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Ethylene and Auxin-Ethylene Interaction in Adventitious Root Formation in Mung Bean (*Vigna radiata*) Cuttings

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Abstract. The role of ethylene in adventitious root formation and its involvement in auxin-induced rooting were investigated in cuttings of *Vigna radiata* (L.). Treatment with 30 μM indole-3-acetic acid (IAA) for 24 h slightly inhibited rooting, whereas the same concentration of indole-3-butyric acid (IBA) significantly stimulated it. Ethylene derived from 1-aminocyclopropane-1-carboxylic acid (ACC) increased the number of adventitious roots but inhibited their emergence and elongation. Endogenous levels of ethylene, ACC, and malonyl-ACC (MACC) were initially higher in cuttings treated with IAA. This trend was quickly reversed, and cuttings, particularly hypocotyls, treated with IBA produced higher levels of ethylene and had more ACC and MACC during most of the rooting process. Aminoethoxyvinylglycine significantly inhibited rooting, but its inhibitory effect could not be reversed by ACC. The data suggest that the stimulating effect of IBA on rooting is closely associated with its induction of ACC and ethylene biosynthesis.

Indole-3-acetic acid (IAA) and a number of synthetic auxins, particularly indole-3-butyric acid (IBA), usually stimulate root initiation in cuttings (Hartmann and Kester 1983). In general, IAA is less effective than IBA (Hartmann and Kester 1983), and in some species IAA even decreases rather than promotes the number of roots formed (Eliasson 1980). The higher rooting ability of IBA is generally attributed to its greater stability within the plant tissues (Hartmann and Kester 1983). However, it was recently found that the rate of metabolism of IBA resembles that of IAA (Wiesman et al. 1988).

Several investigators tried to relate the lower rooting ability of IAA to the

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inhibitory effect of IAA-induced ethylene. Mullins (1972) was the first to suggest that the promotive effects of auxins on induction of rooting are opposed by the inhibitory effects of auxin-induced ethylene. Since IAA is more effective than IBA in inducing ethylene production, he suggested that this might explain why IAA is less effective than IBA in inducing root formation. This hypothesis could not be verified in later works, since no correlation was observed between levels of auxin-induced ethylene and the number of roots formed in mung bean hypocotyl sections or cuttings treated with various auxins (Batten and Mullins 1978, Geneve and Heuser 1982).

Recently, Nordström and Eliasson (1984) reported that ethylene production may be responsible for the decrease in root number caused by treating pea cuttings with low concentrations of IAA. Studies of the effects of ethylene—supplied as free ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC), or ethephon—on rooting of cuttings have yielded conflicting results (literature cited by Robbins et al. 1985). Even when cuttings of the same species were used, e.g., mung beans, it was reported that ethylene increased (Krishnamoorthy 1972, Robbins et al. 1983, 1985, Roy et al. 1972), decreased (Batten and Mullins 1978, Geneve and Heuser 1983, Jusaitis 1986, Mullins 1972), or had no effect on (Mudge and Swanson 1978) rooting. Based on the effects of ethylene and inhibitors of ethylene biosynthesis, it has been suggested that low concentrations of ethylene are required for rooting, whereas high concentrations are inhibitory (Jusaitis 1986).

There are a few reports on the role of auxin-induced ethylene in rooting of mung bean cuttings. Some of them utilized hypocotyl sections of etiolated seedlings (Batten and Mullins 1978, Mullins 1972), whereas others used the common whole rooting system—i.e., cuttings of light-grown seedlings that consisted of a terminal bud, two primary leaves, epicotyl, and hypocotyl. However, in the latter case the ethylene evolved in response to auxin was measured in the whole cutting and not in the hypocotyl where roots are formed (Geneve and Heuser 1982). This study reexamined the role of ethylene and auxin-induced ethylene in rooting of whole mung bean cuttings.

Material and Methods

Plant Material

Mung bean (*Vigna radiata* L.) seeds were soaked in aerated tap water for 18 h and then sown in moistened Vermiculite. Seedlings were grown at 25°C and 70% relative humidity and a 16-h photoperiod with a quantum flux density of 170 $\mu E\ m^{-2}\ sec^{-1}$ (cool white fluorescent tubes).

