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CHANGES IN FREE GALACTOSE, *MYO*-INOSITOL AND OTHER MONOSACCHARIDES IN NORMAL AND NON-RIPENING MUTANT TOMATOES

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Key Word Index—*Lycopersicon esculentum*, Solanaceae, tomato fruit, *nor* and *rin* mutants, monosaccharides, development

Abstract—Using high-resolution GC, changes in total free galactose, *myo*-inositol, arabinose, xylose, rhamnose and mannose have been studied in pericarp tissue from ripening (cv Heinz 1350 and Rutgers) and non-ripening mutant (*rin* and *nor*) tomato fruit Free galactose increased 3.8–6.3 times in normal ripening lines but not in the mutants. The amount of free *myo*-inositol in the mutant fruit declined after maturity, whereas the content decreased and then increased slightly in ripening tomatoes Arabinose, xylose, rhamnose and mannose either decreased slightly or did not change significantly in the four tomato lines during the developmental stages studied. Thus, the increase in free galactose in ripening tomatoes is apparently not simply the result of a general increase in soluble cell wall-related sugars

INTRODUCTION

A substantial loss of galactose and arabinose residues occurs from cell walls of ripening tomato fruit [1, 2]. Similar losses also occur from cell walls of other fruits during ripening and senescence [3–6]. Although the decrease in tomato cell wall galactan seems to result from a reduced rate of *de novo* galactan synthesis [7], the mechanism by which neutral sugar polymers are solubilized and the fate of the released material remain unclear [8, 9].

In tomato fruit, a large increase in free galactose was detected during ripening using galactose dehydrogenase (D-galactose:NAD^+ 1-oxidoreductase; EC 1.1 1.48) from *Pseudomonas fluorescens* as an assay system [8]. The increase in free galactose did not occur in non-ripening mutant tomato fruit. Although the source of the free galactose is unknown, evidence suggests that it may result from the progressive inability of tomato pericarp tissue to metabolize galactose residues released from cell walls during ripening [8].

Although commercial galactose dehydrogenase from *P. fluorescens* will not oxidize D-galactose-6-phosphate, D-galactosamine, D-galacturonic acid, D-xylose, D- or L-glucose, D-ribose, or D-mannose, it will react with L-arabinose [Sigma Chemical Co, personal communication]. Therefore, a more specific determination of the amount of free galactose in tomato fruit during ripening is essential to understanding the metabolism and significance of this monosaccharide, which is normally toxic to plant cells [10–15].

The objective of this study was to estimate, by high-resolution GC, the amount of free galactose and other cell wall-related neutral monosaccharides in tomato fruit during ripening. In this paper, the concentrations of free galactose, arabinose, xylose, rhamnose, mannose, and of *myo*-inositol, an important precursor for cell wall biosynthesis, in normal and non-ripening mutant tomatoes are reported.

RESULTS AND DISCUSSION

Free galactose was detected in normal and non-ripening mutant tomato fruit by high-resolution GC (Fig. 1). These data substantiate a previous report on the detection of free galactose in tomatoes which had utilized an enzymatic assay for galactose [8]. The amount of free galactose in 'Heinz 1350' and 'Rutgers' fruit increased 3.8 and 6.3 fold, respectively, during ripening (Fig. 1A) However, the free galactose content of *rin* and *nor* fruit remained constant between 28 and 54 days post-pollination (Fig. 1B). Although the source(s) of the free galactose is unknown, one possibility is that it results from cell wall galactan turnover. Its increase in ripening tomatoes may be a result of their progressive inability to metabolize solubilized galactose [8]. In non-ripening

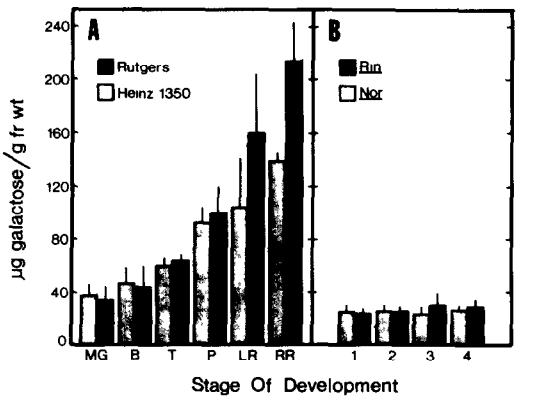

Fig. 1. The amount of free galactose in pericarp tissue from normal (A) and non-ripening mutant (B) tomato fruit during development Developmental stages are defined in the Experimental Each value represents the mean of six replicate fruits Vertical lines at the top of each bar represent the s d

mutant fruit, free galactose may be kept at a low level due to its continued ability to metabolize this sugar. Further studies using radioactive cell wall precursors are necessary to determine if the free galactose that accumulates in ripening tomato fruit tissue is from the catabolism of cell wall polymers.

