

## RELATIONSHIP BETWEEN IAA CONTENT AND ADVENTITIOUS ROOT FORMATION IN BEAN SEEDLINGS TREATED WITH CCC

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### Abstract

We investigated the connection between the IAA content and IAA distribution in intact bean seedling, stem- and primary leaf-cuttings, originating from seeds treated with CCC.

The promotive effect of CCC on the rooting process in intact plants and stem-cuttings correlates with a higher IAA-content in the basal part of hypocotyls of the treated plants.

After the treatment with CCC the IAA content of the primary leaves increases, whereas that of the petiole is lower than the control. Formation of adventitious roots in CCC-treated leaf-cuttings is inhibited. This inhibition can be released by IBA, ABA and ethylene-generator treatment. In all three cases, with the STS pretreatment, the initiation of root primordia can be prevented. These results indicate, that ethylene is the hormone that can play a key role in this system, in the induction of adventitious roots.

*Key words:* abscisic acid, adventitious roots, bean, CCC, indole-3-butyric acid, IAA content, IAA distribution, ethylene, *Phaseolus vulgaris*, rooting, STS.

### Introduction

The change in the shoot/root ratio in the CCC (chlorocholine-chloride)-treated seedlings is not only the result of the inhibition of shoot growth, but CCC also stimulates the formation (GIRI et al., 1982) and elongation of roots, too (HOFFMAN, 1973).

The retarding effect of CCC on shoot growth is generally explained by the inhibition of certain steps of the mevalonic acid pathway (GRAEBE, 1987). The stimulating effect of CCC on rooting can be correlated with its effect on IAA metabolism, and it is generally accepted that IAA has an important role in rooting (TORREY, 1976; HARTMAN and KESTER, 1983).

In our work we investigated the relationship between the rooting process and IAA content in intact beans, stem- and primary leaf-cuttings, originating from seeds treated with CCC.

## Materials and Methods

In our experiments we used *Phaseolus vulgaris* L. cv. Juliska seedlings. After the seeds had been soaked in a CCC (Merck-Schuchardt) solution of 500 mg/l in a thermostat at 25°C, they were sowed in garden mould. Plants were grown under controlled conditions (CONVIRON Cabinet model EF7, equipped with 4x50 W Sylvania incandescent lamps, at 25/20°C day/night temperatures respectively, 16 h illumination with 60 Wm<sup>-2</sup> and 65% relative humidity). The IAA content of the hypocotyls was measured in six-day-old plants, while that of the primary leaves in fourteen-day-old ones.

Measurement of IAA content: the IAA contents of 100 hypocotyls and that of 100 primary leaves were determined after extraction with 80% cold methanol. The extract was evaporated to dryness under reduced pressure, then 0,5 M K<sub>2</sub>PO<sub>4</sub> solution was added (pH 8,5).

The purification and fractionation were carried out according to the methods of KAMISAKA and LARSEN (1977). The amount of IAA present in the final acidic ether fraction was measured by the indole- $\alpha$ -pyrone fluorescence method (KNEGT and BRUINSMA, 1973; HEMBERG and TILLBERG, 1980) with a Perkin-Elmer spectrofluorimeter.

Stem- and primary leaf-cuttings were rooted in a fourth strength Hoagland nutrient solution supplemented with 1 ml of complex solution of micronutrients.

In order to promote rooting the primary leaves were evenly moistened with 1  $\mu$ l of 1 mg/l indole-3-butyric acid (IBA, Reanal), tryptophan (TRY, Reanal), abscisic acid (ABA, Sigma), and 1  $\mu$ l of 10 mg/l gibberellic acid (GA<sub>3</sub> Reanal) solution containing 0,05% TWEEN 80 as a detergent.

The ethylene treatment was carried out by "Rol-Fruct" an ethylene-generator, containing 40% 2-chloroethyl-phosphonic acid (Chinoin Co., Budapest). 0,04  $\mu$ g of agent was spread over the entire surface of one leaf.

All treatments were repeated twice within an hour.

0,1 mM silver thiosulfate (STS, 0,1 mM AgNO<sub>3</sub> + 0,1 mM sodium thiosulfate) was applied to inhibit the effect of ethylene.

## Results and Discussion

The effect of CCC-treatment on the growth of bean seedling is presented in Table 1. and Figure 1.

These data show that besides retarding seedling growth and increasing stem-thickening, CCC results in thicker and longer roots. The stimulating effect of CCC on rooting can be noticed, not only in intact beans, but also in stem-cuttings (Fig. 2).

