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Effects of Abscisic Acid and Benzyladenine on Fruits of Normal and *rin* Mutant Tomatoes[1, 2]

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ABSTRACT

Since ethylene application did not induce ripening in detached fruits of the nonripening mutant *rin* we initiated studies to determine possible involvement of other hormones. We proposed that the lack of ripening in mutant *rin* tomato fruit may result from a lack of abscisic acid or from excessive endogenous levels of cytokinin. Application of abscisic acid (3×10^{-5} M and 10^{-3} M) to detached fruits of a normal strain (*Lycopersicon esculentum* Mill. cv. 'Rutgers') reduced the time to initiate ripening by about 50%. This acceleration of the onset of ripening appeared not to be due to an increased rate of ethylene production. Abscisic acid did not alter respiration or ethylene production or induce ripening in *rin* fruit. Ripening in Rutgers fruit was not influenced by treatment with 6-benzyladenine (4.44×10^{-6} M, 4.44×10^{-5} M or 1.8×10^{-4} M). Fruits of the mutant *rin* showed no response to exogenous BA. However, senescence rates of leaf disks of both Rutgers and *rin* were significantly inhibited by as little as 10^{-7} M exogenous benzyladenine. The results are discussed in relation to previous studies of the physiology of *rin* fruits and it is concluded that endogenous levels of ABA and cytokinins do not account for the lack of ripening in *rin* fruit.

Several plant hormones have been shown to be effective in altering the pattern and/or sequence of the ripening processes in tomato fruits (5, 9). While ABA plays a role in leaf senescence, any such role in altering fruit ripening has been unclear. Applications of exogenous ABA as well as measurements of endogenous levels of this plant hormone in tomato fruit support the thesis that ABA may play a role in the ripening process (5), while cytokinins seem to antagonize ABA (9). Varga and Bruinsma (11) suggested that ripening is progressively retarded at increasing levels of endogenous cytokinins.

It has been established that the morphology of a tomato mutant can be altered by hormone application. Tal and Imber (10) demonstrated that leaves of a wilty mutant of tomato contain only about one-tenth of the extractable ABA found in a normal cultivar. These same investigators were able to alter the morphology of this mutant to a normal appearance of ABA application (4).

This study was conducted to determine whether ABA applications would enhance ripening of normal fruit, and to ascertain whether ABA application might induce a normal ripening pattern in fruit of the *rin* mutant. Secondarily, we wanted to know whether the application of cytokinins might result in inhibition of the ripening pattern of normal tomato fruit, according to the suggestion of Varga and Bruinsma (11).

MATERIALS AND METHODS

Uniform populations of fruits of Rutgers (*Lycopersicon esculentum* Mill.) and of a partially isogenic strain of *rin* were produced in a greenhouse as described previously (2, 6). Fruits were harvested at 80% of development. The upper layers of tissue of each stem scar were carefully removed with a scalpel. The fruits were then transferred to a jar in which 1 ml of solution per 100 g fresh weight of fruit was infiltrated under a vacuum of about 160 mm Hg.

A racemic mixture of *cis,trans*- and *trans,trans*-ABA (Burdick Laboratories, Muskegon, Mich.) was applied at 3×10^{-5} M and 10^{-3} M in 0.1% (v/v) aqueous methanol. The control solution was 0.1% aqueous methanol.

BA at 4.44×10^{-4} M was prepared by autoclaving in distilled H_2O. Concentrations of 1.8×10^{-4} M, 4.44×10^{-5} M and 4.44×10^{-6} M BA were applied to the fruit. Autoclaved distilled H_2O was used as a control.

Each treatment was replicated five times. CO_2 and ethylene evolution rates were measured daily (7). The time of first red color appearance was also noted for each fruit.

