Effects of Low O₂ Root Stress on Ethylene Biosynthesis in Tomato Plants (*Lycopersicon esculentum* Mill cv Heinz 1350)¹

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**ABSTRACT**

Low O₂ conditions were obtained by flowing N₂ through the solution in which the tomato plants (*Lycopersicon esculentum* Mill cv Heinz 1350) were growing. Time course experiments revealed that low O₂ treatments stimulated 1-aminocyclopropane-1-carboxylate (ACC) synthase production in the roots and leaves. After the initiation of low O₂ conditions, ACC synthase activity and ACC content in the roots increased and reached a peak after 12 and 20 hours, respectively. The conversion of ACC to ethylene in the roots was inhibited by low levels of O₂, and ACC was apparently transported to the leaves where it was converted to ethylene. ACC synthase activity in the leaves was also stimulated by low O₂ treatment to the roots, reaching a peak after 24 hours. ACC synthase levels were enhanced by cobalt chloride and aminooxycetic acid (AOA), although they inhibited ethylene production. Cobalt chloride enhanced ACC synthase only in combination with low O₂ conditions in the roots. Under aeration, AOA stimulated ACC synthase activity in both the roots and leaves. However, in combination with low O₂ conditions, AOA caused a stimulation in ACC synthase activity in the leaves and no effect in the roots.

Ethylene production is observed in all higher plants, where it is involved in numerous aspects of plant growth and development but most notably as a response to stress conditions (1).

Waterlogging of higher plants causes petiole epinasty, chlorosis, stem hypertrophy, reduced growth, and adventitious root growth (15). Epinastic growth of tomato petioles has been shown to occur within 12 h following flooding of the soil (12). Exposure to ethylene will also cause petiole epinasty in a variety of plants (9).

Adams and Yang (2) identified ACC as an intermediate in the conversion of methionine to ethylene. Ethylene biosynthesis requires the conversion of AdoMet to ACC, which gives rise directly to ethylene. The cleavage of AdoMet to produce ACC plus 5′-(methylthio)adenosine is catalyzed by ACC synthase (19, 20).

Low O₂ conditions in the root zone of tomato plants have been reported to cause elevated ethylene levels and epinasty in the shoot regardless of whether the condition is imposed by waterlogging or by flushing with N₂ (4, 12, 14). It has been shown that ethylene production by maize (*Zea mays* L.) roots exposed to 5 kPa oxygen was inhibited by AVG supplemented to the nutrient solution and this also reduced aerenchyma formation (13). This work provided evidence that the stimulation in ethylene production was probably due to an enhancement in ACC synthase and that ethylene was involved in the promotion of aerenchyma in adventitious roots of maize. Cohen and Kende, utilizing an *in vivo* assay for this enzyme (8), showed that ACC synthase levels can be enhanced in deep-water rice internodes (*Oryza sativa* L.). Because they were unable to detect ACC synthase in homogenates of deep-water rice internodes, they utilized *in vivo* methods that assayed ACC accumulation in tissue under N₂. Their conclusion was that the stimulation of ACC synthase activity by low O₂ pressures was one of the first biochemical events leading to internodal growth in deep-water rice. The activity of ACC synthase in homogenates of vegetative tissues is often either much lower than expected or not measurable at all *in vitro* (8, 20). To overcome this problem, we used the method described by Tsai *et al.* (18) to assay for ACC synthase. In the present study, we surveyed the changes in ACC synthase, ACC, and ethylene in whole tomato plants by flushing the roots with N₂ and also evaluated the effects of ethylene biosynthesis inhibitors.

