Association between Elemental Content and Fruit Ripening in *rin* and Normal Tomatoes

Received for publication July 19, 1977 and in revised form February 5, 1978

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ABSTRACT

Analysis of Ca and other inorganic ions in the pericarp of *rin*, a nonripening mutant, and normal tomato (*Lycopersicon esculentum* Mill) fruits revealed significant differences in their accumulations at advanced stages of fruit development. During early stages of fruit development, soluble Ca was higher in Rutgers and there were no detectable changes in the accumulation patterns of the other inorganic ions. In the mutant *rin*, bound Ca continued to increase with age and it was twice as high as compared to earlier stages. In the normal tomato, bound Ca decreased about 3-fold at later stages of development. Mg and Mn also showed some changes similar to Ca. K continued to increase with age and the mutant *rin* had lower levels than Rutgers throughout development. Other ions such as P, Zn, Cu, and Co were similar in the mutant and normal fruits. These results are interpreted as indicating that high levels of bound divalent cations in the mutant *rin* may be associated with an altered membrane and cell wall and play a role in fruit ripening.

*rin* was first described as a spontaneous mutation in 1968 (21). Since then, the mutant has been used as a tool for the study of fruit ripening and ethylene biosynthesis (7, 18). Even though levels of internal ethylene in attached 45 to 75% mature fruits of the *rin* and normal tomatoes are similar (12), fruits of the mutant monitored for up to 120 days after harvest show little or no change in ethylene or CO₂ production; ethylene production is not stimulated by treatment with ethylene or propylene, while stimulation of CO₂ production persists only in the presence of the exogenous olefins (7).

Inorganic solutes have been reported to play structural and regulatory functions in different physiological systems. Divalent cations contribute to membrane stability by altering the structure of the membrane (13, 24). For example, Jones and Lunt (8) in their review pointed out that electron microscope studies have shown extensive disintegration of mitochondria, ER, and cytoplasmic membranes in various Ca-deficient plants. Ca can also delay ethylene-stimulated leaf abscission and senescence (15), enhance the effect of GA and cytokinin of leaf senescence (15), and decrease the ethylene stimulation of the swelling index in etiolated peas (10). In climacteric fruits, Ca treatment depressed ethylene and CO₂ production during ripening (6, 25). Hence, this investigation was initiated to determine the levels of calcium and other inorganic solutes in normal and *rin* tomatoes.

MATERIALS AND METHODS

Rutgers and isogenic *rin* tomatoes (*L. esculentum* Mill) were grown in greenhouses in a mixture of soil, vermiculite, and peat moss in the ratio of 2:1:1 (v/v), respectively. Plants were drip-irrigated for 5 min daily, using a complete Hoagland nutrient solution (full strength) alternated with plain water throughout the growing season. Day (25 C) and night temperatures (19 C) were also maintained. Plants were trained to one stem, and fruits tagged at the time of anthesis allowing for two to three fruits/flower cluster and a maximum of seven to 10 fruits/plant.

Composite samples of three fruits each representing the two varieties at four different ages (30, 40, 50, and 60 days) after anthesis were picked at random. Fruits were cleaned with deionized water. The pericarp was separated from the jelly and the seeds, blotted dry, and homogenized in a Waring Blender in a cold room (4 C). Representative samples were freeze-dried and finely pulverized to pass through a 40-mesh screen. Pulverized samples were shaken after addition of distilled H₂O for 1 hr and centrifuged at 15,000g for 15 min. The supernatant was filtered using Whatman No. 541 ashless filter paper and the filtrates were analyzed for soluble Ca, K, Mg, and P. The pellet was washed three times with distilled H₂O and the supernatant was discarded. The pellet was ashed at 550 C overnight, dissolved in 5% HCl, and used to determine the bound Ca, P, and Mg. For the determination of Cu, Zn, Mn, and Co, freeze-dried samples were ashed directly.

