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COMPOSITIONAL CHANGES IN CELL WALL POLYSACCHARIDES FROM CHILLED AND NON-CHILLED CUCUMBER FRUIT

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Key Word Index—*Cucumis sativus*; Cucurbitaceae; cucumber; chilling injury; carbohydrates; galactose; galacturonic acid.

Abstract—Cell wall carbohydrate composition and 1-aminocyclopropane-1-carboxylic acid (ACC) content have been determined in chilled (2.5°) and non-chilled (12.5°) cucumber fruit. The major compositional change that accompanied the increased capability for ACC synthesis during chilling was a diminished loss of galactose residues, relative to the loss which occurred at 12.5°. However, the loss of galactose residues increased markedly when fruit were transferred from 2.5° to 20°, and wall galactose levels eventually declined to similar levels in both chilled and non-chilled fruit. Rhamnose, arabinose, xylose, mannose and cellulose content of walls was similar in chilled and non-chilled fruit and did not change substantially upon transfer of fruit to 20°. Upon transfer of chilled fruit from 2.5° to 20°, an increase in the relative amount of galacturonic acid in cell walls occurred; this change did not occur in non-chilled fruit. Thus, chilling stress results in a rapid change in the neutral sugar and galacturonic acid composition of cell wall pectic polysaccharides upon warming.

INTRODUCTION

Stimulation of ethylene production is often observed after exposure of chilling-sensitive plants to low temperatures [1, 2]. Wang and Adams [3] showed that chilling-induced ethylene production by cucumbers occurs via the same pathway as in ripening fruit and involves an increased capacity of fruit to synthesize 1-aminocyclopropane-1-carboxylic acid (ACC). They further demonstrated that the increase in endogenous ACC content involves an increase in ACC synthase activity, possibly through an induction of *de novo* synthesis [4]. The increase in endogenous ACC during low temperature stress provides an effective index for chilling exposure in cucumbers [5].

Although various chilling injury symptoms may be partially related to cell wall integrity and metabolism, such as internal breakdown, surface pitting, growth inhibition and decay, little research has been conducted on cell walls in relation to chilling stress. Fukushima *et al.* [6] concluded that chilling injury in cucumber fruit is a type of water-stress injury and that water-stress injury only occurs in fruits with 'rigid' cell walls. It was further suggested that a de-esterification of pectin and increase in polymeric (hot-water-insoluble) pectin during low temperature stress may result in cell walls with greater rigidity, leading to susceptibility to chilling [7, 8].

In an effort to more clearly establish changes in the cell wall during chilling injury, we have examined the carbohydrate composition of cucumber cell walls during low temperature stress and have used endogenous ACC content as an index of chilling exposure.

RESULTS AND DISCUSSION

As in previous studies [3, 4], the endogenous level of ACC in skin tissue from chilled cucumber fruit increased rapidly after transfer from 2.5° to 20° (Fig. 1). The effect of chilling was evident within 24 hr at 2.5°. The level of ACC increased from 0.2 to 1.9 nmol/g fr. wt during this period, indicating that rapid physiological changes in cucumbers were induced within 24 hr of exposure to chilling stress. The synthesis of ACC during warming continued to increase with increasing length of chilling, up to 8 days. Levels of ACC in non-chilled fruit were low and showed little change during 12 days at 12.5° (Fig. 1). The level of ACC in chilled tissue which was not warmed for 6 hr prior to analysis remained low and did not increase during the

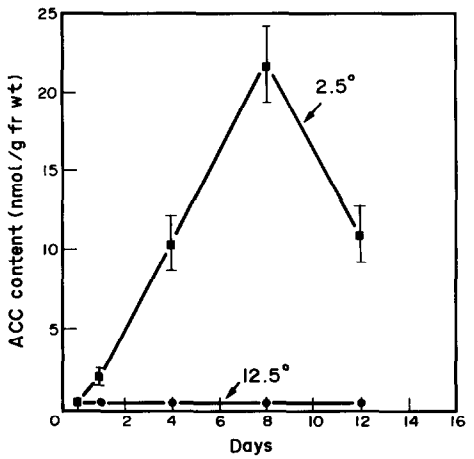

Fig. 1. ACC content of skin tissue from cucumbers held at chilling (2.5°) and non-chilling (12.5°) temperatures for up to 12 days. Fruit were warmed at 20° for 6 hr prior to analysis. Data points represent the mean ± s.d.