**MATERIALS AND METHODS**

**Plant Preparation**

Tomato (*Lycopersicon esculentum* Mill cv Heinz 1350) plants were grown for 4 weeks. Seeds were germinated in a slant board sandwich which consisted (in order) of a plexiglass board, a layer of KimPak, nylon cloth, seeds (single row 1 inch from the top), another layer nylon cloth, KimPak, and plexiglass. The sandwich was wrapped with aluminum foil and taped to assure that the seeds were firmly held. The sandwich was stood upright, seed side on the top, and watered daily. After a 2-week growth period, the seedlings were transferred to aerated full-strength Hoagland solution No. 1 (11) in large tubs where they were grown for 1 week and then transferred to 50-mL Erlenmeyer flasks for another week before treatment. The plants were grown under continuous

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² Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AdoMet, S-adenosylmethionine; AOA, aminooxycetic acid.
cool-white fluorescent light (70.3 ± 2.78 μmol m⁻² s⁻¹) at 25 ± 2°C.

Plant Treatment

Low O₂ conditions were accomplished by bubbling N₂ instead of air through the nutrient solution of the plants. The activity of ACC synthase, ACC content, and ethylene production were assayed at 0, 6, 12, 20, 24, and 30 h following treatment initiation.

The plants were pretreated for 4 h in growing solution (5) containing either 220 μM CoCl₂ or 200 μM AOA, after which air or N₂ treatment to the growing solution was initiated. The changes in ACC synthase and ACC were assayed 12 or 24 h after the 4-h pretreatment.

Assay of ACC Synthase, ACC, and Ethylene

Leaves (including petioles) from the first node above the cotyledonary node and roots from the first primary root below the cotyledonary node were extracted. Unless otherwise specified, the stems were not used.

Twelve plants per treatment were used to evaluate changes in ACC synthase and ACC in the roots and shoots. Tissues were extracted according to the method described by Tsai et al. (18) with slight modifications. Tissues from tomato seedlings were extracted in 1 volume of 1.0 m potassium phosphate buffer (pH 8.0) containing 4.0 mm DTE, 0.5 mm pyridoxal-5-phosphate, and a small amount of sea sand.

Three plants per treatment were used to evaluate ethylene. The procedures of measurement were similar to those of Bradford and Yang (7). The samples were weighed, and ethylene production was expressed on a fresh weight basis.

Ethylene was analyzed with a Hewlett-Packard 5830 dual column gas chromatograph equipped with a 3.175-mm o.d. × 1.2 m columns packed with activated alumina. Injector port, column, and flame ionization detector temperatures were 100, 100, and 300°C, respectively.

RESULTS

Time Course of Ethylene Biosynthesis

In preliminary experiments, ACC synthase activity was not detectable in stems. Therefore, the following experiments were conducted on roots and leaves.

ACC synthase activity in the roots of plants that were treated with N₂ reached a peak 12 h after beginning the treatment (Fig. 1RA). In the leaves, ACC synthase activity increased only slightly after 24 h and then declined (Fig. 1LA).

ACC content in the roots reached a peak 20 h following the initiation of N₂ treatment to the roots (Fig. 1RB). The ACC content in the leaves increased similarly to ACC synthase activity, reaching a peak after 24-h N₂ treatment (Fig. 1LB).

The ability of the roots to produce ethylene was very low during the entire N₂ treatment and was not obviously different from the air treatment (Fig. 1RC). On the other hand, the ability of the leaves to produce ethylene reached a peak after the peaks of ACC synthase and ACC (Fig. 1LC).

Effect of CoCl₂ 12 h Following Initiation of Treatment

ACC synthase activity in the roots exposed to the N₂ treatment plus 220 μM CoCl₂ for 12 h was twice as high as that from roots exposed to N₂ alone. However, there was no effect on ACC synthase activity in the leaves (Fig. 2A). The addition of CoCl₂ did not affect ACC synthase activity in either roots or leaves under the air treatment (Fig. 2A).

The ACC content in the roots of the N₂-treated plants was unaffected by the CoCl₂ treatment 12 h following treatment (Fig. 2B). In the leaves, the ACC content was very low regardless of the N₂ or N₂ plus CoCl₂ treatments (Fig. 2B).
leaves, although ACC synthase activity was enhanced greatly with the N₂ plus AOA treatment, the ACC content was still very low and only slightly higher than the air plus AOA treatment (Fig. 4B).