Table 1. Effect of CCC on the growth of bean plants (Data of six-day old plants)

	hypocotyl				root		
	length (mm)	thickness (mm)	fresh weight (g)	dry weight (mg)	fresh weight (g)	dry weight (mg)	length of primary root (mm)
Control	67,5 $\pm$ 3,2	3,4 $\pm$ 0,25	1,325 $\pm$ 0,3	56,42 $\pm$ 4,06	0,176 $\pm$ 0,014	15,47 $\pm$ 2,45	80,5 $\pm$ 9,5
Treated	35,2 $\pm$ 2,3	4,8 $\pm$ 0,23	1,038 $\pm$ 0,19	50,16 $\pm$ 4,50	0,310 $\pm$ 0,012	18,56 $\pm$ 2,22	125,5 $\pm$ 11,5

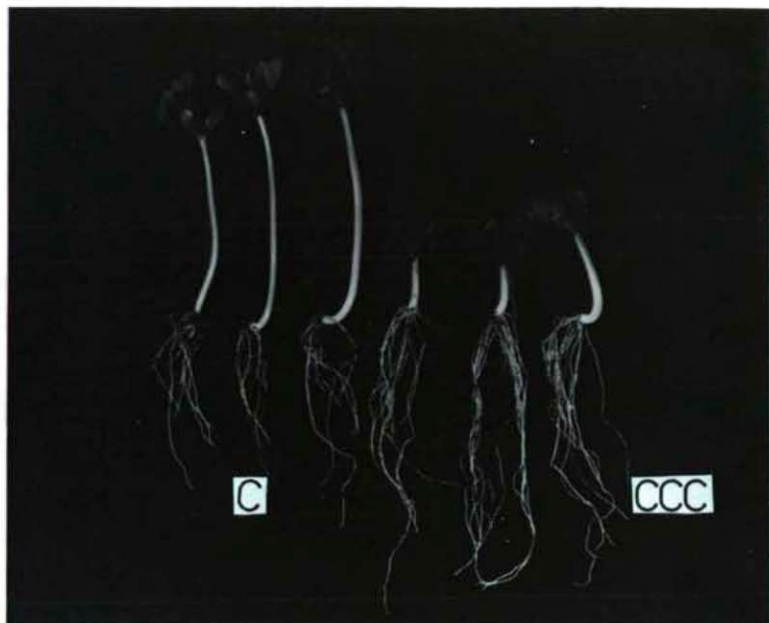


Fig. 1. Effect of CCC on the growth of six-day old *Phaseolus vulgaris* cv. Juliska seedlings

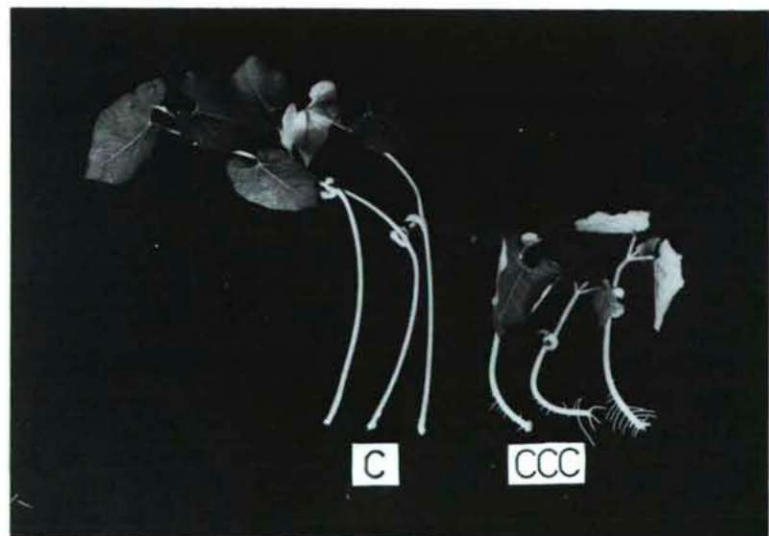


Fig. 2. Adventitious rooting in hypocotyls of bean. Left: control, right: treated with CCC.

CONNECTION BETWEEN THE IAA CONTENT OF HYPOCOTYLS AND THE EXTENT OF  
ROOTING

It is well-known that plant hormones play an important role in the rooting process. Auxins seem to be the most important of these, since in the case of exogenous application, IAA or synthetic auxins, stimulate formation of adventitious roots (TORREY, 1976; HARTMAN and KESTER, 1983).

