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Effect of Homobrassinolide on *In Vitro* Growth of Apical Meristems and Heat Tolerance of Banana Shoots

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ABSTRACT

The effect of homobrassinolide (hBR) alone or in combination with IAA or 2-ip on induction and elongation of shoots regenerated from apical meristems of banana cultured *in vitro* was investigated. hBR (0.2 µM) had a pronounced effect on stimulating shoot proliferation compared with control. IAA alone in media at a concentration of 1µM was less effective in stimulating shoot elongation than hBR. Supplying medium with 2-ip (10 µM) had the highest stimulating effect on the number of shoots regenerated from apical meristems. However, its activity on shoot elongation was less than hBR or IAA. Sequential treatment of apical meristems with hBR followed by IAA resulted in synergistic enhancement of shoot elongation and was superior to other treatments. When hBR was added simultaneously or sequentially with 2-ip, induction and elongation of shoots was markedly improved compared with each of them alone, showing an additive effect of the two growth regulators. hBR had a remarkable anti-stress effect and greatly reduced the percentage of heat injury.

Key Words: Homobrassinolide; Banana; *In vitro;* Shoot elongation; Heat stress

INTRODUCTION

Brassinosteriods (BRs) represent a group of naturally occurring steroidal lactones widely distributed in the plant kingdom (Mandava, 1988). These compounds, which include the highly bioactive brassinolide (BR) and its analogues, have been regarded as new plant growth regulators essential for normal plant growth and development (Mandava, 1988). BR was first discovered and isolated from *Brassica napus* pollens (Grove *et al.,* 1979). Physiological responses of BR include effects on elongation, bending, cell division and vascular development (Wada *et al.,* 1981; Yokota *et al.,* 1982, 1985; Arteca, *et al.,* 1983; Katsumi, 1985; Tominaga *et al.,* 1994; Fujioka *et al.,* 1995).

Several reports have been published on the pronounced effects of BR and homobrassinolide (hBR), which is a close analogue to BR (Mori, 1980), on the elongation of young stem tissues. These included maize mesocotyl, pea, Azuki bean and mung bean epicotyls (Gregory & Mandava, 1982; Mandava, 1988; Yopp *et al.,* 1981), bean and cucumber hypocotyls (Mandava *et al.,* 1981; Katsumi, 1985) and wheat coleoptile (Sasse, 1985). Epibrassinolide (eBR), another analogue to BR was also reported to promote stem elongation of sweet pepper (Franck-Duchenne *et al.,* 1998).

BRs were also found to have an activity *in vitro*. They were reported to increase the rate of cell division and colony formation of Chinese cabbage mesophyll protoplasts (Nakajima *et al.,* 1996) and *Petunia hybrida* protoplasts (Oh & Clouse, 1998). BRs were also found to be essential for the differentiation of isolated *Zinnia* mesophyll cells into tracheary elements (Iwasaki & Shibaoka, 1991) and in the morphogenesis of *Arabidopsis* (Li *et al.,* 1996). Moreover, BRs promoted adventitious shoot regeneration from segments of cauliflower hypocotyls (Sasaki, 2002) and to improve embryogenic tissue initiation in conifers and rice (Pullman *et al.,* 2003)

It has been suggested that the effect of BRs is mediated through auxins or probably through increasing tissue sensitivity to endogenous auxins (Mandava, 1988). Numerous physiological studies addressed BRs-auxin interaction: they act synergistically with auxin in elongation (Yopp *et al.,* 1981), in lamina joint bending (Takeno & Pharis, 1982) and in ethylene formation (Arteca, *et al.,* 1983). Additive effect of BRs with cytokinins was also demonstrated (Sasaki, 2002).

In addition to stimulating growth, brassinosteroides were found to help plants overcoming biotic and abiotic stresses (Mandava, 1988). Brassinosteroid-treated tomato and rice plants grew better than control plants under low temperature conditions (Kamuro & Takatsuto, 1991). A significant influence of BRs on the growth recovery of maize and cucumber seedlings after chilling has also been demonstrated (He *et al.,* 1991; Katsumi, 1991). BRs also help wheat leaf cells to overcome heat-shock stress (Kulaeva *et al.,* 1991). They protect Chinese cabbage and rice plants from pathogen attack and salt injury, respectively (Cutler, 1991).