In contrast to free galactose, the amounts of free arabinose, rhamnose, mannose and xylose did not increase significantly in tomato tissue during ripening (Table 1) It is interesting that free arabinose content did not increase in ripening tomatoes, since arabinose, like galactose, is a cell wall component that decreases during ripening [1, 2] Possibly the degree to which these cell wall components are solubilized may differ. Ahmed and Labavitch [3] found that essentially all of the arabinose released from the cell walls of ripening 'Bartlett' pears could be recovered in polymeric form in 80% ethanol-insoluble fractions from tissue homogenates. However, although the galactose content of pear fruit cell walls decreased 50% during ripening, only a small amount of polymeric galactose was present in 80% ethanol-insoluble fractions [3] Possibly a larger portion of the solubilized galactose was present in a monomeric form. Thus, the galactan portion of some fruit cell walls may be primarily hydrolysed into its monomeric constituents, whereas the arabinose component may be hydrolysed only into smaller polymers As in normal ripening fruit, the amounts of free arabinose, rhamnose, xylose and mannose either decreased slightly or did not change significantly in *rin* and *nor* fruits during the developmental stages tested (Table 2).

Because *myo*-inositol is an important precursor in the biosynthesis of many cell wall polysaccharides [16–18], the amount of this monosaccharide in tomato fruit was studied A small difference in the change in amount of free *myo*-inositol between ripening and non-ripening tomatoes was evident (Fig. 2) In 'Heinz 1350' and 'Rutgers' tomatoes, the amount of *myo*-inositol decreased until the turning stage and then increased slightly However, *myo*-inositol levels decreased steadily until Stage 3 in *nor* and *rin* fruit. In general, the total amount of free *myo*-inositol was higher in the non-ripening mutant fruit than in the

Table 1 Amount (μg/g fr wt) of free rhamnose, arabinose, xylose and mannose in ethanolic extracts of pericarp tissue from 'Rutgers' and 'Heinz 1350' tomatoes during ripening

Line	Stage*	Rhamnose	Arabinose	Xylose	Mannose
Rutgers					
	MG	77 ± 50	292 ± 196	37 ± 9	102 ± 61
	B	81 ± 50	355 ± 222	49 ± 11	92 ± 58
	T	101 ± 81	336 ± 277	57 ± 33	98 ± 66
	P	80 ± 60	327 ± 196	89 ± 13	119 ± 42
	LR	89 ± 55	331 ± 190	91 ± 20	152 ± 62
	RR	71 ± 50	356 ± 257	83 ± 30	127 ± 54
Heinz 1350	MG	20 ± 14	307 ± 103	58 ± 9	162 ± 66
	B	21 ± 10	304 ± 106	52 ± 16	155 ± 96
	T	31 ± 19	296 ± 138	55 ± 21	114 ± 49
	P	20 ± 10	290 ± 65	61 ± 18	179 ± 121
	LR	24 ± 15	326 ± 106	65 ± 27	156 ± 85
	RR	17 ± 11	349 ± 88	57 ± 17	156 ± 47

*Ripening stages are defined in the Experimental Values represent the mean ± s d of six replicate fruits

Table 2 Amount (μg/g fr wt) of free rhamnose, arabinose, xylose and mannose in ethanolic extracts of pericarp tissue from *rin* and *nor* mutant tomatoes during development

Mutant	Stage*	Rhamnose	Arabinose	Xylose	Mannose
Nor					
	1	19 ± 7	378 ± 64	43 ± 26	193 ± 114
	2	15 ± 4	281 ± 44	39 ± 24	159 ± 72
	3	13 ± 5	270 ± 39	47 ± 22	192 ± 96
	4	12 ± 5	210 ± 32	41 ± 19	147 ± 61
Rin					
	1	29 ± 5	247 ± 45	64 ± 45	123 ± 51
	2	26 ± 14	231 ± 81	62 ± 34	119 ± 71
	3	19 ± 8	209 ± 83	64 ± 36	101 ± 47
	4	18 ± 10	208 ± 90	57 ± 28	106 ± 54

*Stages of fruit development are defined in the Experimental Values represent the mean ± s d of six replicate fruits