Rooting System

Cuttings were made from 9-day-old seedings. A cutting consisted of a terminal bud, two primary leaves, epicotyl, and 4 cm of the hypocotyl. Four cuttings were placed in a 25-ml vial containing 15 ml of distilled water or test solutions.

Solutions were renewed every 24 h. Rooting was performed at 25°C and 75% relative humidity and a 16-h photoperiod with a quantum flux density of 380 $\mu E\ m^{-2}\ sec^{-1}$ (cool white fluorescent tubes and incandescent bulbs). Number of visible roots was determined 7 days after the cuttings were made. Results are reported as means of 4–6 vials.

Treatments

All treatments were made in distilled water. Stock solutions of IAA and IBA (10 mM) were made by dissolving the auxins in an equimolar concentration of NaOH. The auxins were diluted before use as required. Silver thiosulfate (STS) was prepared according to Veen (1983). Cuttings were treated with 1 mM STS for 15, 30, and 60 min. Following treatment, cuttings were rinsed with distilled water.

Ethylene Measurements

Ethylene production rates were determined separately in three different sections of the cuttings: (I) the 1-cm basal portion of the hypocotyl; (II) the remaining 3-cm portion of the hypocotyl; and (III) the upper part of the cutting, including the epicotyl and the two primary leaves. To monitor ethylene production rates, sections I and II were transferred to a 25-ml vial and section III to a 55-ml test tube and sealed with a rubber serum cap. Ethylene accumulated during the subsequent 1 h was determined by withdrawing a 1-ml gas sample with a hypodermic syringe and assaying by a gas chromatograph equipped with an alumina column and a flame ionization detector.

Determination of ACC and MACC

Tissue was extracted with 80% ethanol at 80°C, and after removal of the ethanol, the extract was brought to a volume of 2.5 ml with water. An aliquot was assayed directly for ACC content by the method of Lizada and Yang (1979). For measuring MACC content, a 200-µl aliquot was hydrolyzed with 2 N HCl for 3.5 h. After neutralization with NaOH, the ACC liberated was assayed as described above. MACC content was calculated as the difference in ACC content before and after hydrolysis.

Results

Effect of IAA and IBA on Rooting

Treatment of mung bean cuttings with 30 µM IAA for 24 h somewhat reduced

Table 1. Effect of 30 μM IAA or IBA applied for different periods on rooting of mung bean cuttings.

	Number of roots/cutting[a]		
	Duration of auxin treatment (h)		
Auxin	24	48	72
None[b]		14.2 ± 1.2[c]	
IAA	13.4 ± 0.9	38.4 ± 2.7	62.5 ± 5.9
IBA	72.4 ± 5.3	89.1 ± 4.9	97.8 ± 6.1

[a] Rooting was scored 7 days after the initiation of treatment.
[b] The water control was the same for all periods.
[c] Mean ± SE.

the number of adventitious roots formed compared to the control (Table 1). In some experiments the reduction in root number by IAA reached about 40% (data not shown). Treatment with the same concentration of IAA for longer periods significantly increased root number. As expected, IBA greatly stimulated rooting, and its effect also increased when the duration of treatment was prolonged. Roots in control cuttings and in the cuttings treated with IAA for 24 h appeared mainly in the basal hypocotyl region, whereas those in IBA-treated cuttings were distributed over the entire hypocotyl section regardless of the duration of treatment.

Ethylene Production in Auxin-Treated Cuttings

For studying the pattern of auxin-induced ethylene in the cuttings, we selected the 24-h treatment, which showed the greatest difference in the capability of the two auxins to induce rooting (Table 1). Since as outlined above, the distribution of roots differed between treatments, the hypocotyl was divided into two sections—a 1-cm basal section, and the above-3-cm section. The upper portion of the cutting was also included in this study.