Table 2. illustrates the distribution of the IAA content in the apical and basal parts of hypocotyls. As these data show, in the hypocotyls of the treated plants there is less IAA than in the control. However, the IAA concentration of the hypocotyls (the amount of IAA per gram fresh weight) is higher, because the hypocotyls of the treated plants are much shorter than those of the untreated ones, and their fresh weight is also smaller. It is the amount of IAA in the basal part of the hypocotyls that is of importance in the rooting process. Our results demonstrate that the distribution ratio of IAA between the apical and basal parts of the treated plants is 1:3,69, whereas that of the control is 1:1,41.

Table 2. Effect of CCC on the IAA content and quantitative distribution between the apical (A) and basal (B) parts of hypocotyls

IAA content	Control		Treated	
	A	B	A	B
ng IAA/hypocotyl part	5,1±0,8	7,2±0,8	2,3±0,6	8,5±0,55
ng IAA g <sup>-1</sup> fresh mass	7,76	9,23	5,75	17,45
Distribution% of the total IAA quantity	41,46	58,54	21,29	78,71

We found a similar tendency in respect of IAA concentration, the ratios are A:B = 1:1,90 and A:B = 1:3,03 in the control and in the treated plants respectively.

These results unambiguously indicate that the stimulating effect of CCC on rooting can be correlated with the higher amount of IAA in the basal part of hypocotyls. At the same time the basal parts of the hypocotyls of treated plants exhibited a higher ethylene production (NAGY and TARI, 1986).

EFFECT OF CCC TREATMENT ON ROOTING OF PRIMARY LEAVES

Since the primary leaves of bean seedlings can root very well and are excellent subjects to study the hormonal regulation of adventitious rooting, it was interesting to investigate whether the stimulating effect of CCC on rooting would prevail in leaves as well. For rooting experiments primary leaves of fourteen-day-old bean

plants were used. Results are presented in Fig. 3. In the case of bean leaves we could not observe any stimulating effect of CCC on rooting, on the contrary, the formation of root primordia were definitely inhibited in petioles.

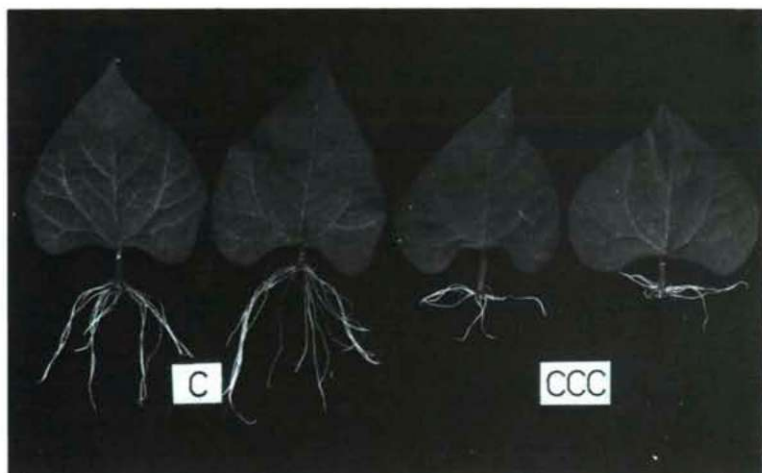


Fig. 3. Adventitious root formation of detached primary leaves of CCC treated bean plant

#### CONNECTION BETWEEN FORMATION OF ADVENTITIOUS ROOTS AND IAA CONTENT OF PRIMARY LEAVES

The IAA content of the primary leaves was measured at the time of their placement in rooting solution. Our results are summarized in Table 3.

We concluded that the IAA content of the treated leaves is higher than that of the untreated, but the majority of this IAA as contrasted with the control, can be found in the blade. After CCC treatment the distribution of IAA changed in the case of leaves as well, but this change is disadvantageous for adventitious rooting.

Table 3 Effect of CCC on the IAA content of the primary leaves of bean

IAA content	Control		Treated	
	Lamina	Petiole	Lamina	Petiole
ng IAA/leaf part	1,57±0,16	2,31±0,22	3,0±0,21	1,52±0,17
ng IAA g <sup>-1</sup> fresh mass	3,2	28,5	4,15	20,4
Distribution% of the total IAA quantity	40,46	59,54	66,37	33,63

#### THE EFFECT OF VARIOUS HORMONE TREATMENTS ON ADVENTITIOUS ROOT FORMATION OF PRIMARY LEAVES

Knowing the physiological fact that growth and development of plants are regulated by hormones which are in interaction with each other, we applied various hormone treatments to promote the rooting process of primary leaves of CCC-treated seedlings. Data represent the results on the 10th day following the treatment. Later, to estimate the effectiveness of these treatments, we shall consider leaves of CCC-treated plants as the control.