Leaf senescence was measured as the rate of Chl breakdown in 7-mm diameter leaf disks taken from the tip leaflet of the newest fully expanded leaf. All procedures were carried out under sterile conditions. Each treatment contained 12 disks which were incubated in the dark at 22 C. Chl was extracted at 6 days after detachment by placing 2 disks into 5 ml of dimethylformamide. Each extraction was replicated 6 times.

RESULTS

ABA at 3×10^{-5} and 10^{-3} M reduced the time to initiate ripening in Rutgers fruit by about 50% as indicated by the onset of the respiratory climacteric and the associated rise in ethylene production and the development of red color (Fig. 1). Treatment with ABA also tended to increase the maximum rates of CO_2 and ethylene production during ripening but these noted differences were not significant. Treatment of *rin* fruits with ABA did not significantly alter the rates of either CO_2 or ethylene production or cause any changes in color (Fig. 2). BA treatment did not effect any significant changes in the time to the onset of ripening or the rates of CO_2 and ethylene production in Rutgers (Fig. 3), nor did

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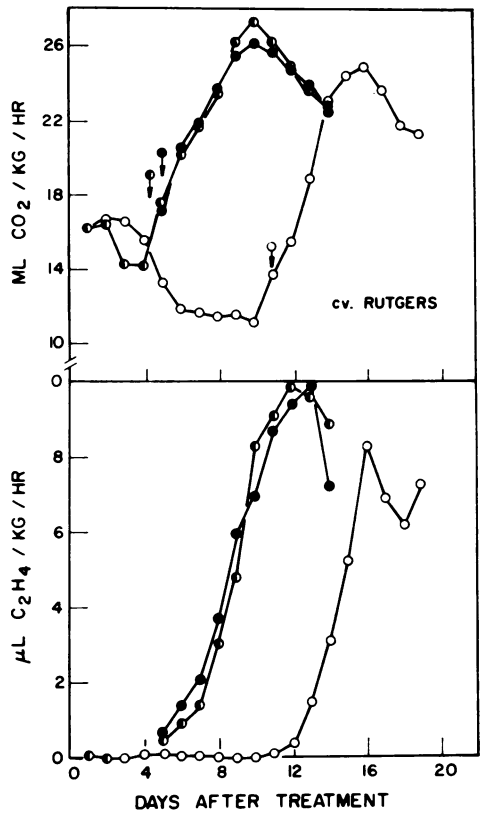

FIG. 1. Effect of ABA treatment on the rate of CO_2 and ethylene evolution by Rutgers fruits. Fruits were harvested at 80% of development (day = 0) and treated with control solution (○——○), 3×10^{-5} M (◐——◐), and 10^{-3} M (●——●) ABA. One ml of solution/100 g of fruit fresh weight was infiltrated into each fruit. To provide a more accurate presentation of the rates of ethylene production during ripening, composite curves were prepared by matching the data for individual fruits in each treatment. The day of the first significant increase in ethylene production was taken as day 1 of ripening. Arrows indicate the first day of red color appearance.

BA treatment alter the physiological behavior of *rin* fruit (Fig. 4). However, when BA was applied to senescing leaf disks of Rutgers and *rin*, Chl breakdown was inhibited by 10^{-7} M BA (Fig. 5).

DISCUSSION

In agreement with Khudairi (5), our data show that ABA accelerated the onset of ripening in a normal strain of tomato (Fig. 1). The magnitude of this acceleration was similar to that obtained by treating fruit of similar maturity with a saturating concentration of ethylene or propylene (3, 7). However, ABA apparently accelerated ripening other than by prolonged stimulation of endogenous ethylene production prior to the onset of the climacteric rise (Fig. 1). It is noteworthy that ABA did not stimulate ethylene production or initiate any ripening process in *rin* fruits. The response of Rutgers fruits to ABA is interesting since it has been shown that fruits of approximately the same maturity as those used in this study contained a high endogenous level of ABA (1). Preliminary studies on endogenous ABA levels in Rutgers fruit (McGlasson, unpublished data) have confirmed previous work (1) and have also shown that developing *rin* fruits accumulate ABA to levels similar to those found in Rutgers.