Ethylene production rates in all three sections of control cuttings were relatively low and did not change significantly during the entire experimental period (Fig. 1). Ethylene production rates in sections I and II of cuttings treated with both auxins peaked 8 h after the initiation of auxin treatment, while those in section III peaked after 4 h and declined sharply thereafter. Cuttings treated with IAA produced initially, after 4 h, more ethylene than cuttings treated with IBA. This response was particularly noted in sections I and III. After 8 h, this trend was reversed in sections II and III, where IBA treatment resulted in higher levels of ethylene than did IAA treatment. In section I, both auxins induced similar levels of ethylene between 8 and 24 h. After 24 h, ethylene levels induced by IBA exceeded those induced by IAA in all three sections.

ACC and MACC Levels in Auxin-Treated Cuttings

The pattern of changes in endogenous ACC levels in sections II and III of all

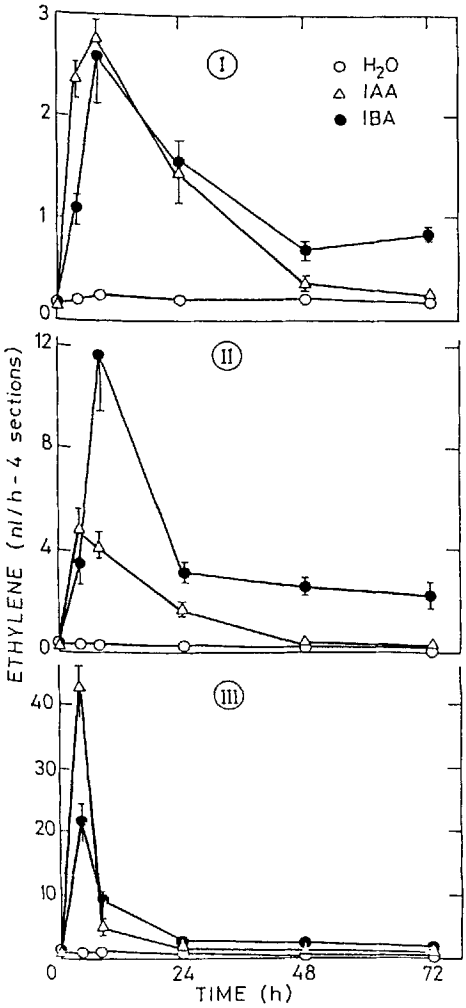

Fig. 1. Time course of ethylene production rates in various sections of mung bean cuttings treated with IAA or IBA. The cuttings were treated with 30 μM IAA or 30 μM IBA for 24 h and then transferred to distilled water for additional 48 h. At various periods after the initiation of auxin treatment, the cuttings were excised into three sections (I, II, III), as described in the Materials and Methods, and their ethylene production rates were immediately determined. Bars indicate 1 SE.

treatments (Fig. 2) resembled that of the changes in the ethylene production rates (Fig. 1). However, there was no direct quantitative relationship between the endogenous ACC levels and the ethylene production rates. Thus, section III produced the highest level of ethylene 4 h after the initiation of auxin treatment, but its ACC content was relatively low. Also, in section II, IBA induced about a 10-fold increase in ACC content over IAA 8 and 24 h after the initiation of auxin treatment, but it induced only a two- to threefold increase in ethylene production rates. In section I, levels of endogenous ACC were somewhat higher in IAA-treated seedlings during the first 8 h of the treatment (Fig. 2). Later on, ACC levels in section I of IAA-treated cuttings declined, whereas those in IBA-treated cuttings increased, reaching a peak after 24 h. From this time, ACC levels in IBA-treated cuttings were higher than those in IAA-treated cuttings in all three sections.