Since gibberellins and cytokinins generally inhibit adventitious root formation, these hormones were not applied as an independent treatment.

##### *Effect of IBA treatment on formation of adventitious roots*

The inductive phase of the rooting process is characterised by high IAA-oxidase activity and low IAA content; in the second, initiative phase the IAA-oxidase activity decreases and the IAA content rises (GASPAR, 1981; MALDINEY et al. 1986; MATO et al. 1988; MONCOUSIN et al. 1988). In the initiative phase rooting needs a high auxin level (WELANDER, 1983; MALDINEY et al. 1987).

IBA, a compound generally used to stimulate adventitious rooting, acts as the auxin reserve at the time of high auxin oxidase activity – as it is not oxidized by the peroxidases –, when in the second phase of rooting the IAA-oxidase activity decreases, the IBA is metabolised into IAA (EPSTEIN and LAVEE, 1984; ALVAREZ et al. 1989).

Consequently, it is not the IBA that stimulates rooting, but the IAA formed from it. Furthermore IBA – like other synthetic auxins – alters the IAA-oxidase activity, in this way also increasing the endogenous auxin content for rooting (KEVERS et al. 1981; DRUART et al. 1982; MATO and VIEITEZ, 1986).

Besides those mentioned so far, IBA treatment modifies the distribution of cytokinins in the leaves as well which results in less cytokinin in the petiole of the treated leaves than that of the control; this circumstance is advantageous for rooting (BRIDGALL and VAN STADEN, 1985). In our case this effect can be of great importance since the CCC treatment increases the cytokinin level (SKENE, 1968).

The effectiveness of IBA in stimulating rooting is probably in connection with high ethylene production in the region of adventitious root formation, throughout the majority of the rooting process (RIOV and YANG, 1989).

The result of IBA treatment is presented in Figure 4. The rooting process of the primary leaves was effectively promoted by IBA. In our experiments pretreatment with STS prevents the effect of IBA in root initiation.

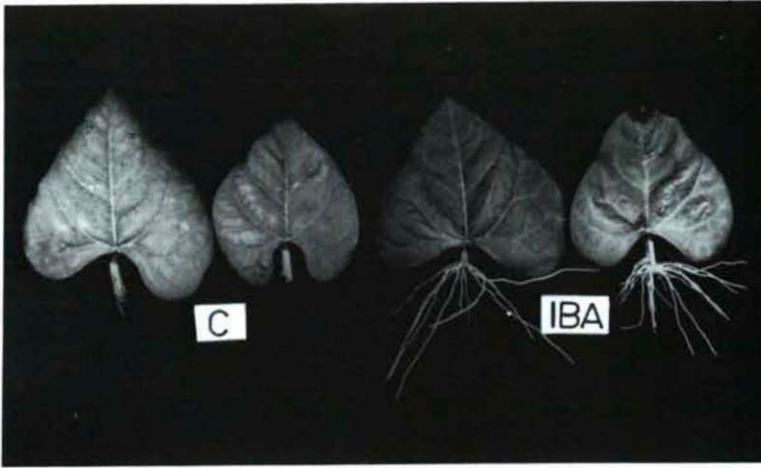


Fig. 4. Effect of IBA on the adventitious root formation of detached primary leaves of bean plant pretreated with CCC

*Effect of tryptophan treatment on adventitious root formation of primary leaves*

In the inductive phase a favourable auxin level can be achieved by a tryptophan treatment, a precursor of IAA biosynthesis, the result of which is presented in Fig. 5. Tryptophan treatment has no effect on the process of root formation in this case.