Since the physiological effects and mode of action of hBR still need further understanding, the present work was an attempt to give insights in this connection. Therefore, the present investigation reports on the interaction of homobrassinolide (hBR) with an auxin (indole-acetic acid,

IAA) or a cytokinin (iso-pentyladenine, 2ip) in stimulating shoot induction, proliferation and elongation of *in vitro* apical meristems of banana (*Musa* spp. Cv. Grande). The anti-stress effect of hBr was also evaluated after exposing banana shoots to heat stress.

MATERIALS AND METHODS

Banana (*Musa* spp. Cv. Grande) apical meristems used, were taken from Tissue Culture and Biotechnology Lab., Faculty of Science, Ain Shams University, Cairo, Egypt. Bacteriological Agar was purchased from Meron, Marine Chemicals, Cochin, India. Culture medium, IAA and 2-ip are Sigma products.

Preparation of culture media. Medium (Murashige & Skoog, 1962) was used as basal medium including MS vitamins and 30g/L sucrose. Media were solidified with 0.8% Bacteriological Agar after adjusting the pH to 5.8±0.1 and autoclaved for 15 min at 121°C and 15 psi. Plant growth regulators, IAA, 2-ip and hBR were added to the medium after autoclaving using sterile fine filters of 0.2µm pore size (Flow Lab. UK).

Effect of hBR on growth. Apical meristems of banana, in the form of short clusters (Fig. 1a), were cultured on hormone-free medium (control) or media containing homobrassinolide (0.2 µM) and/or IAA (1 µM). Apical meristems were also cultured on media containing 2-ip (10 µM) alone or in combination with hBR (0.2 µM). In a separate experiment, apical meristems were pretreated with 0.2-µM hBR for 24 h before transferred to media containing either IAA (1µM) or 2-ip (10 µM). All cultures were incubated at 27±1°C and 16 h photoperiod. Results were recorded after four weeks of incubation as the mean length of shoots (cm), percentage of regenerated shoots per jar and the mean of total fresh weight. Morphological characteristics of the shoot systems in response to different treatments were also described.

Effect of hBr on heat stress tolerance. Heat stress tolerance of banana shoots was tested as percentage of membrane injury caused by exposing these plantlets to high temperatures (40 or 50°C). Thermo-stability of leaf cell membranes was determined by the electrolyte leakage from stressed cells according to Wu and Walter (1983) with modifications. Banana shoots produced on media containing hBR alone along with the control samples (untreated with hBR) were subjected to heat stress. Both samples were incubated for 15 or 30 min at 40 or 50°C. Leaves were detached from the plantlets after heat exposure, dissected into squares (1x1 cm^2) and weighed. Equal weights (1g each) of leaf squares treated with hBR were transferred to flasks containing 50 mL deionized water. Flasks were shaken at 120 cycles/minute for 10 min. The electric conductivity (H_1) of the solution was measured using "Jenway 3310 Conductivity Meter". Tissues of leaf squares were then killed (10 min at 95°C), allowed to cool and the electric conductivity (H_2) was measured again. The same procedure was carried out with the control samples. Injury was expressed as:

$$\%\, of\ injury = 1 - \frac{1-(H_1/H_2)}{1-(C_1/C_2)} \times 100$$

Where C_1 is the conductivity reading of heat stressed control samples and C_2 is the value of killed control samples (10 min. at 95°C).

Data were subjected to Student's t-test for statistical analysis.