Ca and K were analyzed by flame photometry, and Mg, Zn, Cu, and Mn were determined by flame atomic absorption. Flameless atomic absorption was used for Co determination. P was determined by colorimetry (1) using ammonium molybdate and hydroquinone. Results are presented on dry wt basis with the standard deviations.

RESULTS

Soluble Ca was significantly higher in Rutgers, especially at 50 days (Fig. 1A), the anticipated time of ripening in normal tomatoes. Bound Ca contents in both varieties were not different at 30 and 40 days of age (Fig. 1B). Between 40 and 50 days, bound Ca in the Rutgers dropped significantly and remained at a low level, while in *rin* a significant increase was observed. The total Ca contents (Fig. 1C) were similar in both varieties at early stages, but greatly increased in *rin* after 50 days of anthesis.

In Rutgers fruits, the soluble Mg contents at 30 and 40 days were similar to that of *rin*, but they had significantly increased by 50 and 60 days and were significantly higher than those of *rin* (Fig. 2A). Soluble Mg in *rin* did not change throughout development. The bound Mg contents (Fig. 2B) were higher in *rin* than in Rutgers. Bound Mg contents of Rutgers significantly decreased at later stages of fruit development. Total Mg followed a pattern similar to that of the bound Mg.

Levels of soluble K increased with age in both varieties (Fig. 3). At early stages, *rin* and Rutgers were similar in soluble K contents, but the levels were higher in the Rutgers at advanced stages of fruit development.

Phosphorous levels, both bound and soluble, and total Co, Cu,
Figs. 1. A–C: Ca content of rin (●) and Rutgers (○) fruits at 30, 40, 50, and 60 days after anthesis. A: soluble; B: bound; C: total Ca in pericarp tissue (dry wt basis).

and Zn were similar in rin and Rutgers fruits at all stages of development.

**DISCUSSION**

The rin mutant contains higher levels of bound Ca during advanced stages of fruit development compared to normal fruits. These results are suggestive of better preserved membranes which may contribute to the delay in senescence observed in rin mutant. Ca has been reported to maintain the integrity of membranes (6, 8, 13, 14) and delay senescence (14, 25). The mutant failed to show any increase in membrane permeability during fruit development and maturation (18) which normally occurs during ripening (2, 22) of fruits. In apple fruits, respiration was inversely related to Ca content of the flesh (6) while in avocado fruits, treatment with Ca depressed respiration during both preclimacteric and climacteric phases (25).

Firmness of tomato fruits is related to the nature of pectic substances (5) associated with Ca (11). In tomato leaves, Ca deprivation decreased the synthesis of radioactive cell wall polysaccharides (20) while an increase in Ca stimulated the intense formation and outflow of pectic substances from the leaves into the fruits (23). At the same time, activity of pectolytic enzymes was reduced (23). Hence, the lack of polygalacturonase activity in rin (3, 19) and the higher levels of bound Ca at the advanced stages of fruit development may contribute to stronger cell walls as compared to Rutgers. In contrast, Ca in Rutgers was solubilized during ripening contributing to the large drop in bound Ca and the concomitant rise in the soluble form. These results correlate with the increase in polygalacturonase activity of Rutgers tomatoes during ripening (19).

Our results also indicated no significant differences in Co, Cu, and Zn content in fruit tissues between these two varieties. This
excludes the possibility of a heavy metal (4) deficiency contributing to the reduced levels of ethylene production reported in the mutant (7). The possibility of Co as an antiethylene agent (9) in rin is also ruled out.

Our special interest in the solute alteration of membrane functions lies in the area of hormonal action; if hormones act through binding to a site of action on a membrane, then solute alterations of membrane thickness (13), permeability (16), and other characteristics might be expected to alter hormonal effectiveness (10, 17). Earlier reports have indicated that the effectiveness of each of the five known plant hormones is altered by Ca and other cations (10) including the binding of auxin to the membrane (17).

Acknowledgments—The authors wish to thank M. E. Patterson and C. G. Woodbridge for their assistance in various aspects of this research.

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