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The Effect of IAA, IBA, NAA, and 2,4-D on Root Promotion and Ethylene Evolution in *Vigna radiata* Cuttings[1]

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Additional index words. plant propagation, mung bean, adventitious root initiation

Abstract. **Ethylene liberated from control and auxin-treated cuttings of *Vigna radiata* (L.) R. Wilcz cv. Berken was monitored for 14 hours. For root initiation, naphthaleneacetic acid (NAA) and indolebutyric acid (IBA) were the most effective with indoleacetic acid (IAA) intermediate and 2,4-dichlorophenoxyacetic (2,4-D) the least effective. No correlation was observed between the quantity of auxin-induced ethylene evolved and the number of roots formed. Decreasing the NAA solution pH from 7.0 to 3.0 reduced the evolution of ethylene but did not alter the rooting response of the cuttings. It was concluded that stimulation of adventitious root initiation by auxin is not mediated by ethylene.**

The mechanism of auxin action in adventitious rooting is fragmentary although it was identified as a root-forming substance in 1934 by Thiamann and Went (13). Zimmerman et al. (14, 15, 16) reported increased root and root hair production by exogenous supplies of ethylene and the similarities between the effects of auxin and ethylene were noted early. Both exogenous and endogenous supplies of auxin are known to stimulate ethylene production leading to the suggestion that auxin action may be mediated through the release of ethylene (1, 4, 5, 6, 8, 12). Ethylene and ethylene-releasing compounds have been reported to promote, inhibit or have no effect on rooting. In mung bean, a positive correlation between added ethrel and rooting has been noted (7, 11). Mullins (10) proposed that the promotive effects of auxin

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on root initiation are opposed by inhibitory effects of auxin-induced ethylene; and that root initiation is promoted when the rates of ethylene production are low relative to auxin concentration.

Our purpose was to examine further the relation among auxin, ethylene and rooting. The amount of ethylene evolved by mung bean cuttings treated with IAA, NAA, IBA, and 2,4-D was compared to the number of adventitious roots formed on the same.

Materials and Methods

Plant material and bioassay procedures. Mung bean seeds cv. Berken were surface-sterilized in 10% clorox (v/v) for 10 min and rinsed in tap water. After aeration for 24 hr in tap water, they were sown 1 cm deep in plastic trays (29 × 18 × 5.5 cm) containing vermiculite. The growth chamber was maintained at 24°C during germination and a 16-hr day quantum flux density of 165 $\mu Em^{-2}sec^{-1}$.

Uniform cuttings were made from 9-day-old seedlings and placed in autoclaved distilled water prior to use (3). Distilled water used for reagents and cutting treatments was autoclaved to remove microbial contamination (5). Each cutting consisted of a 3-cm hypocotyl, two primary leaves, and the unexpanded trifoliate bud. Ten cuttings were selected at random and placed in a 19 × 65 mm shell vial containing 1 ml of test solution. The initial test solution was absorbed within 3 hr and was replaced with 6 ml of distilled water. Additional distilled water was added as needed during the 5-day rooting period.

The vials were arranged in a completely randomized design in the growth chamber. After 5 days, roots were counted and average number of roots per cutting was computed.

Auxin effects on root initiation and ethylene evolution. The effect of various concentrations (10^{-7} to 10^{-3}M of IAA, IBA, NAA, and 2,4-D on root initiation was tested. Cuttings were pulsed with 1 ml of test solution buffered to pH 7.0 with 1mM citrate-potassium phosphate buffer. Each experiment was duplicated with 3 vials containing 10 cuttings each per concn. NAA (10^{-4}M) was selected from preliminary experiments to test the effect of pH on the average number of roots initiation. NAA was buffered at pH 3.0, 4.0, 5.0, 6.0, and 7.0 with 1 mM citrate-potassium phosphate buffer.

