Ripening of tomato fruit locule gel tissue in response to ethylene

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Received 10 September 1999; accepted 8 March 2000

Abstract

Ethylene production by locule gel tissue excised from immature tomato fruit was inhibited by exposure to 4.5 μmol l⁻¹ C₂H₄, and 1-aminocyclopropane-1-carboxylic acid (ACC) content was reduced. In contrast, CO₂ production, ethylene forming capacity (EFC), red colour development, tissue liquefaction and seed maturation as measured by subsequent germination rate were stimulated by C₂H₄, and, in immature gel, the onset of autocatalytic C₂H₄ production was hastened. The autoinhibition of C₂H₄ production in immature locule gel tissue required continuous exposure to C₂H₄, as transfer to air and then back to C₂H₄ resulted in C₂H₄ production first increasing to control levels, then decreasing again. Locule gel tissue from pink fruit however, responded to C₂H₄ treatment with increased C₂H₄ and CO₂ production, but ACC levels were unchanged. Inhibition of C₂H₄ action by pretreatment with STS inhibited both autoinhibition and autostimulation of C₂H₄ production in immature and mature gel tissues, respectively. These results indicate that there is a transition from a negative to a positive feedback mechanism of C₂H₄ on C₂H₄ biosynthesis in locule gel during ripening. Control of this feedback mechanism, which involves ACC synthesis, may be separate from that of other manifestations of C₂H₄ action. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Tomato locule gel; Ripening; Ethylene; Ethylene feedback

1. Introduction

Tomato fruit is composed of distinct tissue types including pericarp, placenta, septa and locular gel tissues (Brecht, 1987). Locular gel develops prior to ripening of the pericarp and eventually, towards the terminal stage of ripening, exhibits an almost liquid nature (Kader and Morris, 1976). A climacteric rise in C₂H₄ production has also been observed in gel tissue prior to other tissue types (Brecht, 1987).

When tomato fruit are roughly handled during early ripening stages, symptoms of internal bruising may occur that are noticeable during later ripening stages (Sargent et al., 1992). Exposing tomato fruit to such mechanical injury may enhance fruit respiration and C₂H₄ production, this
diminishing 2 days later (Atta-Aly et al., 1987; Atta-Aly, 1992). Although fruit ripening was enhanced under such conditions, fruit gel tissue failed to ripen normally and developed abnormal colour, as indicative of internal bruising (Hatton and Feeder, 1963). It is possible therefore, that tomato locule gel tissue has a unique or different ripening behaviour. In addition, little is known about in vitro ripening of locular gel tissue and in vitro seed maturation.

The present work, therefore, was carried out for the following objectives: to study in vitro ripening of tomato locule gel tissue; to determine the impact of in vitro gel ripening on seed maturation as measured by subsequent seed germination; to examine C₂H₄ feedback mechanisms in locular gel tissue and the response of such tissue to C₂H₄ action.

2. Material and methods

2.1. Plant material

Full size tomato (Lycopersicon esculentum Mill cv. Sunny.) fruits were harvested from a commercial field in south Florida at immature and pink stages. Fruits were transferred to the laboratory on the same day. Upon arrival, fruits were washed with chlorinated water (3.4 mM NaOCl), sorted, re-graded in terms of size and developmental stage, and kept at 15°C and 95% RH overnight. Experiments were repeated three times using fruits from the same source.

2.2. Gel tissue preparation

Immature fruit were those with firm, green gel tissue showing no evidence of red colour development or tissue liquefaction, while pink fruit showed pink or red colour on 30–60% of the fruit surface. The outer pericarp of one of the locules was removed, and the locular gel excised and placed in a sterile Petri dish. A cork borer (1 cm diam.) was then used to excise a gel disc with 1 mm of central placental tissue attached (one disc/fruit). Discs were placed, placental surface down, inside glass tubes (17 ml vol.; 2 cm diam.; one disk/tube) over 0.5 mm glass beads at the bottom of each tube to protect the tissue base from anaerobic conditions, especially when gel liquefaction had occurred in the fruit at pink stage. Tissues were then distributed among chemical solution treatments and exposed thereafter to C₂H₄ using a gas flow system as described below.