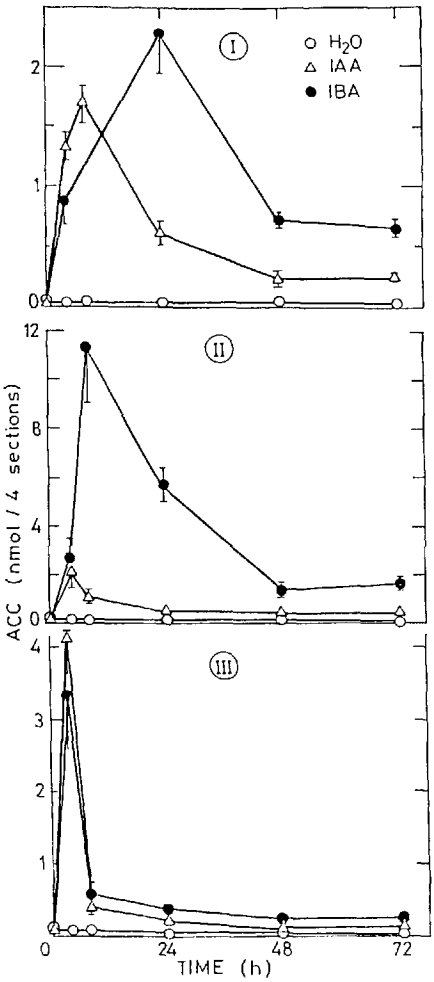

Fig. 2. Time course of ACC accumulation in various sections of mung bean cuttings treated with IAA or IBA. ACC was extracted immediately after section excision. Details as in Fig. 1.

MACC levels in control cuttings did not change much during the entire experimental period (Fig. 3). In IAA-treated cuttings, MACC levels increased in all sections up to 24 h after the initiation of auxin treatment and then almost leveled off. MACC levels in all three sections of IBA-treated cuttings were initially slightly lower than in IAA treatment, but they increased continuously up to 72 h, when the experiment was terminated. At that time, MACC levels in IBA-treated cuttings were two- to fourfold higher than those in IAA-treated cuttings.

Effect of ACC on Rooting

Cuttings were treated with different concentrations of ACC for various times.

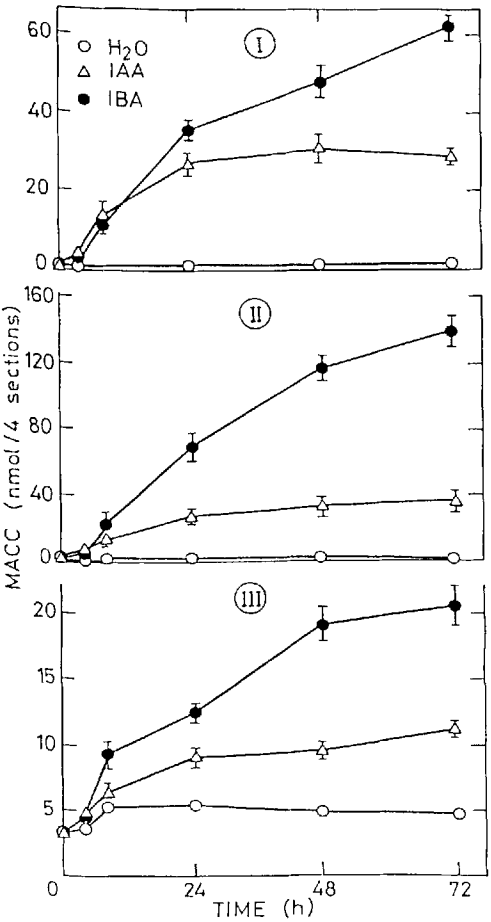

Fig. 3. Time course of MACC accumulation in various sections of mung bean cuttings treated with IAA or IBA. MACC was extracted immediately after section excision. Details as in Fig. 1.

Treatment with 0.01 and 0.1 mM ACC for 24 h was almost ineffective (Table 2). Extending the duration of treatment to 72 h resulted in an increase in the number of adventitious roots formed. Treatment with 0.1 mM ACC for 72 h almost doubled the number of adventitious roots. ACC at 0.3 mM slightly increased root number when applied for up to 48 h but decreased it significantly after 72 h of treatment. Although the latter treatment decreased root number, the basal section of these cuttings showed a strong meristematic activity as evidenced by the marked swelling of this region. ACC treatment also affected the distribution of roots (Table 2). Most roots in control cuttings were located in the basal section, whereas in ACC-treated cuttings, a large number of roots appeared also in section II.