12 day period at 2.5° (data not shown). These data confirm previous studies which showed that stimulation of ACC synthesis was a response to chilling and that the increase in endogenous ACC level was associated with increasing severity of chilling injury.

The total non-cellulosic neutral sugar content of cell walls from cucumbers decreased substantially when fruit were held at both 2.5° and 12.5° (Table 1). The loss of neutral sugar residues from cell walls during storage at a non-chilling temperature is contrary to a recent study on cell wall composition during cucumber fermentation [9]. The difference in results is probably due to the fact that in the previous study, fruit were held at 10° for 4 days prior to analysis. In the present study, a large decrease in neutral sugar content occurred during the first 4 days after harvest (Table 1 and Fig. 2).

Similar to the change in ACC level, the effect of chilling on non-cellulosic neutral sugar composition of cell walls was evident within 24 hr of chilling exposure (Table 1). The decrease in total neutral sugar content was reduced from 11.5% in non-chilled tissue to 7% in chilled tissue within 1 day of exposure. The difference became larger with increasing length of chilling. Substantially larger amounts of neutral sugar were retained in walls at 2.5° (23.3 ± 3.1 mg/100 mg wall) than at 12.5° (15.4 ± 2.9 mg 100 mg/wall) after 12 days.

Unlike the increase in ACC content during warming, total cell wall neutral sugar in chilled tissue remained the same 6 hr after transfer from 2.5° to 20° (data not shown); changes in neutral sugar composition occurred during chilling, rather than during warming. After transfer of cucumber fruit to 20°, following 12 days at 2.5° or 12.5°, the total cell wall neutral sugar content of fruit from both temperatures stabilized at 13% (Table 1).

The loss of neutral sugar residues from walls involved primarily galactose-containing polysaccharides (Fig. 2).

Table 1. Total non-cellulosic neutral sugar composition of cell walls from cucumbers held at chilling (2.5°) or non-chilling (12.5°) temperatures for 12 days and then transferred to 20° for 3 days

Storage condition	Time (days)	Total non-cellulosic neutral sugar	% decrease from day 0
		(mg/100 mg wall*)	
Control	0	31.4 ± 5.4	—
Chilled			
2.5°	1	29.2 ± 4.9	7.0
	4	28.5 ± 2.9	9.2
	8	26.3 ± 3.7	16.2
	12	23.3 ± 3.1	25.8
20°	13	16.6 ± 2.9	47.1
	14	12.6 ± 2.1	60.0
	15	13.6 ± 2.9	56.7
Non-chilled			
12.5°	1	27.8 ± 2.6	11.5
	4	19.6 ± 2.8	37.6
	8	16.5 ± 2.2	47.5
	12	15.4 ± 2.9	51.0
20°	13	13.9 ± 2.8	55.7
	14	12.8 ± 2.7	59.2
	15	12.6 ± 2.5	60.0

*Data represent the mean of three analyses ± s.d.

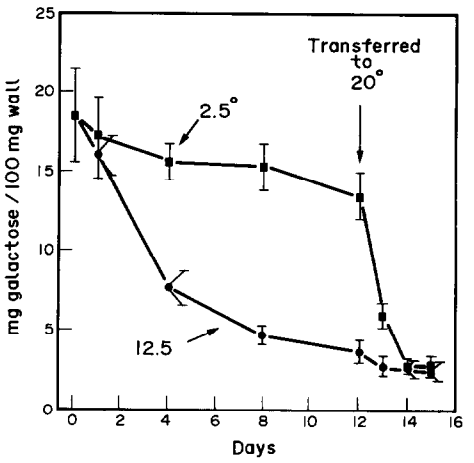

Fig. 2. Galactose content of cell walls from cucumbers held at chilling (2.5°) and non-chilling (12.5°) temperatures for 12 days and then transferred to 20° for 3 days. Results from fruit which were warmed for 6 hr prior to analysis were similar to fruit analysed immediately after removal from storage. Thus, only data for unwarmed fruit are shown. Data points represent the mean ± s.d.

The loss of galactose residues occurred at a much greater rate in non-chilled fruit than in chilled fruit. The difference was apparent after 1 day and became more pronounced as storage progressed. Whether or not the reduced loss of galactose from the wall is a physiological response of cucumber fruit to chilling stress is not clear. The loss of galactose residues in chilled fruit increased sharply after transfer from 2.5° to 20°, subsequent to 12 days chilling, and wall galactose content eventually declined to similar amounts in fruit held at both temperatures (Fig. 2). The change in galactose loss from walls of chilled fruit after transfer from 2.5° to 20° is a response to temperature and not a sign of recovery from chilling injury since the fruit had developed irreversible symptoms of chilling injury, i.e. pitting and shriveling (data not shown).

