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Short communication

In vitro root induction by 24-epibrassinolide on hypocotyl segments of soybean [*Glycine max* (L.) Merr.]

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Abstract. Soybean hypocotyl segments were treated in the dark with 24-epibrassinolide (BR) at a range of concentrations for different durations. The maximum effect on adventitious root induction, both in terms of number and length was obtained at very low concentration (0.0001 ppm) of BR applied for 8 h. Higher concentrations were supraoptimal unless applied for a shorter period (4 h). BR was ineffective when applied at low concentration in continuous light.

1. Introduction

Brassinolide is a natural phytohormone which has powerful growth accelerative effects when applied to young plants; it may be regarded as a sixth group of phytohormones [1]. Since the discovery of 'brassin' [4], subsequently renamed brassinolide [2], promotive effects have been reported on cell elongation, cell division and growth of callus. In some biological systems, brassinolide has been shown to have from 10 to 100 times more plant growth regulating activity than gibberellic acid [6]. Since Mandava et al. [3] reported BR inhibition of adventitious root development in hypocotyls of mung bean, dwarf bean and cucumber, little further information has been obtained on the root-inducing efficiency of BR either in callus or in hypocotyl. This paper reports the in vitro induction of adventitious roots in hypocotyl segments of soybean at different concentrations of brassinolide.

2. Materials and methods

Seeds of soybean [*Glycine max* (L.) Merr.] were germinated in vermiculite and grown in 16 h light and 8 h dark photoperiods or in continuous light

Table 1. Effect of 24-epibrassinolide on the number and mean length of adventitious roots in hypocotyl segments of soybean. Values are mean of 10 replicates ± S.D

Time	Concentration (ppm)	No. of adventitious roots initiated	Mean length of adventitious roots (mm)
0	Control	12 ± 2.8	40 ± 0.2
4 h	0.0001	17 ± 2.0	53 ± 0.1
4 h	0.001	31 ± 3.3	84 ± 0.1
4 h	0.1	28 ± 2.4	61 ± 0.2
8 h	0.0001	41 ± 2.8	144 ± 0.8
8 h	0.001	23 ± 2.0	63 ± 0.1
8 h	0.1	11 ± 1.6	50 ± 0.3
Continuous	0.0001	19 ± 2.8	47 ± 0.2

(2000 lux) or full dark conditions at 20 ± 1 °C for 15 days. Roots were removed from the 15-day-old seedlings and hypocotyl segments treated with 24-epibrassinolide (BR) for either 4 or 8 h in the dark; one treatment at the very low concentration (0.0001 ppm) was treated continuously in light for 10 days. The explants, consisting of two cotyledons and 10–12 cm of hypocotyl, were sterilized with 2.5% antiformine for 2–3 minutes, washed in distilled water and then treated with different concentrations of BR as indicated in Table 1. Each treatment including the untreated control consisted of triplicates of ten segments. After treatment, the segments were removed from the test solutions and submerged to half their length (5–6 cm) in distilled water contained in test tubes. The distilled water in these tubes was replaced every 24 h to avoid any microbial contamination. The tubes were kept in a growth chamber at 20 ± 1 °C with a light intensity of 2000 lux. The number of adventitious roots initiated per hypocotyl and their length were measured ten days after treatment.

3. Results and discussion

In both the etiolated hypocotyls obtained from dark grown seedlings and hypocotyls from the continuous light treatment, BR treatment failed to induce adventitious roots at any of the concentrations employed. Hypocotyl segments produced in 16 h light and 8 h dark photoperiods and then treated with eiher 0.1 ppm BR for 8 h in the dark or 0.0001 ppm BR continuously in the light did not respond to treatment (Table 1 and Figure 1). Although 0.1 and 0.001 ppm BR treatment for 4 h were significantly different from the control, the most effective treatment was 0.0001 ppm BR for 8 h, which gave the maximum number and length of adventitious roots. Brassinolide treatment for 4 h with 0.001 ppm was also comparatively effective in inducing