Adventitious roots in ACC-treated cuttings were significantly shorter than those of control cuttings (Table 3). In general, there was a negative correlation between root length and ACC concentration or the duration of ACC treatment. In addition, roots in ACC-treated cuttings appeared later than those of control

Table 2. Effect of ACC concentration and the duration of ACC treatment on number and distribution of adventitious roots in mung bean cuttings.

ACC concentration (mM)	Number of roots/cutting[a]		
	Duration of ACC treatment (h)		
	24	48	72
0[b]		14.7 ± 0.9 (8)[c,d]	
0.01	13.4 ± 1.2 (17)	16.5 ± 0.9 (24)	18.9 ± 1.3 (26)
0.1	16.1 ± 1.1 (23)	18.7 ± 1.2 (45)	25.3 ± 2.0 (54)
0.3	18.8 ± 1.4 (32)	17.1 ± 1.3 (41)	8.9 ± 0.7 (21)

[a] Rooting was scored 7 days after the initiation of treatment.
[b] The water control was the same for all periods.
[c] Mean ± SE.
[d] Figures in brackets indicate the percentage of roots above the 1-cm basal section.

Table 3. Effect of ACC concentration and the duration of ACC treatment on emergence and length of adventitious roots in mung bean cuttings.

ACC concentration (mM)	Root length (mm)[a]		
	Duration of ACC treatment (h)		
	24	48	72
0[b]		8.1 ± 0.5 (70)[c,d]	
0.01	8.0 ± 0.4 (70)	8.6 ± 0.6 (78)	6.3 ± 0.5 (78)
0.1	8.4 ± 0.3 (78)	6.4 ± 0.4 (82)	4.8 ± 0.2 (86)
0.3	5.9 ± 0.3 (92)	2.1 ± 0.1 (98)	1.1 ± 0.04 (118)

[a] Root length of 10 representative roots was determined 7 days after the initiation of treatment.
[b] The water control was the same for all periods.
[c] Mean ± SE.
[d] Figures in brackets indicate hours required for root emergence in at least 50% of the cuttings.

cuttings (Table 3). Again, there was a negative correlation between the time required for root emergence and ACC concentration or the duration of ACC treatment.

Effect of Inhibitors of Ethylene Biosynthesis and Action

Aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis (Lieberman 1979), significantly reduced adventitious root formation in mung bean cuttings (Table 4). Treatment with 50 μM AVG for 3 days almost completely eliminated root formation in the cuttings. ACC at 10 μM was unable to reverse the inhibitory effect of AVG, although cuttings treated with ACC at this concentration produced much higher levels of ethylene than control cuttings (data not shown).

Treatment with 1 mM STS, an inhibitor of ethylene action (Veen 1983), for up to 1 h did not affect adventitious root formation in mung bean cuttings (data

Table 4. Effect of a 3-day exposure to AVG and/or ACC on rooting of mung bean cuttings.

Treatment	Number of roots/cutting[a]
Experiment 1	
H_2O	17.4 ± 1.0[b]
10 μM AVG	5.9 ± 0.3
10 μM ACC	21.3 ± 0.8
10 μM AVG + 10 μM ACC	8.3 ± 0.6
Experiment 2	
H_2O	17.0 ± 1.3
50 μM AVG	0.7 ± 0.03
10 μM ACC	20.1 ± 0.8
50 μM AVG + 10 μM ACC	0.9 ± 0.1

[a] Rooting was scored 7 days after the initiation of treatment.
[b] Mean ± SE.

not shown). Increasing STS concentration or the duration of treatment resulted in phytotoxic symptoms.