Following test solution uptake, cuttings were enclosed in 1-liter jars with an airtight serum cap inserted into the lid. Each treatment jar contained 40 cuttings in 4 ml of distilled water. At 1-hr intervals, for 14 hr, 1-ml gas samples were analyzed using a Hewlett-Packard 5830A dual column gas chromatograph with 3.175 mm o.d. × 1.2 m columns packed with activated alumina for ethylene determination. Injection port, detector, and column temperatures were 75, 150, and 50°C respectively. After each gas determination, the jars were flushed with air and recapped for the next determination. Results were recorded as nanoliters of ethylene per gram of fresh weight per hour.

Results

Effect of auxins on root promotion. IAA, IBA, NAA, and 2,4-D promoted adventitious root initiation in mung bean cuttings (Fig. 1). Increasing the concentration of NAA and IBA between 10^{-7}M and 10^{-3}M increased the number of roots per cutting. IAA and 2,4-D were less effective in promoting root initiation; IAA was effective only at 10^{-4} M and 10^{-3}M and 2,4-D stimulated rooting only at 10^{-3}M. Varying solution pH from 7.0 to 3.0 did not alter the rooting response of cuttings to NAA at 10^{-4}M. Rooting remained constant over the pH range (78.3 roots at pH 7 verses 77.9 at pH 3).

Ethylene evolution in response to auxin treatment. Detectable amounts of ethylene were evolved from mung bean cuttings for all

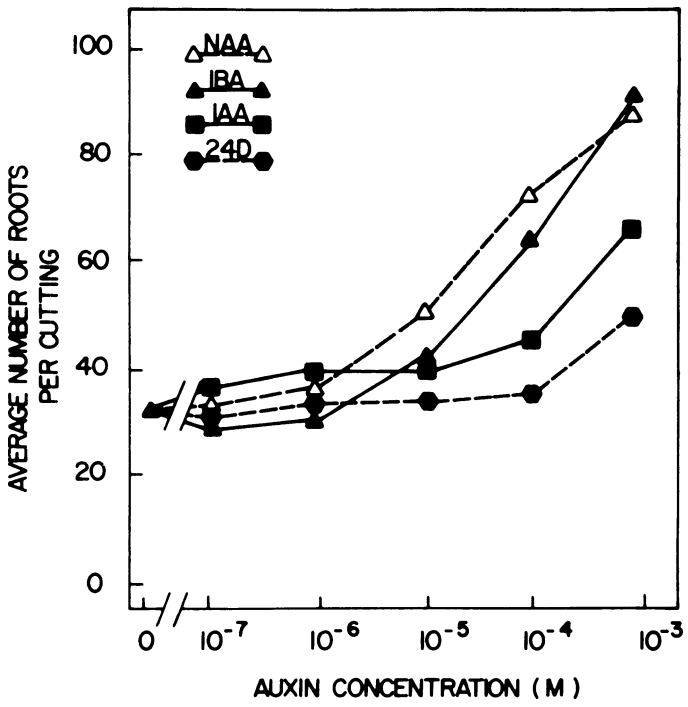

Fig. 1. The relative effectiveness of IAA, IBA, NAA, and 2,4-D on adventitious root initiation in mung bean.

auxins at concentrations of 10^{-5}M, 10^{-4}M, and 10^{-3}M (Fig. 2, 3, 4, 5). The higher the auxin concentration, the greater the quantity and duration of ethylene evolution. No measurable amounts of ethylene were detected in the buffer control or at 10^{-7}M and 10^{-6}M auxin treatments. Of the auxin-treated cuttings, those treated with 2,4-D produced the greatest total amount of ethylene and those treated with IBA produced the least. NAA- and IAA-treated cuttings produced similar quantities of ethylene, intermediate between 2,4-D and IBA-treated cuttings.