DISCUSSION

We have shown that ACC synthase levels in tomato roots under low O₂ conditions reached a peak after 12 h (Fig. 1RA) which was followed by an increase in ACC content (Fig. 1RB). ACC produced in the roots was apparently transported to the leaves (Fig. 1LB) where it was converted to ethylene (Fig. 1LC), which is in agreement with previous reports (6, 7). Other researchers (8, 13) have provided evidence indicating that ACC synthase increases in response to low O₂ pressures, and our work now confirms these earlier findings.

Low levels of ACC synthase are also induced in the leaves of tomato plants when their roots are subjected to low O₂ conditions (Fig. 1LA). It is possible that this effect is due to autocatalytic ethylene production which has been reported to induce ACC synthase activity in leaves (16).

ACC synthase activity in tomato roots was promoted by CoCl₂ plus N₂ 12 h after beginning the treatment; however, by 24 h this difference had disappeared (Fig. 3A). CoCl₂ in

Effect of CoCl₂ 24 h following Initiation of Treatment

Twenty-four hours after beginning treatments, N₂ treatment increased ACC synthase activity in the roots and leaves above the air treatment levels, and the addition of CoCl₂ did not affect ACC synthase activity in either roots or leaves under air treatment (Fig. 3A).

N₂ treatment caused an increase in ACC content of roots and leaves, but inclusion of CoCl₂ reduced this increase only in the roots (Fig. 3B). CoCl₂ had no effect on ACC content in combination with the air treatment (Fig. 3B).

Effect of AOA Treatment

When roots were aerated in the presence of AOA, ACC synthase activity in the roots was stimulated to a level similar to that of roots under low O₂ conditions with or without added AOA (Fig. 4A). In the leaves, the addition of AOA enhanced ACC synthase activity under either the air or N₂ treatments, and the N₂ plus AOA treatment showed higher activity than the air plus AOA treatment (Fig. 4A).

The N₂-stimulated increase in ACC content in the roots was inhibited by AOA, but AOA had no effect on ACC content of roots exposed to the air treatment (Fig. 4B).
combination with the air treatment had no effect on ACC synthase activity (Fig. 2A). We were unable to show that CoCl₂ plus air treatment enhanced ACC synthase activity in either roots or leaves. It is possible that not only ethylene autocatalysis but also some unknown factors may stimulate the production of ACC synthase.

AOA treatment stimulates ACC synthase in low O₂-treated tomato plants (Fig. 4A), but AOA inhibited the conversion of AdoMet to ACC (Fig. 4B). This is in agreement with others who have shown that AOA stimulates IAAs-induced ACC synthase activity in etiolated mung bean hypocotyl segments while inhibiting the conversion of AdoMet to ACC (3). Also, under aeration, AOA stimulated ACC synthase activity in both roots and leaves (Fig. 4A). AOA is a nonspecific inhibitor of ACC synthase (10, 17) and can cross the mitochondrial membrane (17). Moreover, the addition of AOA did not enhance the ACC synthase activity under low O₂ conditions in the roots (Fig. 4A). Because low O₂ treatment affects O₂ utilization of mitochondria and AOA may affect the mitochondria, it is possible that both low O₂ and AOA treatment may induce ACC synthase by the same mechanism and the effect of low O₂ stress on ethylene biosynthesis in tomato plants may be caused by an energy problem.

ACC synthase levels were enhanced by CoCl₂ and AOA; however, these treatments inhibited ethylene production in tomato plants under low O₂ treatment. Many stresses, such as metal ions, chemical stress, and others can stimulate ethylene production in plants by stimulating ACC synthase. Therefore, it is difficult to make the interpretation of results with these inhibitors. Further studies to better understand how ACC synthase is regulated by stress in plants should utilize new molecular techniques currently available.

LITERATURE CITED