The thesis that cytokinins inhibit fruit ripening (11) as they do in leaf senescence (9) and that *rin* fruits do not ripen due to high cytokinin concentration has not been supported by our findings. The application of exogenous BA ($1.8 \times 10^{-4} - 10^{-6}$ M)

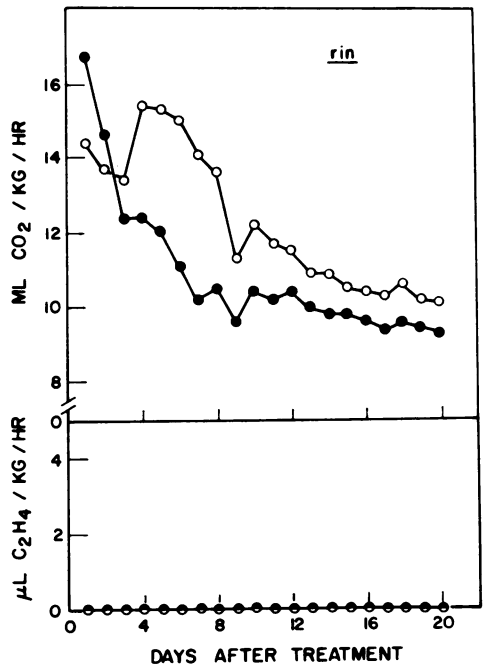

FIG. 2. Effect of ABA treatment on the rate of CO_2 and ethylene evolution by *rin* fruits. The legend is as for Fig. 1.

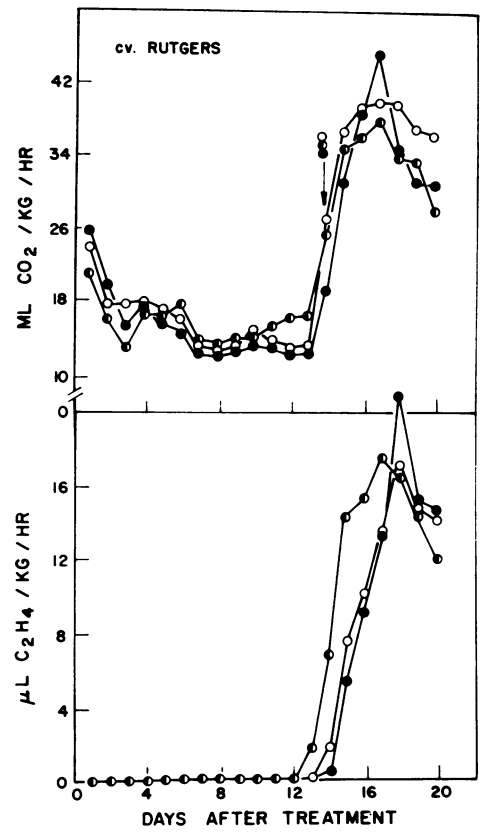

FIG. 3. Effect of BA treatment on CO_2 and ethylene evolution rates from Rutgers fruits. Fruits were harvested at about 80% of development (day 0) and treated with control solution (○——○), 4.44×10^{-6} M BA (◐——◐), and 4.44×10^{-5} M BA (●——●). Results with 1.8×10^{-4} M BA (not shown) were not different. One ml of solution/100 g fruit fresh weight was infiltrated into each fruit. Arrows indicate the first day of red color appearance.