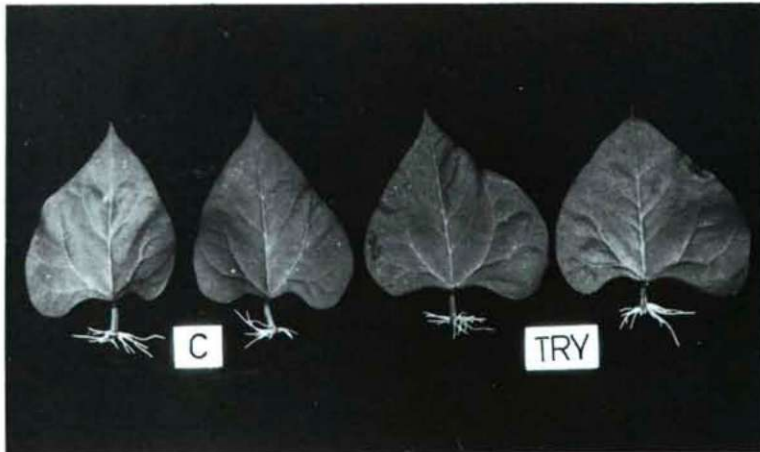


Fig. 5. Effect of tryptophan on the adventitious root formation of detached primary leaves of bean plant pretreated with CCC

*Effect of tryptophan and gibberellin treatment on adventitious root formation of primary leaves*

It is known that the transformation of tryptophan into IAA is stimulated by GA (JINDAL and HEMBERG, 1976; LAW and HAMILTON, 1984). According to KUTAČEK (1985) the GA treatment stimulates the activity of the enzyme of IAA biosynthesis, the indole-3-acetaldehyde oxidase; LAW (1987) reported that the GA transforms the L-tryptophan into D-tryptophan, which will become the precursor of auxin synthesis. The effect of the tryptophan and gibberellin treatment on the rooting of leaves is presented in Fig. 6. This treatment was also ineffective in initiating root primordia.

From the ineffectiveness of the tryptophan and tryptophan plus gibberellin treatments we can conclude that the increasing IAA synthesis due to the increasing tryptophan level, does not take place, or is negligible with regard to rooting in treated leaves.

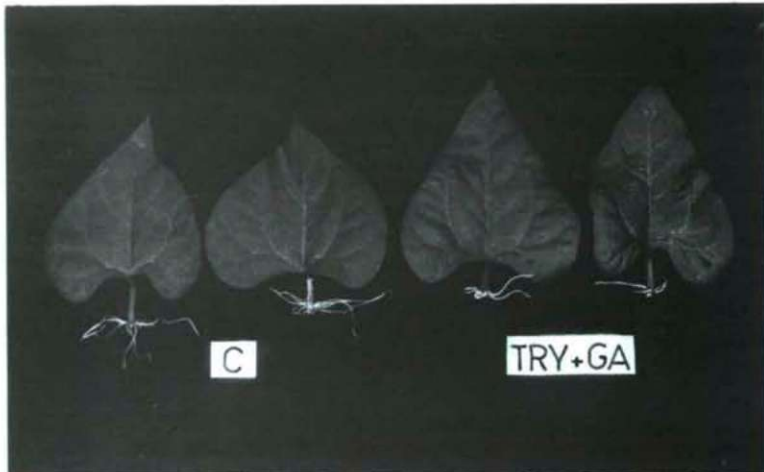


Fig. 6. Effect of tryptophan and gibberellin treatment on the adventitious root formation of detached primary leaves of bean plant pretreated with CCC

*Effect of ABA treatment on adventitious root formation of primary leaves*

ABA treatment is also effective in initiation of adventitious roots (BATTEN and GOODWIN, 1978). In the natural process of adventitious rooting the endogenous level of ABA rises (FOONG and BARNES, 1981), since the leaf tissues synthesise ABA under the influence of stress (WALTON, 1980; SOTTA et al. 1985; BERTHRON et al. 1989).



The effect of ABA treatment on the rooting of bean leaves is presented in Fig. 7. In our experiments the promoting effect of ABA can be prevented by STS pretreatment.

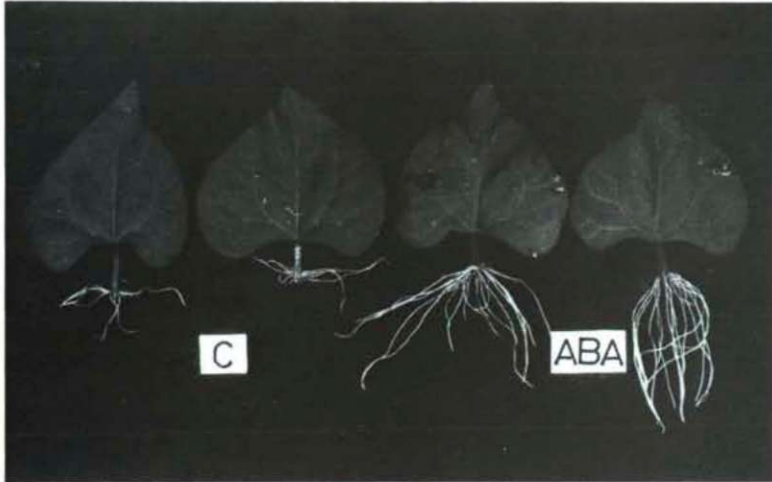


Fig. 7. Effect of ABA treatment on the adventitious root formation of detached primary leaves of bean plant pretreated with CCC