RESULTS AND DISCUSSION

Effect of hBR on growth. A pronounced increase in shoot length was observed when homobrassinolide was added to the nutrient MS media compared with the control (Table I, Fig. 1b). hBR was also found to have a noticeable effect on the morphology of regenerated banana shoots. Thicker, swelled stems with enlarged leaves were produced (fig. 1b, left). This was accompanied by an increase in the percentage of shoots induced from apical meristems as well as an increase in the fresh weight (Table I). Shoots of the control were thin with coiled narrow leaves compared with those produced with hBR (Fig.1b, right).

This result obtained with hBR was supported by several investigations. Yopp *et al.* (1981) reported that BRs greatly improved the elongation of maize mesocotyl, pea and azuki epicotyl sections. The same effect was also observed with bean and cucumber hypocotyls and bean seedlings (Mandava *et al.*, 1981; Gregory & Mandava, 1982). Similarly, BR stimulated leaf elongation of wheat (*Triticum aestium*) and mustard (*Sinapis alba*) plants (Braun & Wild, 1984 a, b). An increase in fresh weight of slices of Jerusalem artichoke tissues in response to BR was also reported by Yopp *et al.* (1981).

In addition, Clouse *et al. (*1992) reported that BR (0.1 µM) induced measurable increase in length of soybean epicotyl. Frank-Duchenne *et al.* (1998) found that epibrassinolide, at a concentration of 0.1 µM, promoted stem induction and elongation, and produced large leaves of two cultivars of sweet peppers.

Table I. Effect of different treatments on shoot induction in banana. Data are mean ± SD

Treatment	Shoot length (cm)	Shoot induction (%)	Total f.wt. (g/jar)
Hormone-free medium (control)	6.8 ± 0.09	65.3	14.4 ± 1.3
hBR (0.2µM)	9.7 ± 0.3	87.7	23.2 ± 0.8
IAA (1µM)	8.5 ± 0.2	72.1	19.8 ± 0.6
2-ip (10µM)	7.3 ± 0.0	93.2	21.3 ± 1.4
hBR + IAA	8.9 ± 0.1	82.5	22.1 ± 0.7
hBR + 2-ip	10.7 ± 0.	89.4	26.5 ± 1.1
Pretr. hBR then IAA	13.4 ± 0.4	85.1	24.9 ± 0.1
Pretr. hBR then 2-ip	10.9 ± 0.03	93.6	26.3 ± 0.8

Fig. I. (a) Cluster of banana apical meristems used as starting materials. (b) Right: Banana plantlets produced on hormone-free medium (control); Left: Banana plantlets produced on medium containing hBR (0.2 µM). (c) Banana plantlet produced after treating the apical meristems with 0.2µM hBR for 24 h. then transferred to medium containing 1µM IAA

The stimulatory effect of BRs on stem elongation and swelling has been explained its effect on both cell expansion and cell division (Meudt & Thompson, 1983). Clouse *et al.* (1992) found that in cultured parenchyma cells of *Helianthus tuberosus*, application of even nanomolar concentrations of BR stimulated cell division by at least 50% in the presence of auxins and cytokinins. BR, However, promoted cell elongation but not cell division in cell suspension cultures of carrot (Sala & Sala, 1985; Bellincampi & Morpurg, 1998). BR also promoted the rate of cell division and enhanced cluster and callus formation of Chinese cabbage protoplasts (Nakajima *et al.*, 1996).

Results were in contrast with Wilen *et al.* (1995) who reported that growth of bromegrass cell culture was inhibited by the application of 10 or 30µM of epibrassinolide. Moreover, growth of both callus and suspension cultures of *Agrobacterium tumefaciens*-transformed tobacco cells was inhibited by exogenous application of brassinosteroids (Bach *et al.*, 1991).

In the present study, when IAA was used as a sole growth regulator in the culture media, it was less effective in enhancing shoot elongation than BR, but its effect was higher than that of the control and 2-ip (Table I). This result was in contrast with the finding of Katsumi (1985) who reported that both IAA and hBR stimulated the elongation of hypocotyl sections of cucumber, but hBR was not as active as IAA in bringing about this effect. When the activity of 2-ip was tested, it had a lower effect than hBR and IAA in stimulating shoot elongation, although it produced the highest percentage of shoot induction from the apical meristems (Table I).

