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# Abscisic acid and Ethrel abolish the inhibition of adventitious root formation of paclobutrazol-treated bean primary leaf cuttings

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## Abstract

Paclobutrazol (PB), a triazole growth retardant and an inhibitor of gibberellin biosynthesis, reduced at 17 μM concentration the adventitious root formation of bean primary leaf cuttings. Treatments with 5 μM ABA or 4 μM Ethrel, an ethylene-releasing compound, restored the rooting of PB-treated cuttings. Ethylene production and the content of the precursor 1-aminocyclopropane-1-carboxylic acid (ACC) were enhanced in root-forming tissues of PB-treated petioles 48 h after ABA application. The effect of ABA could be abolished by 10 μM $CoCl_2$, an inhibitor of ACC oxidase. Thus, ABA might stimulate rooting through its effect on ethylene release. 2 mM silver thiosulphate, an inhibitor of ethylene action, decreased the rooting of PB-treated cuttings similarly to $Co^{2+}$, but failed to negate the ABA effect. These data indicate that the effect of PB on rhizogenesis is not associated directly with the inhibition of the biosynthesis of gibberellins.

*Additional key words*: 1-aminocyclopropane-1-carboxylic acid, ethylene production, *Phaseolus vulgaris* L.

## Introduction

Plant growth retardants are frequently used in vegetative propagation to promote rooting in cuttings (Davis *et al.* 1985, Šebánek *et al.* 1991). The stimulation of adventitious root initiation is a possible consequence of the inhibition of gibberellin biosynthesis by growth retardants (Grossmann 1990) since gibberellins inhibit the rooting of cuttings (Smith and Thorpe 1975).

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*Abbreviations:* ABA - abscisic acid, ACC - 1-aminocyclopropane-1-carboxylic acid, IAA - indole-3-acetic acid, MACC - (1-malonylamino)cyclopropane-1-carboxylic acid, PB - paclobutrazol, STS - silver thiosulphate.

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A triazole retardant, paclobutrazol (PB) (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol), can bring this about by a gross interference with indole-3-acetic acid (IAA) transport and/or metabolism (Šebánek *et al.* 1991, Nagy *et al.* 1991) and results in an increase in IAA content and consequently in the number of root initials of stem cuttings. The effect of PB on the initiation of adventitious roots, however, varies greatly with the plant part used for cutting formation. In an earlier report (Nagy *et al.* 1991), it was found that primary leaf cuttings of bean rooted very poorly after 17 µM PB treatment. At the cut end of PB-treated petioles callus formation occurred, which was not observed in control cuttings. This is in good agreement with the high cytokinin content of retardant-treated leaves (Grossmann *et al.* 1987), which may shift the auxin/cytokinin ratio in the direction of callus formation. Moreover, high cytokinin level results in a juvenile status of treated plants (Grossmann 1990).

Primary leaf cuttings of bean were very effective with respect to root formation even in the mature phase, and cuttings prepared from 30-d-old plants produced more roots than did those from 10-d-old seedlings (Featonby-Smith and Van Staden 1981). The reduction in rooting capacity of PB-treated primary leaf cuttings occurred during the maturation of primary leaves. As concerns leaf explants, it is very likely that cytokinins within the leaves, which are considered to be inhibitors of adventitious root formation (Bollmark and Eliasson 1986), can affect rooting.

It was presumed that treatments which shifted the hormonal status of PB-treated cuttings towards that of the mature leaves would also normalize the rooting capability. Abscisic acid (ABA) has long been known to accelerate leaf blade senescence and to stimulate ethylene production in senescing tissues (Zacarías and Reid 1990). Involvement of ethylene in senescence is also well documented (Mattao and Aharoni 1988) and PB is an effective inhibitor of ethylene production in plant organs (Tari and Nagy 1994). Both of these growth regulators promoted adventitious root formation of stem cuttings (Chin *et al.* 1969, Liu *et al.* 1990).

In this paper we demonstrate that ABA or the ethylene releasing compound Ethrel restore the adventitious root formation of PB-treated primary leaf cuttings, and the possible involvement of ethylene in the action of ABA will also be discussed.