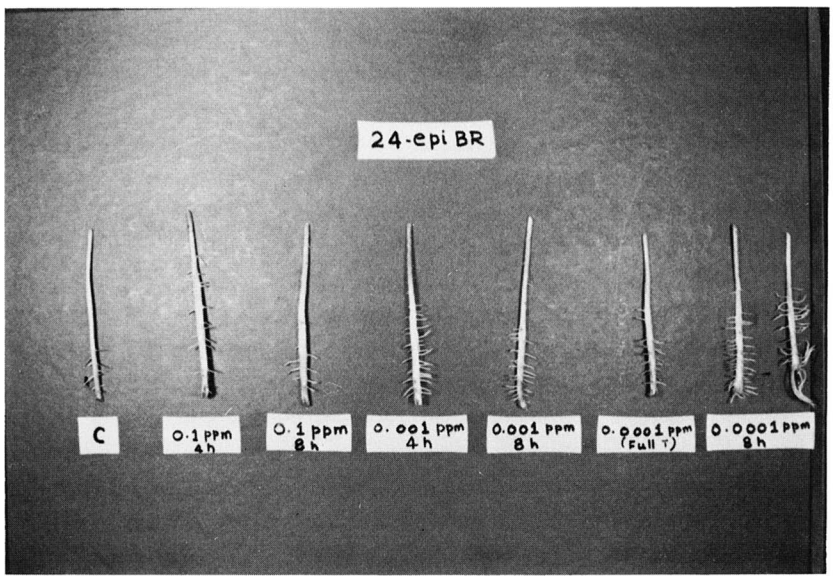

Fig. 1. In vitro rooted hypocotyl segments of soybean treated with different BR concentration. (Cotyledons and a portion of hypocotyl were removed before photography.) C – control; full T – continuous treatment.

adventitious roots and increasing their mean length of lateral roots. The significant differences in root number were due to the formation of adventitious roots over a long distance along the hypocotyl and appear to be due to the induction of more root primordia in hypocotyl segments by BR treatment at very low concentrations (Figure 1); this may be associated with the distribution of BR over a greater length of the hypocotyls [7]. The previous observations of increased root length of spinach, stimulation of root growth in maize and increased yield of sweet potato by brassinolide treatments are consistent with our findings ([5], JRDC-694, 695 24-epibrassinolide group personal reports, Japan).

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References

1. Adam, G. and Marquavdt, V. 1986. Brassinosteroids. Phytochemistry 25: 1787–1799
2. Grove, M.D., Spencer, G.F., Rohwedder, W.F., Mandava, N.B., Worley, J.F., Warthen,

J.D., Jr., Steffens, G.L., Flippen-Anderson, J.L. and Cook, J.C., Jr. 1979. Brassinolide, a plant growth-promoting steroid from *Brassica napus* pollen. Nature 281: 216–217

3. Mandava, N.B., Sasse, J.M. and Yopp, J.H. 1981. Brassinolide, a growth promoting steroidal lactone. II. Activity in selected gibberellin and cytokinin bioassays. Physiol Plant 53: 453–461
4. Mitchell, J.W., Mandava, N.B., Worley, J.F., Plimmer, J.R. and Smith, M.V. 1970. Brassins – a new family of plant hormones from rape pollen. Nature 225: 1065–1066
5. Romani, G., Marre, M.T., Bonetti, A., Gerena, R., Lado, P. and Marre, E. 1983. Effect of brassinosteroid in growth and electrogenic proton extrusion in maize root segments. Physiol Plant 59: 528–532
6. Takatsuto, S., Yazawa, N., Ikekawa, N., Takematsu, T., Takeuchi, Y. and Koguchi, M. 1983. Structure – activity relationship of brassinosteroids. Phytochemistry 22: 2437–2441
7. Wiesman, Z., Riov, J. and Epstein, E. 1988. Comparison of movement and metabolism of indole-3-acetic acid and indole-3- butyric acid in mung bean cuttings. Physiol Plant 74: 556–560