

# An exception of synergistic interaction between brassinosteroids and auxin

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## ABSTRACT

We observed growth promoting effects of brassinolide, a natural brassinosteroid, in the absence of auxin in a standard elongation assay system with hypocotyl segments from dark-grown 4-day-old soybean (*Glycine max*) seedlings. The elongation growth of soybean hypocotyls was not further increased by a combined treatment with brassinolide and IAA. This finding is not consistent with the data appeared in old literatures reporting synergistic interaction of brassinosteroids and auxin. Our observation indicates that the synergism of brassinosteroids and auxin appears not always, but condition-dependently.

**Key words** : brassinosteroids, auxin, hypocotyl elongation, synergism, *Glycine max*

## Introduction

Brassinosteroids are substances belonging to a class of plant hormones that evoke characteristic biological responses called brassin activities - elongation, curvature and splitting of the treated tissues in the bean second-internode bioassay (1). The bean bioassay system was successfully used for the isolation of brassinosteroids and later for the determination of the structure-activity relationship. Another characteristic response of plants to brassinosteroids is the lamina-inclination observed in rice (2). Auxin can induce a similar response in rice, but only at much higher concentrations than those of brassinosteroids (1). Gibberellic acid evokes prominent straight growth, but does not induce the lamina-inclination in rice (3). Such responses indicate the existence of brassinosteroid-specific biological responses.

*In planta*, brassinosteroids induce biological responses not

only by itself, but also by interaction with other plant hormones, most prominently with auxin in cell elongation and ethylene production (4, 5). The mode of interaction has been known as synergistic. For example, when the two hormones were included in the same incubation medium, the elongation growth was much higher than the sum of that induced by each hormone. Furthermore, brassinolide stimulated the growth only in the presence of auxin in some plant species, stimulating a debate on auxin-dependency of brassinosteroid activities in the stimulation of elongation (6).

We investigated elongation growth induced by brassinolide and auxin in soybean hypocotyls segments, and obtained some results inconsistent with old reports.

## Materials and Methods

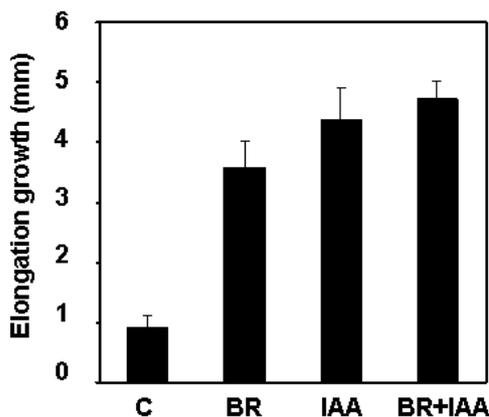
Seeds of soybean (*Glycine max*) were soaked in distilled water for 30 min, spread on wet vermiculite and incubated in darkness at 28°C for 4 days. The segments (10 mm) of hypocotyls were excised by double-edged razor blades and

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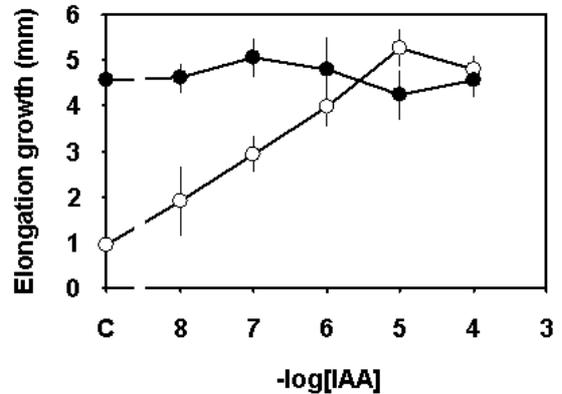
floated on distilled water for 2 hours to reduce spontaneous growth response after excision. Then, 8 - 10 segments were randomly collected and moved to an incubation medium (5 mM potassium phosphate buffer, pH 6.8; 5 ml) with or without plant hormones for standard elongation tests. In all the treatments, the concentration of ethanol, used for dissolving plant hormones, in the medium was controlled and generally set to 0.1%. The length of the segments before and after incubation was measured and analyzed.

## Results and Discussion

Brassinolide, a natural brassinosteroid, stimulated the growth of hypocotyl segments of soybean (*Glycine max* L.) in the absence of auxin, demonstrating the independency of brassinosteroids (Fig. 1). IAA (indole-3-acetic acid; 1  $\mu$ M), a natural auxin, stimulated the growth also in the same test system. When the two hormones were included in the same growth medium together, no further stimulation was observed; i.e. the previously proven synergism did not appear. The currently observed elongation of soybean hypocotyls is very different from that of maize coleoptiles, where brassinolide alone shows only weak growth-promoting activities (Park *et al.*, unpublished).



**Fig 1.** Elongation growth of soybean hypocotyl segments. Values ( $M \pm SE$ ,  $n = 9$ ) show increase in length of 10-mm segment after over-night incubation in the presence of plant hormones as indicated. C: control, BR: 0.1  $\mu$ M brassinolide, IAA: 1  $\mu$ M IAA



**Fig 2.** Dose-response curves of IAA in the elongation growth in soybean hypocotyl segments. The growth after over-night incubation is presented as  $M \pm SE$  ( $n = 10$ ) in the absence (open circle) and presence (closed circle) of 1  $\mu$ M brassinolide. Data originate from a representative experiment in similar repeats.

To scrutinize the detailed characteristics of the interaction between brassinolide and IAA in soybean hypocotyls, we tested the effect of brassinolide on the IAA dose response curve. Because the changes in dose-response curves could reveal the mode of sensitivity variation (7), we tried to determine the type of modification of auxin activities caused by applied brassinolide. In soybean hypocotyl segments, IAA dose-response was increased proportional to the concentrations (Fig. 2). However, the IAA of any concentration did not induce any further increase of elongation in the presence of brassinolide; i.e., the extent of elongation was always similar to that induced by 1  $\mu$ M brassinolide alone. In contrast, brassinolide and auxin revealed a synergistic stimulatory effect on maize coleoptile elongation (Park *et al.*, unpublished).

An interesting aspect of this result is that the maximum elongation, which could be reached by 10  $\mu$ M IAA, was achieved by 1  $\mu$ M brassinolide. Furthermore, the shape of IAA dose-response curve suggests that the auxin response could be saturated by exogenously applied brassinolide in soybean hypocotyls. The level of endogenous IAA might be high enough to evoke maximal growth when 1  $\mu$ M brassinolide was applied. In the same context, somewhat lower levels of free IAA might be expected in maize coleoptiles, where brassinolide alone did not show clear growth-stimulating ef-

fect, than that in soybean hypocotyls. Therefore, it warrants further studies to understand the correlations between the mode of brassinolide effects on auxin action and the endogenous level of IAA in plant tissues.

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