## Materials and methods

**Plant material:** Seeds of *Phaseolus vulgaris L.* cv. Juliska were soaked for 2 h in 17 µM PB solution (research grade, *ICI Agrochemicals*, Bracknell, UK) and then germinated in a dark thermostat at 25 °C. After 48 h they were grown in garden mould, in the growth cabinet with a photoperiod of 14 h (*Tungsram F29* white fluorescent lamps, irradiance 100 µmol $m^{-2}$ $s^{-1}$) at 25/20 °C day/night temperatures and 65 % relative humidity. Three-week-old plants were used to prepare primary leaf cuttings. 30 cuttings were immersed in 10 $dm^3$ of a quarter-strength Hoagland nutrient solution (pH 6.2) supplemented with micronutrients without $Co^{2+}$ (Hoagland and Arnon 1950). The culture medium was replaced every 2 d.

**Treatments of cuttings:** Leaf blades of cuttings were sprayed to runoff with a solution of 5 μM (+)-cis,trans ABA (*Sigma*) or 4 μM Ethrel (2-chloroethyl-phosphonic acid, *Amchem Products Inc.*, Ambler, USA) containing 0.05 % Tween 20 (pH 6.0). $Co^{2+}$ was applied in the culture medium, as 10 μM $CoCl_2$. Silver thiosulphate (STS) was prepared according to Veen and Van de Geijn (1978), and petioles were immersed in 2 mM STS for 30 min before being placed in the nutrient solution. Higher STS concentrations resulted in phytotoxic symptoms.

**Measurements of ethylene, ACC and malonyl-ACC:** Ethylene production of detached blades and petioles was determined by a Hewlett-Packard gas chromatograph equipped with a FID and an alumina column, as described earlier (Tari and Nagy 1994). ACC and MACC were measured following the methods described by Jiao *et al.* (1986).

## Results

**Adventitious root formation of PB-treated cuttings: effects of ABA and Ethrel:** Inhibition of adventitious root formation by 17 μM PB treatment was well-defined on the basis of the rooting percentage, but it was not so strong on the basis of the root number per petiole. Treatment of leaf blades with 5 μM ABA or 4 μM Ethrel, an ethylene generator, restored the rooting of the PB-treated cuttings. The effectiveness of Ethrel in reversing the inhibition of adventitious root formation in this system raises the possibility that the action of ABA is also mediated by ethylene (Table 1).

Table 1. Effect of 5 μM ABA or 4 μM Ethrel on adventitious root formation (rooting percentage and number of roots per cutting) of paclobutrazol-treated bean primary leaf cuttings. Primary leaf cuttings were prepared from 21-d-old bean plants treated with 17 μM PB. Cuttings were placed in quarter-strength Hoagland solution. 5 μM ABA or 4 μM Ethrel prepared with 0.05 % Tween 20 was sprayed onto the leaf blade at the beginning of incubation. Roots were counted 8 d after the treatments. Values with different letters are significantly different ($P = 0.05$, Tukey's test, $n = 30$).

| Treatment | Rooting [%] | Root number [cutting$^{-1}$] |
|---|---|---|
| Control | 66.7 | 4.9 a |
| Control + 5 μM ABA | 68.0 | 5.8 a |
| Control + 4 μM Ethrel | 60.0 | 5.3 a |
| 17 μM PB | 10.5 | 2.6 b |
| PB + 5 μM ABA | 50.5 | 4.8 a |
| PB + 4 μM Ethrel | 45.5 | 4.5 a |

**Ethylene production by blades and petioles during the rooting process:** The leaf blades of the control cuttings did not exhibit a rapid ethylene burst following detachment, but the laminae of the PB-treated cuttings evolved an excision-related ethylene and later the ethylene release displayed rhythmicity (Fig. 1A). In the blades of the control plants ABA had no significant effect, but stimulated ethylene formation in the

PB-treated laminae (Fig. 1C). In the petioles, ABA treatment resulted in a well-established increase in the ethylene production of the PB-treated cuttings during the early phase of root initiation, which could not be observed during the first 24 h and which was not so sharp later in the control plants (Fig. 1D).