2.3. Chemical solution treatments

For each treatment, 100 µl of each solution was applied to the gel surface of the tissue. With the exception of ACC, which was applied 4 days after gas exposure, all solutions were applied immediately after excision. Chemical solutions were applied to the tissues in five replicates with 15 samples/replicate for each of the following measurements.

2.3.1. Treatments

These tissues were divided into three groups. The first group was used for measuring the initial levels of C₂H₄ and CO₂ production, immediately after excision, with an incubation time of 30 min. This incubation time was sufficient for measuring basal C₂H₄ levels and less than that required for wound C₂H₄ to be initiated (Atta-Aly, 1992). The second and the third groups, however, were treated with water and exposed to gas treatments either for monitoring C₂H₄ and CO₂ production 2, 3, 4 and 5 days after excision or for ACC analysis 4 days after excision to eliminate the interference of wound C₂H₄ with tissue ACC levels. Wound C₂H₄ is negligible 2 days after excision (Atta-Aly, 1992). Ethylene and CO₂ production was also measured 8 days after excision, but only for immature excised gel tissue.

2.3.2. Ethylene forming capacity (EFC)

Tissues were treated with water directly after excision and then exposed to gas treatments for 4 days, when water or 100 µM ACC was added to the tissues 2 h before measuring C₂H₄ production, as an indicator of EFC without wounding interference.
2.3.3. \(\text{C}_2\text{H}_4\) action

This was achieved in two different ways as follows:

1. Tissues were treated with water and then divided into two groups. The first group was exposed to the air for 3 days, then transferred to the 4.5 \(\mu\text{mol l}^{-1}\) \(\text{C}_2\text{H}_4\) atmosphere for 1 day and back to air for another day, while the opposite order of exposure was carried out for the second group. \(\text{C}_2\text{H}_4\) production was analyzed at the time of each atmosphere transfer.

2. Silver thiosulfate (STS) at 0.5 mM, as an inhibitor of \(\text{C}_2\text{H}_4\) action, was applied to the tissues while either water or 0.5 mM sodium thiosulfate \((\text{Na}_2\text{S}_2\text{O}_3)\) was the control treatment for STS application. After 4 days of gas treatments, \(\text{C}_2\text{H}_4\) production by the tissues was analyzed.

2.4. Ethylene treatments

Based on the highest respiratory levels of excised tomato gel tissues, measured 1 day ahead, a flow rate system was calculated and adjusted to an air-flow rate of 2 l h\(^{-1}\) to supply normal \(\text{O}_2\) levels around the tissue. \(\text{CO}_2\) levels in the air-flow were checked twice a day and its concentration was always below 0.5% throughout the experimental time. Air, with or without \(\text{C}_2\text{H}_4\), was passed through water twice before passing through the tissue containers (RH 98%).

After chemical solution treatments, tissues from each treatment were divided into two groups, of either air or \(\text{C}_2\text{H}_4\) treatments. Each group was placed inside 10-l gas-flow containers. The containers were kept throughout the experimental time at 20°C and 95% RH. Time between tissue excision and gas exposure for each treatment was less than 30 min.

2.5. \(\text{C}_2\text{H}_4\), \(\text{CO}_2\) and \(\text{ACC}\) analysis

At each time of analysis, the tubes containing tissue were taken out from the gas-flow containers, thoroughly flushed with \(\text{C}_2\text{H}_4\)-free air for 60 s, and then sealed with rubber serum stoppers. Preliminary tests indicated that less than 0.01% of the exogenously applied gas remained in the tissue following the above procedure. After 30 min of incubation at 20°C, 1 ml gas samples were withdrawn from tube head space and used for \(\text{C}_2\text{H}_4\) and \(\text{CO}_2\) measurements. A Hewlett Packard gas chromatograph Model 5080A with FID was used for \(\text{C}_2\text{H}_4\) analysis, while a Gow Mac Model 60 with TCD (GOW Mac Instrument Co., NJ) was used for \(\text{CO}_2\) measurements. After withdrawing the gas samples, the tubes holding tissues were unsealed and returned back to their gas-flow containers.