Solution pH altered the amount of ethylene evolved from cuttings treated with NAA at 10^{-4}M (Fig. 6). Ethylene evolution

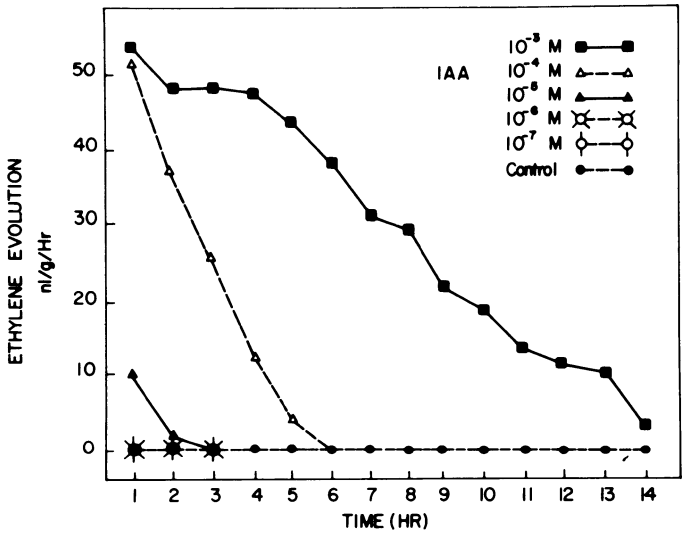

Fig. 2. Fig. 2. The stimulation of ethylene evolution from mung bean cuttings treated with IAA.

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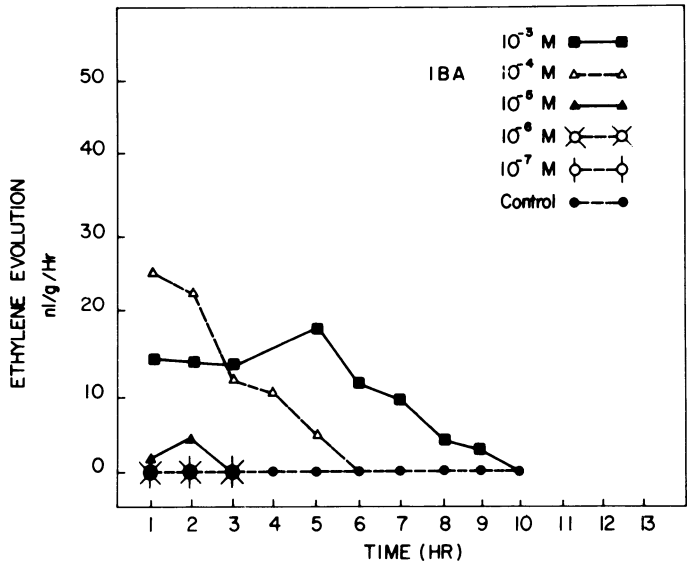

Fig. 3. The stimulation of ethylene evolution from mung bean cuttings treated with IBA.

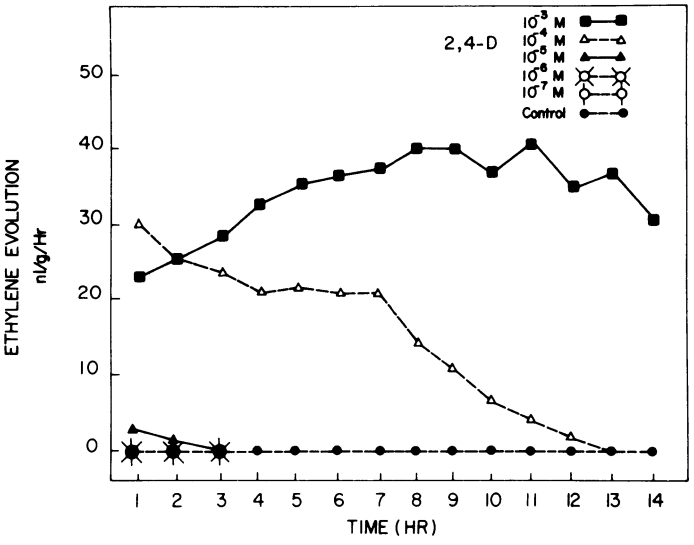

Fig. 5. The stimulation of ethylene evolution from mung bean cuttings treated with 2,4-D.

was greater at pH 7.0 and showed a steady decline as the pH was lowered. Control cuttings, those treated only with the various pH buffers, evolved no measurable quantities of ethylene. The rooting response was not proportional to the relative total amounts of evolved ethylene for cuttings treated with IAA, IBA, NAA, and 2,4-D at 10^{-4}M (Fig. 7). NAA and IBA promoted root initiation to a similar degree, but IBA-treated cuttings produced less than one-half the amount of evolved ethylene. Cuttings treated with NAA and IAA produced the same total amounts of evolved ethylene, yet IAA was less effective in stimulating rooting. The highest total quantity of ethylene evolved was by cuttings treated with 2,4-D; however, it was the least effective as a promoter of root initiation.