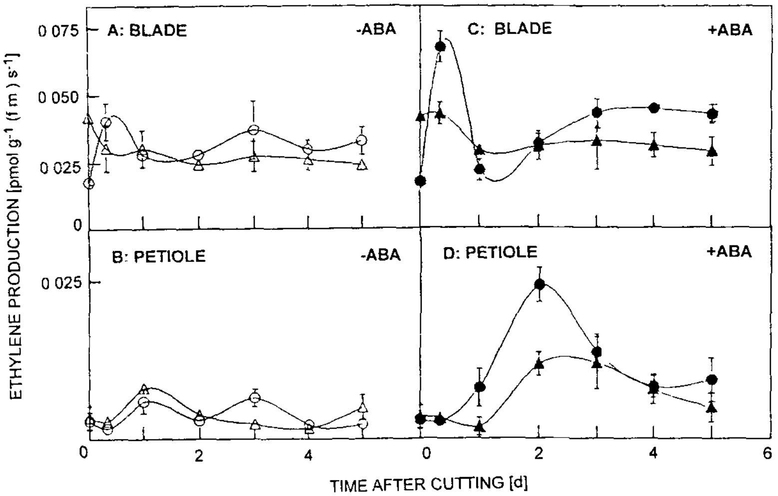

Fig. 1. Effect of 5 μM abscisic acid on ethylene production by blades (*A*, *C*) and petioles (*B*, *D*) of control and PB-treated bean primary leaf cuttings during the first 5 d of the rooting. PB (17 μM) was applied to seeds. ABA was sprayed onto the leaf blades in a 5 μM solution containing 0.05 % Tween 20. Control - *open triangles*; 17 μM PB - *open circles*; control + 5 μM ABA - *closed triangles*; PB + 5 μM ABA - *closed circles*. Each point represents the mean value of 5 samples ± SE.

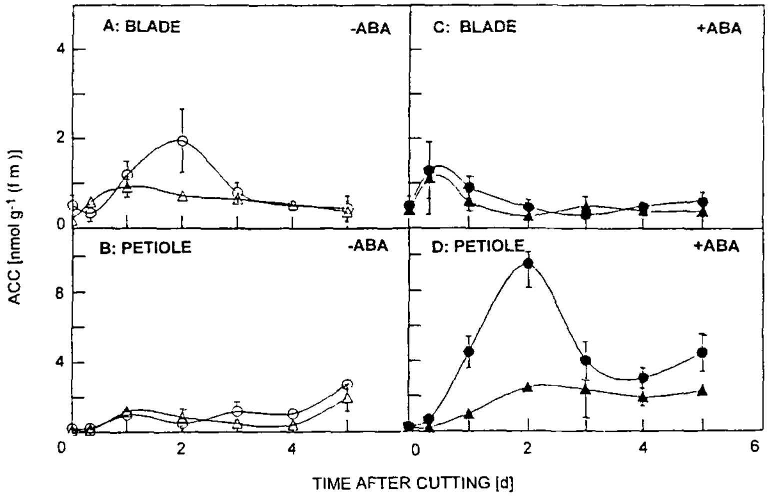

Fig. 2. Effect of 5 μM abscisic acid on ACC contents of blades (*A*, *C*) and petioles (*B*, *D*) of control and PB-treated bean primary leaf cuttings during the first 5 d of the rooting. Control - *open triangles*; 17 μM PB - *open circles*; control + 5 μM ABA - *closed triangles*; PB + 5 μM ABA - *closed circles*. Means ± SE, *n* = 4.

The ACC content followed the pattern for ethylene in the petioles (Fig. 2B, D). In the presence of ABA, the PB-treated petioles accumulated a significant amount of ACC.

**Effects of $Co^{2+}$ and STS on the ABA-induced rooting of the PB-treated cuttings:** To answer the question of whether the effect of ABA was mediated by ethylene, 10 μM $CoCl_2$, an inhibitor of ACC oxidase, was added to the rooting solution. This concentration of $Co^{2+}$ reduced the ethylene production by about 30 - 50 % in the leaf blades and petioles during the first 2 d of incubation (data not shown).

In the presence of $Co^{2+}$ the effect of ABA on adventitious root formation was not manifested. $Co^{2+}$ alone inhibited the rooting of the PB-treated cuttings (Fig. 3).