For \(\text{ACC}\) analysis, fruit gel tissues were removed from their gas-flow containers, frozen in liquid nitrogen and kept at \(-20°C\). Two grams of frozen tissues were homogenized in 10 ml 0.2 mM trichloroacetic acid (TCA) (Atta-Aly et al., 1987). The mixture was centrifuged at 1000 \(\times\) g for 10 min and the supernatant decanted. Aliquots were assayed for \(\text{ACC}\) using a modified version of the procedure used by Lizada and Yang (1979).

2.6. Analysis of gel colour development and seed germination

Colour of the excised gel tissue was measured directly after excision at the gel surface using a Hunterlab ColorQuest Sphere Spectrocolorometer and the Hue angle was calculated as tangent\(^{-1}\) \(\frac{b}{a}\) (Francis, 1970). Seeds were then water-extracted, air-dried and stored in the laboratory at 23°C and 45% RH for the germination experiment.

The same procedures were also carried out for gel tissues excised from immature fruit after 10 days of continuous air or 4.5 \(\mu\text{mol l}^{-1}\) \(\text{C}_2\text{H}_4\) exposure. During the germination experiment, seeds were placed on Whatman No. 1 paper in Petri dishes, irrigated with water as needed, and kept in the dark at 25°C and 95% RH. Seed germination was recorded daily for 7 days, when the highest germination percentage was obtained, and the percentage seed germination was calculated. The germination experiment was carried out in five replicates, each consisting of 100 seeds.

Experiments were designed as factorial arrangements in completely randomized designs with five replicates, each consisting of 15 (locule gel) or 100
(seed) samples. Means were analyzed for statistical significant differences using the LSD test at 5% level (Little and Hills, 1978).

3. Results and discussion

Exposing tomato gel tissue excised from immature fruit to continuous air-flow $\pm 4.5 \mu$mol l$^{-1}$ C$_2$H$_4$ significantly reduced tissue C$_2$H$_4$ production in comparison with that exposed to C$_2$H$_4$-free air. The opposite trend however, was observed when gel tissue excised from fruit at the pink stage was exposed to C$_2$H$_4$ or when excised immature gel tissue showed red colour development after 8 days of continuous C$_2$H$_4$ exposure (Fig. 1). The inhibition of C$_2$H$_4$ production in immature gel is due mainly to the reduction in ACC (Fig. 2), because exogenous C$_2$H$_4$ application induced EFC (Fig. 3) regardless of tissue physiological age. The high level of ACC consumption in gel tissue excised from pink fruit during C$_2$H$_4$ application may be the reason behind the insignificant difference in ACC levels between C$_2$H$_4$ and air-treated gel tissues (Fig. 2), because C$_2$H$_4$ application strongly induced C$_2$H$_4$ production by such tissue (Fig. 1).

Based on these findings, it seems that gel tissue excised from immature tomato fruit exhibits C$_2$H$_4$ autoinhibition while gel tissue excised from pink fruit shows C$_2$H$_4$ autocatalysis. Ethylene autocatalysis was also found in immature excised gel tissue, but after 8 days of excision when red colour had developed under continuous C$_2$H$_4$ exposure (Fig. 1). It is also possible that ACC synthesis is the limiting step in the C$_2$H$_4$ feedback mechanism. These findings are in agreement with

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Fig. 1. Effect of exogenous ethylene treatment on ethylene production by tomato gel tissue excised from immature and pink fruits.

