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Effect of Rhizobitoxine, Cycloleucine and Cycloheximide on Wound Ethylene Production by Fruit Pericarp Tissue of *Rin* Mutant Tomato

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ABSTRACT

Both l-aminocyclopropane-l-carboxylic acid (ACC) and S-adenosylmethionine (SAM) increased wound ethylene production by fruit pericarp tissue of *rin* mutant tomato but ACC was more effective than SAM. Rhizobitoxine or cycloleucine given along with ACC stimulated more wound ethylene production. Rhizobitoxine inhibited SAM-stimulated wound ethylene production but cycloleucine did not. This suggests that cycloleucine may have different mode of action from rhizobitoxine. Cycloheximide inhibited wound ethylene production by tomato disks treated and non-treated with ACC.

INTRODUCTION

Methionine has been shown to be in the *in vivo* precursor of ethylene biosynthesis in both wounded and senescing tissues including flowers, fruits,leaves, stems and roots (Abeles and Abeles, 1972; Adams and Yang 1974; Aharoni *et al.*, 1979, Burg and Thimann, 1959; Hanson and Kende, 1976 a; Hanson and Kende, 1976 b; Hyodo, 1978). Plant tissues convert only C-3 and C-4 of methionine to ethylene (Abeles and Abeles, 1972; Hanson and Kende, 1976 a; Hyodo, 1978). Adams and Yang (1974, 1979) have proposed SAM and ACC as intermidiates in the conversion of methionine to ethylene. Subsequent work has led to the isolation of the ACC-forming enzyme from tomato fruit tissue (Boller *et al.*, 1979) and ACC-dependent ethylene forming system from pea stems (Konze and Kende, 1979 a). The biosynthetic pathway of ethylene is thought to proceed as follows: methionine $\longrightarrow$SAM$\longrightarrow$ACC$\longrightarrow C_2H_4$. In this scheme, aminoethoxyvinylglycine (AVG), a well known inhibitor of ethylene biosynthesis (Lieberman *et al.* 1974), has been shown to block the formation of ACC (Adams and Yang, 1979; Boller *et al.*, 1979; Jones and Kende, 1979). ACC formation from SAM is the rate-limiting step of ethylene production in plant tissue (Adams and Yang, 1979). This paper reports the characteristics of inhibitory effect on ACC-stimulated wound ethylene production by rhizobitoxine, cycloleucine and cycloheximide in fruit pericarp tissue of *rin* tomato (ripening inhibitor mutant).

MATERIALS AND METHODS

Plant material and incubation. Plant of *rin* mutant tomato *(Lycopersicon esculentum* Mill*).* were grown and trained to single stem. Flowers were tagged at anthesis; only one flower per cluster being pollinated. All others in a cluster were excised. Disks of pericarp tissue (diameter: 1.5 cm; thickness: 0.25 cm) with intact epidermis from green fruits (40 ± 1 days after anthesis) were prepared from the equatorial part of tomato fruits using a cork borer and and template. Therefore, all disks were uniformly prepared in size and fresh weight. Disks were placed with epidermis on a layer of glass beads

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in a 20-ml scintillation vial. The vials were flushed with ethylene-free air and sealed with a serum cap, then incubated in darkness at 20°C.

Ethylene determination. At the end of every 2-h period, wound ethylene production by tomato disks was withdrawn with a l-ml gas tight syringe and assayed using a gas chromatograph (Varian Aerograph Series 1700) equipped with a flame ionization detector. The vials were returned to the incubation conditions. Each experiment consisted of five to six tomato disks and was repeated three times. Data represent mean values.

RESULTS AND DISCUSSION

In this study, Fig.1 shows that SAM applied to tomato disks sitmulated wound ethylene production but less than ACC. The response to applied SAM showed a lag period while ACC did not. Rhizobitoxine completely inhibited wound ethylene production stimulated by applied SAM but it stimulated more wound ethylene production when it was applied together with ACC. The ability of rhizobitoxine to inhibit wound ethylene production by tomato disks with and without SAM treatment indicates that the methionine and SAM pathway is operative in this wounded tissue from *rin* mutant tomato fruits and similar to that found in the majority of wounded and senescent plant tissues (Adams and Yang, 1979; Aharoni *et al.*, 1979, Hanson and Kende, 1976 a; Hanson and Kende, 1976 b; Suttle and Kende, 1980). The inability of rhizobitoxine to inhibit the conversion of ACC to ethylene by tomato disks consistent with the hypothesis that rhizobitoxine inhibits the formation rather than the utilization of ACC (Adams and Yang 1979, Boller *et al.*, 1979). It is not known how ACC applied togeter with rhizobitoxine produces more wound ethylene than when ACC was applied alone.