Discussion

Although the results of Roy et al. (11) and Krishnamoorthy (7) showed a positive stimulation of adventitious root initiation in mung bean from applied ethrel, Mullins (10) comparing ethylene evolution from IAA- and IBA-treated cuttings proposed that auxin-stimulated rooting is inhibited by ethylene.

Our results with 4 different auxins, however, show no correlation between number of roots formed and ethylene liberated, which agree with those of Mudge and Swanson (9) who found that ethylene liberated from ethephon had no effect on rooting in mung bean cuttings. They also reported that internal ethylene levels of ethephon-treated cuttings were increased by increasing solution pH but that increased internal ethylene failed to increase rooting beyond that obtained by IBA alone.

In the present study, NAA and IBA initiated similar large numbers of roots but produced widely differing amounts of ethylene. IBA induced low levels of ethylene production, as previously shown (9), but produced a comparable number of roots to NAA, which strongly promoted ethylene formation. IAA and 2,4-D

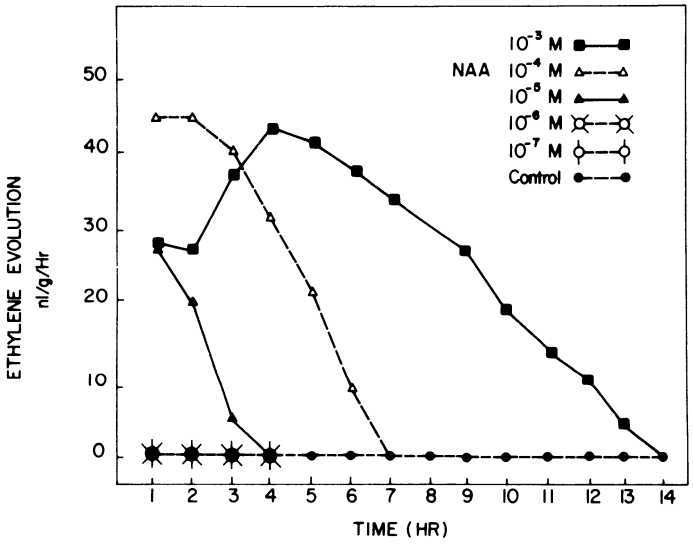

Fig. 4. The stimulation of ethylene evolution from mung bean cuttings treated with NAA.

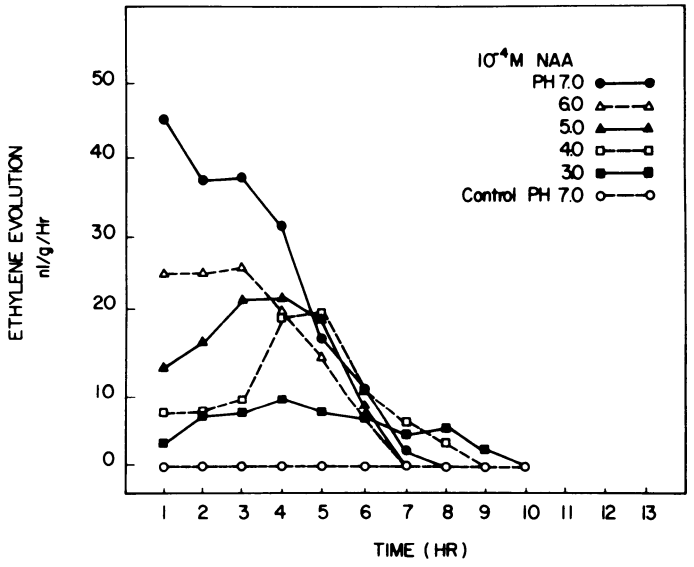

Fig. 6. The effect of increasing solution pH on ethylene evolution from mung bean cuttings treated with NAA at 10^{-4}M.