A loss of galactose residues occurs from cell walls during the ripening of tomatoes [10, 11] as well as other fruit [12]. However, the significance and metabolism of galactose-containing polysaccharides during ripening remains to be established. In tomatoes, the loss of galactose residues seems to be related to an altered rate of cell wall polysaccharide turnover; *de novo* galactan synthesis is diminished [13] while structural galactan is hydrolysed by β-galactosidase [14]. Alternatively, the loss of neutral sugar residues (arabinose) during the ripening of pears is apparently due to the action of polygalacturonase through cleavage of α-1,4-galacturonosyl linkages of a pectic polysaccharide rich in arabinose-containing side chains [15]. Because of the lack of information on cucumber fruit cell wall structure and metabolism, the nature of the difference in rates of galactose residue loss from walls of chilled and non-chilled fruit cannot yet be established. It seems likely that the diminished loss of galactose residues from the cell walls of chilled fruit is a temperature-related inhibition of wall-degrading enzyme activity.

The content of rhamnose, arabinose, xylose and mannose in cell walls was similar in fruit held at 2.5° and 12.5°

Table 2. Rhamnose, arabinose, xylose, mannose and non-cellulosic glucose content of cell walls from cucumbers held at chilling (2.5°) or non-chilling (12.5°) temperatures for 12 days and then transferred to 20° for 3 days

Storage condition	Time (days)	Neutral sugar (mg/100 mg wall)*				
		Rha†	Ara	Xyl	Man	Glc
Control	0	1.0±0.2	2.0±0.7	5.3±1.2	1.4±0.2	3.2±0.1
Chilled						
2.5°	1	0.9±0.3	2.0±0.6	5.0±1.2	1.2±0.2	3.0±0.2
	4	0.9±0.3	2.0±0.5	5.0±0.9	1.4±0.1	3.6±0.1
	8	1.0±0.3	1.9±0.7	4.6±1.0	1.2±0.1	2.2±0.2
	12	0.8±0.2	1.3±0.2	4.5±1.0	1.3±0.1	1.9±0.1
20°	13	1.2±0.4	1.8±0.5	4.5±1.2	1.2±0.1	1.9±0.1
	14	1.0±0.3	2.0±0.5	4.3±1.1	1.1±0.1	1.4±0.1
	15	1.1±0.3	2.3±0.7	4.7±1.4	1.2±0.2	1.6±0.1
Non-chilled						
12.5°	1	0.8±0.2	1.9±0.5	5.2±0.9	1.4±0.1	2.5±0.1
	4	1.0±0.3	1.5±0.4	5.5±1.2	1.5±0.2	2.4±0.1
	8	1.0±0.3	1.6±0.5	5.5±1.3	1.4±0.1	2.0±0.1
	12	0.9±0.3	2.0±0.6	5.5±1.5	1.4±0.2	1.8±0.1
20°	13	1.0±0.3	1.8±0.6	5.2±1.5	1.3±0.1	1.8±0.2
	14	1.0±0.3	1.7±0.6	4.7±1.4	1.1±0.1	1.6±0.1
	15	1.0±0.3	1.8±0.6	4.6±1.2	1.1±0.1	1.5±0.1

*Data represent the mean of three analyses ±s.d.

†Neutral sugar abbreviations: Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Glc, non-cellulosic glucose.

and their relative levels did not change significantly during storage (Table 2). Although the content of non-cellulosic glucose decreased in fruit held at both temperatures (Table 2), it is hard to assess whether this represents a true decrease in wall composition or rather is due to a small, residual amount of starch in wall preparations which decreased during storage. Cellulose content of walls from fruit at both chilling and non-chilling temperatures was similar and ranged from 29.1 to 45.9 mg/100 mg wall; no consistent changes in content were observed.

During storage at 2.5° or 12.5°, no difference in galacturonic acid content of cell walls from chilled and non-chilled cucumbers was evident (Fig. 3). However, upon transfer of chilled fruit to 20°, an increase in the relative amount of galacturonic acid in cell walls occurred. This large change did not occur in fruit which had been held at 12.5°, although a small increase was observed.