The interaction between hBR and IAA was examined, in terms of sequential treatment. When apical meristems of banana were pretreated with 0.2µM hBR for 24 h before transferred to media containing 1µM IAA, a noticeable increase in shoot elongation was observed, which was greater than when each of them was added separately (Table I, fig.1c). This synergism between hBR and auxin was described in many plant systems (Yopp *et al.*, 1981; Meudt & Thompson, 1983; Katsumi, 1985). However, they found that when the tissue was pretreated with exogenous auxin before exposing to hBR, no increase in auxin response (no synergism) was observed. Cucumber hypocotyl sections pretreated with auxin (in the absence of hBR) did not show any sensitivity to auxin. It is likely that hBR serves as a potentiator (or modulator) in enhancing the auxin response. This synergism was observed in several reports (Yopp *et al.*, 1981; Arteca *et al.*, 1983; Katsumi, 1985).

When the interaction between hBR and 2-ip was tested, the results indicated that they had additive effect when applied either simultaneously or in sequential way. This was because they greatly promoted the elongation of banana shoots as well as the fresh weight compared with each of them alone. The percentage of shoot induction in both cases had also no significant difference (Table I). Although the interaction between hBR and 2-ip had the

Table II. Percentage membrane injury of banana plantlets produced on medium containing hBR and control

Treatment	hBR		Control	
Temperature(°C)	40	50	40	50
Time(min)	%injury			
15	11	16	22	37
30	18	27	30	52
LSD	*1.96*	*2.02*	*0.98*	*2.34*

greatest effect in stimulating shoot length than the control, hBR, IAA or hBR+IAA, it was less effective than the sequential treatment of hBR then IAA (Table I). Sasaki (2002) reported that when BR was added together with zeatin and iso-pentyladenine, the maximal regeneration of adventitious shoots of cauliflower hypocotyl segments was greatly improved. The previous author suggested that BR may make more cells competent to respond to the organogenic signal of cytokinin and that these cells become more sensitive to cytokinin. It is proposed that BRs may not always act directly on stem elongation but may be an elicitor and/or an enhancer of elongation in concert with endogenous and other exogenously added growth regulators.

Effect of hBR on heat stress tolerance. The relationship between the degree of injury, the temperature at which the injury is induced and the duration of heat exposure had a sigmoidal response for both control plantlets and plantlets produced on media containing hBR. However, the percentage of injury was much lower in later case compared with the control (Table II). Exposing banana plantlets produced on media containing hBR to 40°C for 15 or 30 min caused 11 and 18% injury, respectively. Elevating the temperature to 50°C increased the percentage of injury to 16 and 27% at 15 and 30 min respectively. In control samples percentage of injury was 22 and 30% at 40°C, while it was 37 and 52% at 50°C. This result showed a clear effect of hBR in ameliorating the damage caused by heat stress and inducing a noticeable thermo-resistance in banana shoots. This result was in agreement with Kulaeva *et al.* (1991) who reported that BR protected wheat leaf cells from heat shock stress. Wilen *et al.* (1995) also reported that epibrassinolide (eBR) markedly enhanced cell viability of bromegrass cell suspension culture following exposure to high temperature stress. They referred this result to the accumulation of a set of heat shock proteins of 90KD. In addition, Wang and Zeng (1993) reported a reduction in electrolyte leakage by eBR application during heat and chilling treatment of rice. The authors attributed this result to the stability of cell membrane caused by eBR treatment. The increase in electrolyte leakage (stress-induced injury) has been attributed by Levitt (1980) to lipid phase transition and to effects on membrane-bound transport proteins. Therefore, it is proposed that hBR may protect the membrane integrity in such a way to overcome the heat effects on membrane stability.

CONCLUSIONS

In conclusion, the study indicated that hBR may have a role in regulation of stem elongation, and that it may protect the membrane against heat stress, thereby confers thermo-tolerance. It is suggested that hBR may be used for practical application in agriculture.

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