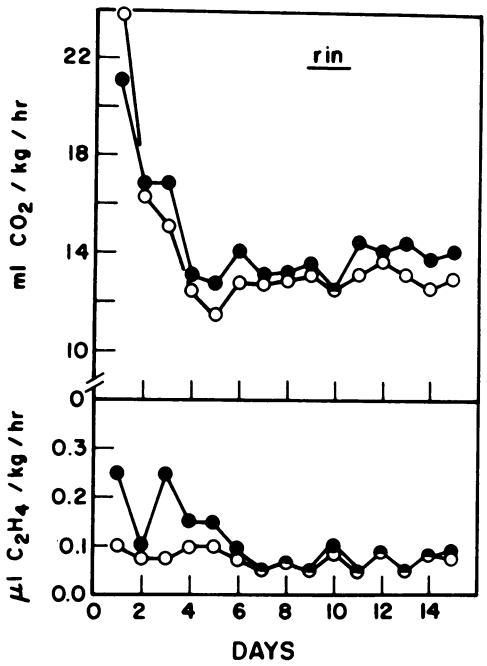

FIG. 4. Effect of BA treatment on CO_2 and ethylene evolution rates from *rin* fruits. The legend is as for Fig. 3.

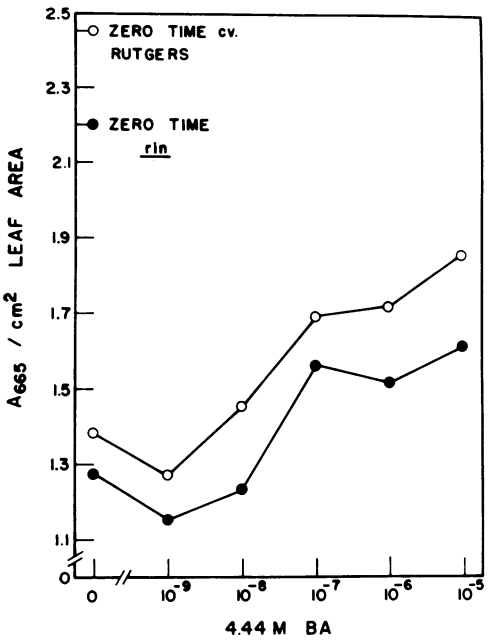

FIG. 5. Effect of BA treatment on Chl content of senescing leaf disks from *rin* and Rutgers strains. Leaf disks were assayed after 6 days at 22 C in the dark.

to fruits of Rutgers and *rin* had no measurable effect on any parameters of their physiological behavior patterns (Figs. 3 and 4), while BA application at 10^{-7} M to leaf disks of these two strains was enough to markedly delay sensecence in these tissues (Fig. 5). If *rin* fruits contain abnormally high levels of cytokinins the onset of ripening in disks of Rutgers fruit tissue transplanted into *rin* fruits would be expected to be retarded. We found that the time to the onset of ripening in Rutgers disks was not altered (8). From these findings we infer that endogenous cytokinin is probably not involved in the initiation of tomato fruit ripening. This seems contradictory to the conclusions of Varga and Bruinsma (11) that increasing levels of endogenous cytokinins retard the rate of ripening of tomato fruits. Their data show that no correlation existed between endogenous levels of cytokinins (Table III in Varga and Bruinsma [11]) and the onset of fruit ripening as measured by days from anthesis to stage 3 of ripening. (Table I in Varga and Bruinsma [11]). While the rate of ripening may be controlled by endogenous cytokinin levels as suggested by these authors, nonetheless cytokinin levels of the seeded fruits were greater by as much as 2 orders of magnitude over cytokinin levels of unseeded fruits; yet the rate of ripening was accelerated by only 1 to 2 days in seeded fruits. We suggest that this magnitude of difference in rate of ripening is not physiologically significant when considered in relation to differences in endogenous cytokinin levels. Furthermore, all data pertaining to endogenous cytokinin levels presented by Varga and Bruinsma (11) were derived from fruits of the same physiological stage of ripening (stage 3). If endogenous cytokinins play a direct regulatory role in the rate of tomato fruit ripening, it is expected that fruits at the same physiological stage would exhibit the same endogenous levels of cytokinins.

The results reported here support our earlier conclusion (8) that the lack of normal ripening in fruit of the *rin* mutant is probably due to the lack of an untranslocatable ripening factor or to the presence of an untranslocatable ripening inhibitor.

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