Exogenous Application of NAA and ACC Cooperatively Inhibits Root Elongation

Exogenous application of NAA as well as ACC inhibited maize primary root elongation in a concentration-dependent manner. In addition, combined treatments of NAA and ACC revealed the auxin-ethylene interaction on root elongation inhibition. The cooperative effect was only appreciated at very low concentrations of both compounds. It was well known that application of the ethylene precursor ACC immediately reduces root growth inhibition, but ethylene is required for root growth, as inhibition of ethylene biosynthesis results on reduced elongation in maize root.

It has also been shown that below certain threshold levels of ethylene the response to auxin is altered. Although the typical effect of external auxin on root elongation is inhibition, exogenous auxin stimulates root elongation in maize root pretreated with ethylene antagonist. In addition, auxin inhibition of maize root elongation can be reversed by ethylene antagonists applied after or before auxin addition, and the inhibitory effect of auxin on the elongation of bean root was also reversed by ethylene synthesis inhibitor. These results supported that auxin and ethylene interact on root regulation. Moreover, combined treatments of auxin and ethylene have provided direct evidence of this interaction. It has been shown that these combined applications cause a cooperative inhibitory effect on root elongation when both compounds were used at very low concentrations.

Crosstalk between auxin and ethylene has been shown at the levels of synthesis, signaling and transport. Application of ACC quickly inhibited elongation in Arabidopsis root associated with apoplastic alkalinization, then it was also proposed that ethylene increases the auxin levels in root cells and subsequently negatively modulates the activity of plasma membrane ATPase regulated by auxin. Many ethylene signaling mutants are also auxin resistant and vice versa. In addition, both hormones require the response of the each other to exert its effect on root growth. However, several differences have been found between the effects caused by auxin or ethylene suggesting that auxin is the most important hormone in controlling root growth.

Maize primary root 60–80 mm long grew at a constant rate of about 3.46 ± 0.32 mm/h in an aerated hydroponic medium.
composed by HEPES 1 mM CaCl2 1 mM and KCl 10 mM for 12 h. The addition of 0.025 µM NAA to the growth medium inhibited root elongation by 38% and 1 µM ACC inhibited by 12% when these compounds were applied separately. The combined treatment of both compounds at the same concentrations produced an inhibition of about 55% (Fig. 1) showing the cooperative action of both compounds in the regulation of elongation root growth.

However in literature we have found that ACC as well as IAA inhibited maize root elongation, but combined treatment of both compounds did not increase the inhibitory effect of IAA.16 These differences could be due to experimental conditions; especially to the ACC and IAA applied concentrations. In our experiments, when the concentration of NAA and ACC were increased, the cooperative effect was not observed.1

Ethylene Synergistically Increases the Inhibitory Effect of Auxin on Cell Length in Epidermal Layer

In order to analyze if these inhibitory effects by auxin and ethylene are mediated by a decrease on cell length in epidermal layer, we measured cell length in the different treatments. The measurements were performed in the elongation zone located about 10 mm away from the root apex where elongation has completely ceased.3,4 At this level cells have reached the final length and no increase in cell length has been measured in more basal zones.

Exogenous supplied NAA reduces epidermal cell length in maize. Epidermal cell length of root treated with 0.025 µM NAA showed a reduction of about 28% compared with untreated root (Fig. 2). External application of ACC increases ethylene level dependent on the ACC concentration. A reduction of cell length was only observed when high concentrations of ACC were applied but it was not observed when low concentration of ACC was applied. One µM ACC added to the incubation medium does not produce a significant reduction on epidermal cell length (Fig. 2). However, 1 µM ACC increased the inhibitory effect caused by 0.025 µM NAA, as the combined treatment 0.25 µM NAA+1 µM ACC) produced an inhibition of about 50%. Thus, the increase in the inhibitory effect from 28 to 50% suggests a synergistic action of ethylene on the auxin-dependent inhibition root growth.

Relationship between Epidermal Cell Length and Root Elongation

To know how auxin and ethylene affect the two processes responsible of root elongation cell division and cell elongation we compared root elongation and cell length in Table 1. First, we estimated the relative epidermal cell length and relative root elongation respect the untreated root in the different treatments, and then we calculated the ratio by dividing relative epidermal cell length by relative root elongation. The results showed that epidermal cell length is responsible for 85–91% of the inhibition of root elongation caused by the treatments. The difference of

Table 1. Relative contribution of epidermal cell length to the root elongation in maize root after treatments with auxin and ethylene and the combination of both

| Treatments     | Epidermal cell length (% vs control) | Elongation root (% vs control) | Ratio of elongation root to epidermal cell length |
|----------------|--------------------------------------|-------------------------------|-----------------------------------------------|
| Control        | 100.00                               | 100.00                        | 1.00                                           |
| 1 µM ACC       | 96.65                                | 87.95                         | 0.91                                          |
| 0.025 µM NAA   | 72.30                                | 61.45                         | 0.85                                          |
| ACC + NAA      | 50.63                                | 44.58                         | 0.88                                          |

Ratio represents the fraction attributable to the elongation of the epidermal cell to the root elongation in the different treatments.
produces inhibition, but it can also stimulate growth depending on environmental stimuli. An increase in ethylene levels normally is responsible for part, but not all, of the inhibition growth by ethylene, auxin or both.

In addition treatments with auxin and ethylene caused different effects depending on environmental condition. Higher concentrations of ACC or NAA inhibited growth whereas low concentration has no effect in P-sufficient white clover root. However, in P-deprived root low concentrations of ACC or NAA stimulated primary root elongation.

Previous reports suggested the interaction auxin-ethylene in regulating root development based in results obtained either in experiment in Arabidopsis mutants in auxin and ethylene or by the treatment with ethylene antagonists and biosynthesis inhibitors. In this work, it has been reported that combined treatment by auxin and ethylene causes a cooperative inhibitory effect on root elongation. Moreover, it is clearly shown that the reduction on epidermal cell length, produced by the synergistic action by auxin and ethylene, results in an inhibition of root elongation. It is well established that auxin is the main hormone in controlling root growth and development, but crosstalk between auxin and ethylene has widely reported in several physiological processes in root. The results presented in this work are in accordance with the hypothesis that ethylene modulates auxin action in developmental processes in roots. Auxin and ethylene have been shown to interact antagonistically or synergistically and this fact could be due to the ethylene level as the biphasic model proposed to explain the differential responses to ethylene suggests. Ethylene has also proposed as the hormone that integrates the environmental changes and it has been suggested as a fine tuning of root elongation.

On the other hand, the differences between the reduction in epidermal cell length by auxin, ethylene or both and the inhibition of root elongation (Table 1) could be produced by the hormone action on cell division as ethylene has been shown to promote endoreduplication1 and to modulate stem cell division in Arabidopsis root. However, further studies in other root zones involved on growth would provide useful information about how auxin and ethylene interact on the regulation of root elongation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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