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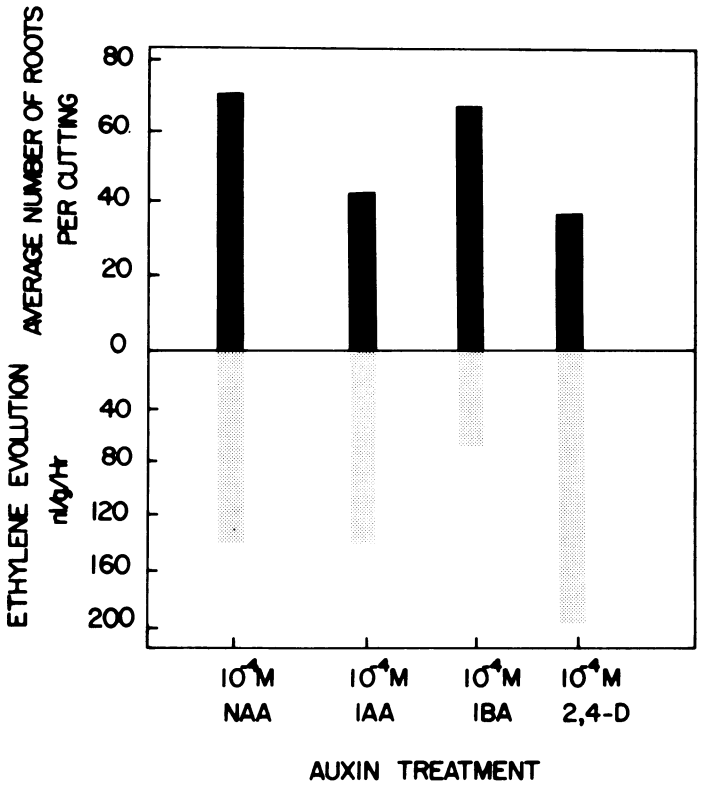

Fig. 7. A comparison of the relative total amounts of evolved ethylene for IAA, IBA, NAA, and 2,4-D at 10^{-4}M with its corresponding rooting response in mung bean cuttings.

were both weak promoters of rooting but 2,4-D produced greater amounts of ethylene than the other auxins.

The patterns of ethylene evolution observed for the 4 auxins were probably a manifestation of auxin metabolism and not associated with root initiation. Lau and Young (8) and Sakai and Imaseki (12) have shown that the magnitude of IAA-induced ethylene production in etiolated mung bean segments parallels the free IAA level. The auxin 2,4-D is comparatively stable in plant tissues (2). In pea roots, growth recovery following treatment with IAA and NAA occurs as these auxins are metabolized, but 2,4-D is not metabolized and root growth remains inhibited (2). The continued production of ethylene by 2,4-D -treated mung bean cuttings in the present study after the 14 hr cutoff period lends support to the idea that varying patterns of ethylene production are related to the presence of free auxin.

The amount of ethylene synthesized in NAA-treated mung bean cuttings was dependent on the initial treatment solution pH. Altering the buffer pH could therefore increase the concentration of ethylene to which the cuttings were exposed. A marked increase in the ethylene level occurred when the pH was changed from 3.0 to 7.0, but as noted, no change in rooting occurred. These results and the lack of correlation between the stimulation of ethylene and the stimulation of root initiation by the 4 auxins argue against the proposal that auxins promote adventitious root initiation through an ethylene intermediate.

Mung bean cuttings, however, may be responsive to a low threshold level of ethylene for rooting, and this needs to be examined. Ethylene concentrations above the threshold level would not further enhance root initiation. Zobel (17, 18), investigating the effects of ethylene on the tomato mutant *diageotropica*, suggested that very low quantities of ethylene may be required for lateral root formation and after a threshold quantity of ethylene has been reached, additional ethylene is surplus. The mung bean cuttings initiate approximately 30 roots per cutting without auxin treatment but evolved no detectable amount of ethylene. If a threshold level exists, it must be very low.

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