It is striking that the content of galacturonic acid in cell walls of chilled cucumber fruit increased upon warming, while galactose, which is a component of pectic polysaccharides, declined. Pitting is the initial symptom of chilling injury in cucumbers and usually develops rapidly upon warming of chilled fruit. Whether or not this is the result of the cell wall changes observed in this study is not known. Nevertheless, the results of this study show that chilling results in a rapid and dramatic change in the composition of pectic polysaccharides in cell walls upon transfer of fruit to warm temperature.

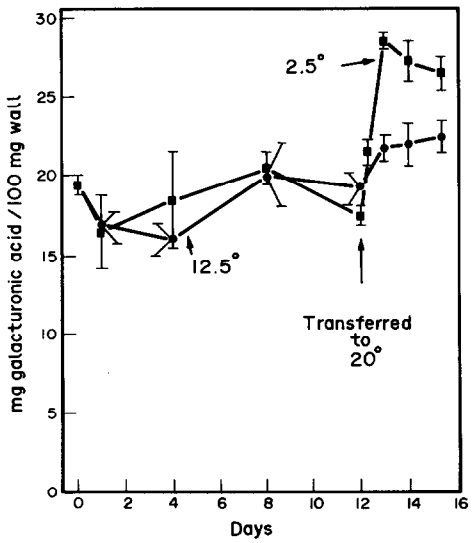

Fig. 3. Galacturonic acid content of cell walls from cucumbers held at chilling (2.5°) and non-chilling (12.5°) temperatures for 12 days and then transferred to 20° for 3 days. Results from fruit which were warmed for 6 hr prior to analysis were similar to fruit analysed without warming. Thus, only data for unwarmed fruit are shown. Data points represent the mean ±s.d.

EXPERIMENTAL

Plant material. Cucumbers (*Cucumis sativus* L., cv. 'Meadowist') were hand-harvested at a local farm and selected for uniformity in size (14–18 cm length) and green color. Fruit were divided into two groups; one group was placed at 2.5° and the other at 12.5°. Six fruit from each group were removed and analysed for ACC content and cell wall carbohydrate composition after 0, 1, 4, 8 and 12 days at the chilling (2.5°) or non-chilling (12.5°) temps. At all sampling times, 3 of the 6 fruit were

analysed immediately and the other 3 fruit were analysed after 6 hr of warming at 20°. Also, at the end of the 12-day sampling period, the remaining fruit from both groups were transferred to 20°; subsequently, three fruit from each group were analysed every 24 hr for 3 days.

ACC determination. Skin samples (1 g) were taken from each fruit and infiltrated with 80% EtOH. The procedure of ref. [16] was used for ACC analysis.

Cell wall carbohydrate analysis. Cucumbers were peeled and the outer 2 cm of cortex tissue removed. Outer cortex tissue (10 g) was placed in 20 ml of 80% EtOH and frozen at −70° for 2 weeks prior to analysis. Cell walls were homogenized in 80% EtOH, filtered and extracted sequentially with 20 mM HEPES–NaOH (pH 7), $CHCl_3$–MeOH (1:1) and Me_2CO as previously described [10], with the addition of an α-amylase treatment before the $CHCl_3$–MeOH extraction to remove starch [15].

The neutral monosaccharide constituents of non-cellulosic polysaccharides were separated and quantified using capillary gas chromatography. Cell wall material was hydrolysed with 2 M TFA using the procedure of ref. [17]. Aldononitrile acetate derivatives of monosaccharides were made according to ref. [18] and were analysed as previously described [19]; *myo*-inositol was used as the internal standard.

Galacturonic acid content of cell walls was estimated by dissolving 10 mg of wall material in H_2SO_4 [20] and assaying aliquots for uronic acid using carbazole [21]; galacturonic acid was used as the standard.

Cellulose content of cell walls was estimated using a procedure similar to that in ref. [22]. The residue remaining after TFA hydrolysis was washed with 1 ml of deionized H_2O. H_2SO_4 (1 ml of 72%) was added, and the samples were incubated at 30° for 1 hr on a shaking waterbath. The samples were diluted to a total vol. of 25 ml with deionized H_2O and autoclaved for 1 hr at 121°. Cellulose content was then estimated using an anthrone determination of total hexose [23]; glucose was used as the standard.

Three individual fruit were analysed at each sampling time. All ACC and cell wall carbohydrate determinations were run in triplicate for each fruit analysed.

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