Cycloleucine (1-aminocyclopentane-l-carboxylic acid) also showed similar results to that of rhizobitoxine when it was applied alone or together with ACC (Fig.2), but it did not inhibit the SAM-stimulated wound ethylene production. The mode of action of the inhibitory effect of cycloleucine on ethylene production may be different from that of rhizobitoxine. If cycloleucine inhibited ethylene production by competing with ACC as suggested by Baker *et al.* (1979), then cycloleucine should reduce the stimulation of wound ethylene production when it was applied together with ACC. There was in fact a stimulation of ethylene production. It is possible that cycloleucine inhibits the conversion of methionine to SAM. When cycloleucine was applied together with SAM, there was no inhibition of SAM stimulation of ethylene production. Therefore, cycloleucine must inhibit the methionine to SAM step. Lombardini *et at.* (1970) showed that cycloleucine inhibits the activities of methionine adenosyltransferase in *E. coli,* rat liver and yeast. In fact, Konze and Kende (1979 b) extracted methionine adenosyltransferase (ATP : methionine S-adenosyltransferase, E.C.2.5.1.6) from the rib segments of flower buds of morning-glory plants (*Ipomoea tricolor* Cav., cv. Heavenly Blue). This enzyme activates methionine in the presence of ATP and forms SAM. ACC applied together with rhizobitoxine and cycloleucine produces more wound ethylene than when ACC was applied alone may be due to rhizobitoxine and cycloleucine suppress malonylation of ACC and more ACC available to the ethylene-forming enzyme and thus increase the ethylene production by tomato disks (Kionka and Amrhein, 1984).

Cycloheximide inhibited wound ethylene production by tomato disks similar to the other inhibitors, but instead of increasing the stimulatory effect of ACC, it inhibited the stimulation (Fig.3). The ability of cycloheximide to inhibit wound ethylene production by both treated

and non-treated tomato disks with ACC indicates that cycloheximide may interfere at more than one site in the biosynthetic pathway. This result also indicates that sustained protein synthesis is a prerequisite for ACC-forming enzyme for wound-induced ethylene biosynthesis (Kende and Boller, 1981; Yu and Yang, 1980). Though Machackova and Zmrhal (1981) reported that 10 mM cycloheximide had no effect on the conversion of ACC to ethylene by wheat coleoptile segments but the result presents here only 0.1 mM cycloheximide was very effective in the inhibition of ACC-stimulated wound ethylene production by tomato disks. In the study, cycloheximide inhibited the ACC to ethylene step may be due to its inhibitory effect on the activity of existing enzyme(s) rather than the inhibition of enzyme induction required for the conversion of ACC to ethylene because enzyme(s) converts ACC to ethylene seems to exist in plant tissues already (Cameron *et al.*, 1979).

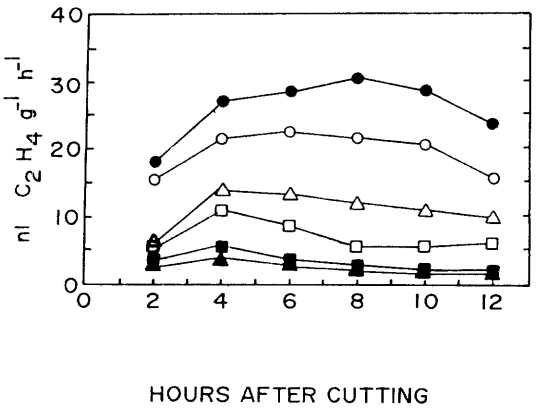

Figure 1. Effect of rhizohitoxine on SAM-and ACC-stimulated wound ethylene production by tomato disks. Twenty ul of H_2O (□—□), 1 mM ACC (o—o), 1 mM SAM (△—△), 1 mM rhizobitoxine (■—■), 1 mM of ACC + rhizo bitoxine (●—●) or 1 mM of SAM + rhizobitoxine (▲—▲) were applied to the cut surface of tomato disks at 0 h after cutting.

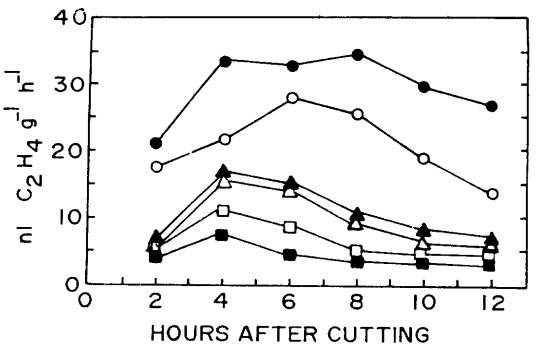

Figure 2. Effect of cycloleucine on SAM-and ACC-stimulated wound ethylene production by tomato disks. Twenty ul of H_2O (□—□), 1 mM ACC (o—o), 1 mM SAM (△—△), 1 mM cycloleucine (■—■), 1 mM of ACC + cycloleucine (●—●) or 1 mM of SAM + cycloleucine (▲—▲) were applied to the cut surface of tomato disks at 0 h after cutting.

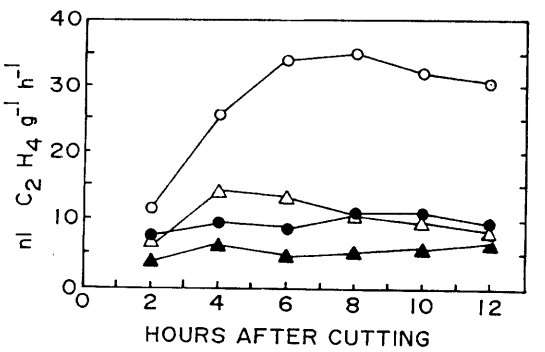

Figure 3. Effect of cycloheximide on ACC-stimulated wound ethylene production by tomato disks. Twenty ul of H_2O (△—△), 1 mM ACC (o—o), 0.1 mM cycloheximide (▲—▲) or 1 mM ACC + 0.1 mM cycloheximide (●—●) are applied to the cut surface of tomato disks at O h after cutting.

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