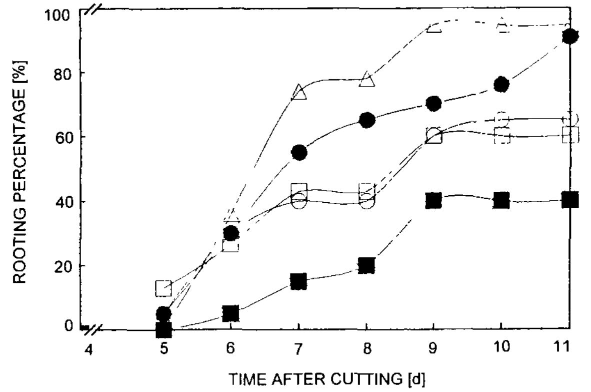

Fig. 3. Effect of 5 μM ABA treatment on the rooting percentage of PB-treated bean primary leaf cuttings in the presence or absence of $Co^{2+}$. The number of rooted cuttings per pot was determined during a week after the emergence of roots. 5 μM ABA was sprayed onto the leaf blades and 10 μM $CoCl_2$ was applied in the nutrient solution. Control - *open triangles*; 17 μM PB - *open circles*; PB + 5 μM ABA - *closed circles*; PB + ABA + 10 μM $Co^{2+}$ - *open squares*; PB + $Co^{2+}$ - *closed squares*.

STS, an inhibitor of ethylene action, did not counteract the effect of ABA on rooting of the PB-treated cuttings, but decreased the root number per petiole to the same extent as did $Co^{2+}$ (control: 18.7 ± 2.2; control + $Co^{2+}$: 25.7 ± 2.8; PB-treated: 14.0 ± 2.0; PB + $Co^{2+}$: 6.0 ± 0.8; PB + STS: 6.1 ± 0.6 roots per petiole, roots were counted on the 11th day).

## Discussion

Primary leaf cuttings prepared from the 17 μM PB-treated plants rooted poorly, in contrast with the control. The inhibition of rooting in PB-treated material may be due to a high cytokinin content or a low ethylene production of the tissues, the former acting as an inhibitor and the latter as an inducer of adventitious root formation.

Inhibition of rooting of PB-treated leaf cuttings could be abolished by 5 μM ABA or 4 μM Ethrel treatment. The laminae and petioles of the PB-treated explants, however, did not produce less ethylene than the control, but the kinetics of ethylene production differed. ABA treatment induced significant increases in the levels of ACC and ethylene in the petioles with root-forming tissues of the PB-treated cuttings. This accumulation occurred during the 2nd day of the incubation and followed the decrease of ethylene production in the leaf blades. The leaf blades might be a source of ACC which could at least partly be transported to and concentrated in the root forming tissues. This suggests that the ABA treatment may reserve ACC and make ACC transport to petioles possible during the transient decrease of ethylene formation in blades.

$Ag^+$, an inhibitor of ethylene binding, in the form of STS, also decreased the root number per petiole to the same degree as that of $Co^{2+}$ in the PB-treated cuttings, but failed to prevent the effect of ABA on root formation. These data are in good agreement with the results of Curtis (1984), who found that the antiethylene properties of $Ag^+$ in the inhibition of petiole abscission were completely canceled or diminished by ABA in bladed explants of *Vigna*. In the abscission system with bladed cuttings, there is also a good possibility for the transport and accumulation of ACC in the petioles to be greatly in excess of those in the control, and those concentrations of $Ag^+$ which are effective in the control fail to diminish the effect of ethylene in the ABA-treated explants.

$Co^{2+}$ completely negated the ABA effect and inhibited the ethylene production of the blades and petioles of cuttings during the first 48 h of root initiation. These results might suggest that the ABA effect is mediated by ethylene.

Since PB-treated cuttings do not evolve less ethylene than control and Ethrel can normalize the rooting of PB-treated explants, these cuttings seem to have a "relative ethylene deficiency". The increase in the endogenous ethylene concentration may stimulate the tissue sensitivity to auxin (Liu and Reid 1992) or may shift the auxin/cytokinin ratio towards a favourable level, *e.g.*, by increasing the cytokinin degradation (Bollmark and Eliasson 1990). Thus, the effect of ethylene on the rooting process may depend on the auxin/cytokinin ratio, rather than the absolute rate of ethylene production. Furthermore, these results indicate that the effect of PB on rhizogenesis does not seem to be associated directly with the inhibition of gibberellin biosynthesis. The initiation of adventitious roots is determined by the new hormonal balance of the plant organ used as a donor for cutting preparation.

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