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Interaction of paclobutrazol and gibberellin in relation to stress protection in azuki bean seedlings

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1. Introduction

Paclobutrazol (PB), a triazole derivative, is one of the most potent plant growth retardants which reduces shoot growth by suppressing internodal elongation in a wide variety of plants (Davis et al., 1988). Inhibition of plant growth, decreased internodal elongation, thicker leaf tissue, increased root to shoot ratio and elevated levels of epicuticular wax formation are some of the morphological changes observed in triazole treated plants (Gao et al., 1987). Triazoles have been shown to protect plants from numerous environmetal stress, and hence have been referred to as "plant multiprotectants" (Fletcher and Hofstra, 1985). PB has been to be highly effective at protecting various plants from heat, chilling, waterlogging, drought and phototoxicity (Kraus and Fletcher, 1995: Gilley and Fletcher, 1998). It has been demonstrated that the morphological and stress protective effects of triazoles are reversed by gibberellic acid(GA_3)(Gilley and Fletcher, 1998), thereby indicating an intimate relationship beween GA_3 and plant stress protection. The present study describes the morphological and biochemical changes associated with PB-induced stress protection and attemps to characterize the effect of GA_3 on PB-induced stress protection in azuki bean seedings.

2. Materials and methods

2.1 Plant material and chemical treatment

Azuki bean seeds(*Vigna angularis*) were imbibed with either distilled water (control), 100㎎ · L^{-1} PB, 50㎎ · L^{-1} GA_3 and a combination of PB and GA_3 for 12 h. Seedlings were grown for 10 days in a controlled environmental growth cabinet maintained at 24: 18℃ day : night in darkness or under a photoperiod of 18 h light : 6 h dark at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2.2 Growth and photosynthetic pigments

Height was measured daily over the first 5 days of growth and dry weights were measured on tissue dried for 48 h at 80℃. Total chlorophyll (a and b) and carotenoid levels were estimated according to the procedures described by Asare-Boamah et al.(1986) using a spectrophotometer. Values were expressed as $\mu g\ g^{-1}$ fresh weight.

2.3 High temperature stress and paraquat injury

Seedlings were exposed to heat stress by raising the temperature from 25℃ to 50±1℃ over a period of 1 h. The seedlings were maintained at this temperature for an additional 3. The secondary leaves of the control and each treatment were excised and a 3 ㎝ segmant removed from each leaf. The segments were placed in 50×60mm petri dishes contained 10 ml of solution containing 0 (control), 1 and 2 $\mu mol \cdot L^{-1}$ paraquat and 0.1%(v/v) tween 20 according to the method of Aono et al.(1991).

3. Results and discussion

PB-treated plants exhibited typical triazole-induced alterations in growth characteristics including reduced shoot and leaf elongation, increased leaf width and darker pigmentation. Shoot fresh and dry weights of treated plants were reduced, while those of roots were increased, resulting in a higher root : shoot ratio. Seedling height was significantly reduced by PB and increased by GA_3, while seedlings treated with a combination of both compounds were very similar to the control. Heat stress decreased fresh mass where changes were minimal in PB-treated leaves, while GA_3 alone and together with PB resulted in values that were not significantly different from the control.

Chlorophyll and carotenoid levels after exposure to heat stress showed similar results with levels for the PB-treatment being much higher than the control, GA_3 or combination treatments after exposure to high temperatures. Incubation in paraquat resulted in chlorosis of leaf segments, especially at the higher concentration of $2\mu mol \cdot L^{-1}$. Previous studies have shown that PB protects wheat seedlings from heat stress and paraquat injury and PB protects plants by increasing antioxidant enzyme activity (Kraus et al., 1995). Leaf segments from control and seedlings treated with GA_3, either alone or in combination with PB, were chlorotic while PB-treated leaf segments remained greener. These visual observations were quantified by measuring the photosynthetic pigment content. Chlorophyll and carotenoid levels were both reduced by paraquat concentration. Incubation in $2\mu mol \cdot L^{-1}$ paraquat reduced chlorophyll and carotenoids in all treatments except PB alone. The application of GA_3 in combination with PB resulted in chlorophyll and carotenoid levels similar to the control. Therefore, GA_3 also interferred with the ability of PB to protect leaves from oxidative damage. This study demonstrates that GA_3, applied in combination with PB, will antagonize the protective effects of PB. It is possible that GA_3 may have a negative influence on the enhanced activity of antioxidant enzymes which have been suggested to be involved in PB-induced protection (Kraus et al., 1995 ; Vettakkorumakankav et al., 1999).

4. Abstract

The growth inhibitory and stress protective effects of paclobutrazol (PB) and gibberellic acid (GA_3) antagonism with PB were investigated in azuki bean(*Vigna angularis*) seedlings. Seedling height and shoot fresh mass were significantly reduced by PB and increased by GA_3. The growth inhibitory effects of PB were reversed by GA_3 whereas the combination of PB and GA_3 resulted in similar values to the control. Heat stress decreased fresh mass, chlorophyll and caroteniod levels of leaves while paraquat induced chlorosis in excised leaf segments. These symptoms of injury were alleviated by PB while seedlings treated with a combination of PB and GA_3 were not significantly different from the control. These results suggest that PB protects azuki bean seedlings from heat stress and paraguat injury and that GA_3, applied in combination with PB, will antagonize the protective effect of PB.

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