with great potential. Similar enhancement in root formation on the stem cuttings of Norway spruce (\textit{Picea abies} Karst.) by the application of homobrassinolide was observed\(^ {17}\).

Brassinosteroids not only affected the root formation but also improved root growth in coleus (Table 1, 2). 28-Homobrassinolide (100 µM) was found to be highly effective in increasing the root growth (Fig. 3). A positive correlation between endogenous brassinosteroids levels and root growth in \textit{Arabidopsis} was established\(^ {18}\). Application of 24-epicastosterone and 24-epibrassinolide promoted root elongation in wild and Br-deficient mutants of \textit{Arabidopsis} such as dwf-1-6 (cbb\(_1\)) and (cbb\(_3\))\(^ {18}\). Similarly, application of Brz 2001, a brassinosteroid inhibitor completely impaired the root growth and application of 24-epibrassinolide totally reversed the inhibitory effect and restored normal root growth\(^ {19}\).

Increase in adventitious root formation in coleus stem cuttings by the treatment with brassinosteroids was further translated into improved growth of the plants as reflected in enhancement in fresh weight and dry weight of the shoot system (Table 2). 28-Homobrassinolide at 100µm was found to be most effective in supporting the growth of the plants. Moreover, the plants obtained from brassinosteroids treated stem cuttings exhibited better foliage growth as compared to that of control plants (Table 2). Increase in leaf area by brassinosteroids as observed in the study amounts to enhancement in photosynthetic area and that might have contributed to increase in growth. Increase in photosynthesis, due to brassinostoroids was reported in case of \textit{Chlorella}\(^ {20}\) and geranium\(^ {21}\). Brassinosteroid application to culture media shown significant increase in growth and biomass production in \textit{Chlorella vulgaris}\(^ {20}\). Similarly exaggerated growth in \textit{Arabidopsis} by brassinolide application was reported\(^ {22}\).

| Table 1—Effect of brassinosteroids on rooting of the stem cutting of Coleus |
|---|---|---|---|
| Treatment | 15\(^{th}\) day | 30\(^{th}\) day | 30\(^{th}\) day |
| | No. of roots/cutting | Fresh weight of roots (mg) | Dry weight of roots (mg) |
| Control | 3.4 ± 0.3d | 240 ± 11e | 32 ± 2e |
| Ebl 50 µm | 4.8 ± 0.5bc | 331 ± 17d | 57 ± 7d |
| Ebl 100 µm | 6.1 ± 0.1a | 670 ± 21ab | 84 ± 9b |
| Hbl 50 µm | 5.2 ± 0.3b | 482 ± 26c | 70 ± 9c |
| Hbl100 µm | 6.3 ± 0.1a | 714 ± 13a | 95 ± 8a |

Ebl: 24-Epibrassinolide; Hbl: 28-Homobrassinolide. The data presented above are Mean ± S.E. (n = 5). Mean followed by the same alphabet in column is not significantly different at \( P = 0.05 \) level.
Table 2—Effect of brassinosteroids on the root, stem and foliage growth of Coleus on 60th day

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot length cm</th>
<th>Shoot Fw (g)</th>
<th>Shoot Dw (g)</th>
<th>Root Fw (g)</th>
<th>Root Dw (g)</th>
<th>Fw leaves/plant (g)</th>
<th>Dw leaves/Plant (g)</th>
<th>No. of leaves/plant</th>
<th>Total leaf area/plant Cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.1 ± 2d</td>
<td>2.66 ± 0.3e</td>
<td>0.25 ± 0.03e</td>
<td>0.52 ± 0.01e</td>
<td>0.07 ± 0.004d</td>
<td>2.90 ± 0.6e</td>
<td>0.23 ± 0.08e</td>
<td>8 ± 0.2c</td>
<td>37.15 ± 4d</td>
</tr>
<tr>
<td>Ebl 50 μm</td>
<td>15.7 ± 0.8c</td>
<td>3.57 ± 0.1d</td>
<td>0.33 ± 0.02d</td>
<td>0.61 ± 0.03d</td>
<td>0.09 ± 0.002c</td>
<td>4.83 ± 0.9d</td>
<td>0.39 ± 0.05d</td>
<td>12 ± 0.5b</td>
<td>64.32 ± 2c</td>
</tr>
<tr>
<td>Ebl 100 μm</td>
<td>16.5 ± 1b</td>
<td>4.39 ± 0.7b</td>
<td>0.40 ± 0.01b</td>
<td>1.26 ± 0.05b</td>
<td>0.20 ± 0.001a</td>
<td>5.59 ± 10b</td>
<td>0.47 ± 0.01b</td>
<td>15 ± 0.1a</td>
<td>81.04 ± 5a</td>
</tr>
<tr>
<td>Hbl 50 μm</td>
<td>15.9 ± 3c</td>
<td>3.95 ± 0.5c</td>
<td>0.38 ± 0.07bc</td>
<td>0.86 ± 0.02c</td>
<td>0.12 ± 0.001b</td>
<td>5.02 ± 0.2c</td>
<td>0.41 ± 0.06bc</td>
<td>13 ± 0.2b</td>
<td>69.64 ± 7b</td>
</tr>
<tr>
<td>Hbl 100 μm</td>
<td>17.4 ± 1a</td>
<td>4.93 ± 1a</td>
<td>0.49 ± 0.01a</td>
<td>1.47 ± 0.08a</td>
<td>0.25 ± 0.007a</td>
<td>9.28 ± 0.7a</td>
<td>0.54 ± 0.1a</td>
<td>16 ± 0.3a</td>
<td>85.67 ± 2a</td>
</tr>
</tbody>
</table>

Ebl: 24-Epibrassinolide; Hbl: 28-Homobrassinolide. Fw = Fresh weight; Dw = Dry weight. The data presented above are Mean ± S.E. (n = 5). Mean followed by the same alphabet in column is not significantly different at P = 0.05 level.
Conclusion
This study has clearly demonstrated the ability of brassinosteroids to improve the overall vegetative growth of Coleus. A great role for brassinosteroids in 21st century agriculture is envisaged. The findings add another dimension to the practical utility of this new group of phytohormones for commercial cultivation of important drugs.

Acknowledgement
The financial support from National Medicinal Plants Board (NMPB), New Delhi, India is gratefully acknowledged.

References

Fig. 3—Effect of 28-Homobrassinolide on root growth of coleus


Davis DT and Hassing BE, Chemical control of adventitious root formation in cuttings, *Bull Plant Growth Reg Soc Amer*, 1990, 18, 1-17.