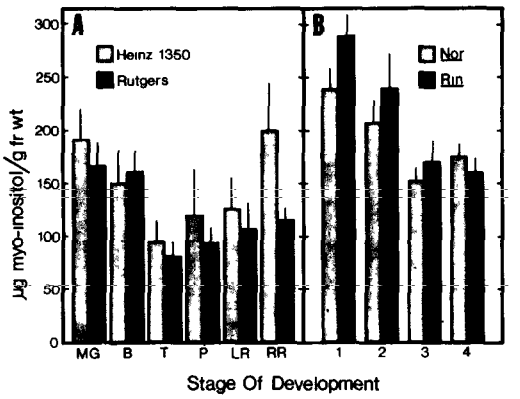

Fig 2 The amount of free *myo*-inositol in pericarp tissue from normal (A) and non-ripening mutant (B) tomato fruit during development Developmental stages are defined in the Experimental. Each value represents the mean of six replicate fruits Vertical lines at the top of each bar represent the s d

ripening fruit. The fact that *myo*-inositol is present in tomato fruit throughout ripening suggests that the apparent decrease in cell wall synthesis that occurs [7] is not due to a lack of availability of *myo*-inositol for wall polymer synthesis. However, the significance of its differing amounts in ripening and non-ripening tomatoes is unclear. In addition to its importance as a cell wall precursor [16–18], free *myo*-inositol also serves as a junction between cyclitol biosynthesis and hexose metabolism via *myo*-inositol-1-phosphate [16]. *Myo*-inositol is also an intermediate in the biosynthesis of phytic acid, IAA esters and their glycosides and phospholipids [16]. Clearly, further studies on the carbohydrate interconversions that occur during fruit ripening are necessary to more fully understand the relationship between cell wall-related carbohydrate metabolism and senescence

EXPERIMENTAL

Plant material. Tomato (*Lycopersicon esculentum* Mill) plants were grown in a greenhouse 'Rutgers' and 'Heinz 1350' fruit were harvested at various stages of coloration and sorted into the following maturity classes: MG, mature green, B, breaker, T, turning, P, pink, LR, light red, RR, red-ripe In order to obtain *nor* (non-ripening, isogenic to 'Heinz 1350') and *rin* (ripening inhibitor, isogenic to 'Rutgers') mutant fruit [19] at known stages of development, flowers were hand pollinated and tagged at anthesis. Fruits were harvested and sorted into maturity classes based on the number of days after pollination as follows Stage 1, 28–32, Stage 2, 36–42, Stage 3, 44–49, Stage 4, 50–54 Normal tomatoes reached the MG and RR stages at *ca* 32 and 50 days post-pollination, respectively Fruits were harvested from groups of 18 plants and stored at −70° for up to 2 months prior to extraction

Extraction Outer pericarp tissue (10 g) was excised from each of six replicate fruit that had first been partially thawed and peeled Each 10 g sample was placed in 15 ml 80% EtOH, boiled for 10 min and homogenized for 1 min (Polytron homogenizer). Homogenates were centrifuged at 27 000 *g* for 15 min and the pellets washed with 5 ml 80% EtOH After re-centrifugation, the two supernatants from each sample were combined and stored at −20° for 16–18 hr EtOH-insoluble polysaccharides that had ppted were removed by centrifuging the extracts at 27 000 *g* for 15 min The extracts were filtered through a column containing 1-ml layers of Dowex 1-X8 and Dowex 50 exchange resins [20]. After washing the column with 80% EtOH, the extracts were made up to 30 ml Aliquots (5 ml) of each sample were placed in vials and evaporated to dryness at 40° in a stream of N_2 Aldononitrile acetate derivatives of the 80% EtOH-soluble sugars were made according to the procedure of ref [21]

GC Aldononitrile acetates were separated and quantified using a single FID instrument equipped with a 12 5 m WCOT fused silica capillary column (0 2 mm i d) coated with OV 101 He was used as carrier gas at a flow of 1 ml/min The oven temp was programmed at 130° for 9 min then increased to 160° at 10°/min After 3 min, the temp was increased to 190° at 3°/min The injection and detection port temp were 200° and 275°, respectively An injection split ratio of 100 1 was used R_fs for sugars from a representative analysis were rhamnose, 10 29 min, arabinose, 11 23 min, xylose, 11 73 min, mannose, 17 21 min, glucose, 17 82 min, galactose, 18 59 min, *myo*-inositol, 21 76 min and dulcitol, 22 29 min The instrument was equipped with an integrator which calculated individual sugar response factors, peak areas and the amount of each sugar in the sample Dulcitol was used as int standard, for calibration, a sugar-standard sample was derivatized and run with each set of samples Triplicate injections were run for each sample

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