Fig. 2. Effect of exogenous ethylene on ACC levels in tomato gel tissue excised from immature and pink fruits. At each developmental stage, significant differences are presented at the 5% level.

Fig. 3. Effect of exogenous ethylene on in vitro EFC and the impact of inhibiting ethylene action with STS application on ethylene production by tomato gel tissue excised from immature and pink fruits. At each treatment, significant differences are presented at the 5% level.
previous findings (Atta-Aly et al., 2000) which indicated that tomato pericarp tissue shifted from C$_2$H$_4$ autoinhibition to C$_2$H$_4$ autocatalysis with the onset of tissue ripening.

The role of C$_2$H$_4$ action in the C$_2$H$_4$ feedback mechanism in gel tissue was examined by either transferring tomato gel tissues between air and C$_2$H$_4$ atmospheres or by STS application (Beyer, 1976). Negative C$_2$H$_4$ feedback (autoinhibition) at the immature stage or positive C$_2$H$_4$ feedback (autocatalysis) at the pink stage in response to exogenous C$_2$H$_4$ application was diminished upon transferring the tissue to air and re-occurred when either tissue was returned back to C$_2$H$_4$ (Fig. 4). In addition, STS application blocked both C$_2$H$_4$ autoinhibition and autocatalysis in response to exogenous C$_2$H$_4$ application in immature and pink gel tissues, respectively (Fig. 3). None of these findings were observed when either water or Na$_2$S$_2$O$_3$ was applied to the tissue as a control (Fig. 3). Similar findings were also evident when tomato pericarp tissues were used (data not shown), indicating that although tomato gel is polygalacturonase-free tissue (Wallner and Walker, 1975), it behaves in a similar fashion to other types of tomato fruit tissues (Atta-Aly et al., 2000) or to the intact fruit (Sisler and Lallu, 1994; Tian et al., 1997) in terms of its climacteric behaviour and its response to C$_2$H$_4$ action and feedback mechanisms.

Exogenous C$_2$H$_4$ application maintained gel tissue respiration at levels higher than control regardless of tissue physiological age (Fig. 5). This was only noticed when CO$_2$ production was reduced to normal levels by the termination of wound stress 2 days after excision (Atta-Aly, 1992). These findings may suggest that the appearance of C$_2$H$_4$ autocatalysis occurred with the onset of ripening is parallel to the climacteric rise in tomato fruit respiration (Tian et al., 1997).

In addition to the rise in C$_2$H$_4$ and CO$_2$ production by tomato gel tissue with ripening initiation and progress, gel liquefaction and red colour development as well as seed maturation in the excised gel are other features which can be used to test the in vitro ripening of tomato gel (Tigchelaar et al., 1978). After 10 days of continuous exposure to either air or C$_2$H$_4$, immature gel tissue showed more liquefaction with C$_2$H$_4$ exposure than air. Red colour development (Fig. 6) significantly increased in the immature gel after 10 days of continuous C$_2$H$_4$ exposure and reached 85% of the in vivo ripe gel colour value, while air-treated gel reached only 25%. It is possible that the inability of gel tissue to develop normal red colour in fruit with internal bruising may, there-
fore, be due to the destruction of C2H4 receptors or gel tissue membranes, and subsequent blocking of C2H4 action.

On the other hand, application of exogenous C2H4 to excised immature tomato gel for 10 days strongly promoted seed maturation, since the germination percentage of seeds extracted from C2H4-treated gel was significantly higher than those extracted from air-treated ones and was almost equal to those extracted from the in vivo ripe gel (Fig. 6). Air storage alone however, also significantly promoted seed maturation, in comparison with seeds directly extracted from immature gel (Fig. 6). These data indicate that C2H4 promotes seed maturation in excised gel tissue, which suggests that C2H4 may play a pivotal role, not only in the in vitro gel ripening, but also in seed maturation.

References