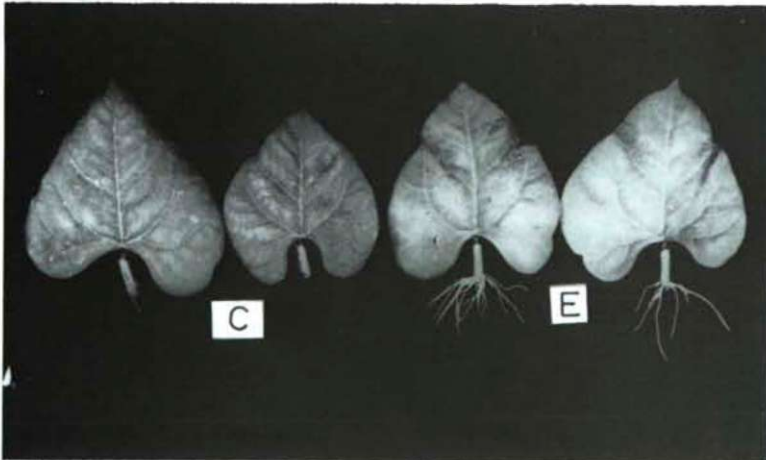


Fig. 8. Effect of ethylene-generator (E) treatment on the adventitious root formation of detached primary leaves of bean plant pretreated with CCC

*Effect of ethylene generator on adventitious root formation of primary leaves*

It is well known that ethylene stimulates the formation of adventitious roots in leaf-cuttings (FABIJAN et al. 1981; ROBBINS et al. 1983; 1985; RIOV and YANG, 1989), if the applied concentration is sufficiently low (JUSAITIS, 1986; LIU et al. 1990).

The effect of "Rol-Fruct" treatment on rooting of primary leaves is presented in Fig. 8. The stimulating effect of the ethylene-generator on rooting is inhibited by the STS pretreatment in our experiments.

### Conclusions

The stimulating effect of CCC on rooting in intact plants and stem cuttings correlates with the higher IAA level and ethylene production in the basal part of the hypocotyls in the treated plants.

The higher IAA level in the primary leaves of bean did not result in an increase in formation of adventitious roots, because the IAA content of the petioles was reduced by the CCC treatment.

In the case of the plants originating from seeds soaked in CCC the rooting process of the primary leaves was effectively promoted by IBA, ABA and ethylene-generator treatments. The common feature of IBA and ethylene is that they are capable of producing a high auxin level for rooting, IBA by metabolising into IAA (EPSTEIN and LAVEE, 1984), the ethylene by means of raising the endogenous auxin content (VARGA et al. 1982; WEIGEL, 1984).

The rise in the endogenous ethylene level increases the endogenous ABA content also (MAYAK et al. 1972; KORABLEVA, 1986). The rise in the ABA level, on the other hand, in other cases results in the elevation of ethylene production (MAYAK and DILLEY, 1976; GOREN, 1979; LIEBERMAN et al. 1977; SAGEE et al. 1980; WRIGHT, 1980; RIOV et al. 1990).

The CCC treatment is likely to inhibit the formation of the appropriate endogenous ABA concentration in bean plants; as this inhibition of ABA biosynthesis has been observed in *Cercospora rosicola* (NORMAN et al. 1983).

It has been known for a long time that auxin increases ethylene production (YANG and HOFFMAN, 1984), so the effectiveness of IBA in initiation of root primordia may be due to the induction of ethylene production by the auxin in the region of adventitious root formation (RIOV and YANG, 1989).

The common feature of our IBA, ABA and ethylene generator treatments is that all three are capable of inducing intensive ethylene production. In all the three cases, in our experiments with the STS pretreatment, the initiation of root primordia in the leaf-cuttings can be prevented. Therefore, on the bases of our results ethylene is the hormone that can play a key role in this system, in the induction of adventitious roots.

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