Discussion

The data obtained in the present study may provide an explanation for the conflicting results on the role of ethylene in adventitious root formation (Robbins et al. 1985). The rooting process consists of at least three distinct stages: initiation, emergence, and elongation (Blazich and Heuser 1979). Data in Tables 2 and 3 indicate that ethylene affects these stages differently. It stimulates root initiation, thus increasing the number of roots formed, but inhibits root emergence and elongation. Inhibition of elongation of both lateral and adventitious roots by ethylene was also observed in previous studies (Chadwick and Burg 1970, Geneve and Heuser 1983, Robbins et al. 1985). In many of the studies on the role of ethylene in adventitious root formation, ACC or ethephon was used as the source of ethylene without monitoring the levels of ethylene in the system. It is possible that in some of these studies, the ethylene concentration was high enough to inhibit the emergence and elongation of the root primordia that were formed, so that fewer roots were observed. This assumption is supported by the observations that when a high concentration of ACC was used, there was a decrease in root number (Table 2), but the base of the cuttings was swollen, indicating that root primordia were indeed formed.

By measuring auxin-induced ethylene production in the site of root formation, we observed that IAA initially induced higher levels of ethylene than IBA (Fig. 1). This is in accordance with previous observations on auxin-induced ethylene production (Mullins 1972). However, this trend was quickly reversed, and ethylene levels induced by IBA in sections I and II, the site of root formation, were significantly higher than those induced by IAA during most of the rooting process. Since ethylene promotes rooting in mung bean cuttings (Table 2), these data indicate that, at least in this species, ethylene is associated with

the greater efficiency of IBA to induce rooting, and not, as previously suggested, with the lower efficiency of IAA (Nordström and Eliasson 1984).

Endogenous levels of ACC and MACC in sections I and II were much higher in IBA-treated cuttings, except for the first 4 or 8 h depending on the section (Figs. 2, 3). ACC synthesis in vegetative tissues is regulated by auxin (Yang and Hoffman 1984). Thus, ACC content may serve as an indicator for auxin level or activity in the tissues (Pengelly et al. 1987, Su and Pengelly 1987). Based on this consideration, it is reasonable to assume that in mung bean cuttings IBA has a higher auxin activity than IAA during most of the rooting process. Since IAA and IBA are metabolized in these cuttings at a similar rate, it has been suggested that the higher and prolonged auxin activity of IBA results from the release of free IBA from its conjugates (Wiesman et al. 1988). Whatever the explanation for the higher auxin activity of IBA may be, our data show that there is a direct relationship between auxin activity, based on ethylene production rates (Fig. 1) or ACC and MACC levels (Figs. 2, 3), and the number and distribution of roots formed in the various treatments (Table 1). IBA showed a relatively high auxin activity as well as root formation along the entire hypocotyl. IAA, in contrast, formed fewer roots, most of which were located only in the basal section, where it showed a significant auxin activity.

Based on experiments with inhibitors of ethylene biosynthesis, it has been concluded that a low concentration of ethylene is required for optimal root production (Jusaitis 1986). AVG has been the most common inhibitor employed in the mung bean system (Geneve and Heuser 1983, Jusaitis 1986, Robbins et al. 1983). However, in one of these studies (Jusaitis 1986) as well as in the present work (Table 4), the inhibitory effect of AVG could not be reversed by ethylene generated from ACC. Even when the inhibitory effect of AVG was significantly reversed by ethylene, the number of roots formed in the AVG + ethylene treatment was much lower than in ethylene alone (Robbins et al. 1983). There is increasing evidence that AVG has nonspecific effects, particularly when it is used in high concentrations (Chappell et al. 1984, Sauerbrey et al. 1987). Added to this are the observations that STS, an inhibitor of ethylene action (Veen 1983), did not affect rooting. These data suggest that AVG affects rooting nonspecifically. Jarvis (1986) has suggested that ethylene is inhibitory to the early stages of root regeneration but stimulatory or essential to a later stage of root initiation. Therefore, application of ethylene biosynthesis inhibitors, such as AVG, at different periods of the rooting process may cause different results. The exact role of endogenous ethylene in adventitious root formation seems to be unclear, and more studies are required.

In conclusion, the present study indicates that auxin and ethylene stimulate rooting of mung bean cuttings. Auxin appears to be more important than ethylene in adventitious root formation in this system. IBA is more effective than IAA in inducing rooting, and this capability is closely associated with its ability to induce ethylene biosynthesis.

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