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MECHANICAL STRESS-INDUCED CHANGES IN SUGAR COMPOSITION OF CELL WALLS FROM CUCUMBER FRUIT TISSUES

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Key Word Index—*Cucumis sativus*; Cucurbitaceae; cucumber; mechanical stress; polysaccharides; sugars; methylation; cell wall.

Abstract—The neutral sugar composition, uronic acid content, esterified methanol content and percent uronic acid methylation of cell walls from the mesocarp and carpel of mechanically stressed and non-stressed (control) 14-day-old cucumber fruit were determined. Forty-eight hr after treatment, mesocarp cell walls from mechanically stressed fruit had significantly less mannose (77% decrease) and galactose (49% decrease) than control fruit stored for the same time, while rhamnose, arabinose, xylose and glucose did not vary appreciably. Concurrently, the per cent uronic acid methylation of mesocarp cell walls did not change following mechanical stress, but increased uronic acid content led to increased uronic acid: neutral pectic sugar ratios. By contrast, carpel cell walls from mechanically stressed fruit 48 hr after treatment exhibited increased uronic acid content, esterified methanol content and percent uronic acid methylation (11, 44 and 33%, respectively) compared to control fruit. The neutral sugar composition of carpel cell walls did not change in response to mechanical stress, although xylose and mannose increased *ca* two-fold during storage. Thus, mechanical stress caused significant cell wall changes in cucumber fruit tissues, but these changes occurred by different mechanisms in the mesocarp and carpel.

INTRODUCTION

Mechanical stress, which simulates harvesting and handling procedures, induces significant degeneration of internal cucumber fruit tissues [1]. Immediately after treatment no visible damage (e.g., carpel separation, bruising, etc.) to the mesocarp and carpel is evident, but degeneration occurs during storage and is associated with increased activity of several cell wall-degrading enzymes including cellulase, pectin methylesterase, polygalacturonase and xylanase [1]. The activity of these enzymes also increases during normal development of cucumbers [2]. Changes of cell wall composition following mechanical stress have not been investigated.

Cucumber cell walls undergo significant changes during development [3, 4], storage, and after chilling treatment [5]. Gross and Wang [5] reported that prolonged storage of cucumbers at non-chilling temperatures decreased the total non-cellulosic neutral sugar content of mesocarp cell walls; most notably galactose and glucose. These same cell wall components decreased only slightly when the cucumbers were stored at chilling temperatures, but declined rapidly upon transfer to 20°. The authors postulated that these rapid changes were due to thermal activation of pre-existing cell wall-degrading enzymes.

The present study was undertaken to further characterize the response of cucumbers to mechanical stress by determining the neutral sugar composition, uronic acid content and uronic acid methylation of cell walls from mesocarp and carpel tissues. It is shown that mechanical stress affects cell walls from both tissues, but that changes appear to occur by different mechanisms.

RESULTS AND DISCUSSION

By utilizing GC for the analysis of neutral sugars and methanol, and a colorometric test for uronic acid determination, 84 and 74% (average from all treatments and experiments) of the cell wall components from cucumber mesocarp and carpel tissues, respectively, were accounted for. The remainder, which was not determined, was probably protein.

Mesocarp cell walls from control (not stressed or stored) cucumbers contained 0.95 μmol of uronic acid and 0.30 μmol of esterified methanol, per mg cell wall, while carpel cell walls had *ca* half these amounts (Table 1). Thus, 31 and 29% of the uronic acid residues present in these tissues, respectively, existed as methyl esters. McFeeters and Lovdal [3] reported somewhat higher percentages of uronic acid methylation for both tissues.

Following mechanical stress, the percent uronic acid methylation of mesocarp cell walls did not change, although as previously reported [5], the uronic acid content increased during storage with a corresponding increase of esterified methanol (Table 1). By contrast, the per cent uronic acid methylation of carpel cell walls had increased 24%, 48 hr after mechanical stress, which was due to a greater increase of esterified methanol levels than uronic acid content (Table 1). This suggests that either a more highly methylated uronic acid polymer was inserted into the existing carpel cell wall or methylation of extant uronic acid residues within the cell wall occurred. As postulated by Knee and Bartley [6], increased uronic acid methylation may decrease calcium cross-linking of pectins within the middle lamella, thereby allowing adja-

Table 1. Uronic acid content, esterified methanol content and uronic acid and mechanically-

Treatment	Storage time (hr)	Mesocarp Uronic acid (μmol/mg cell wall)	Mesocarp Esterified methanol (μmol/mg cell wall)
Control	0	0.95 ± 0.07†	0.30 ± 0.02
No Stress	8	1.18 ± 0.22	0.35 ± 0.01‡
No Stress	48	1.27 ± 0.05‡	0.37 ± 0.02‡
Mechanical stress	8	1.12 ± 0.12	0.33 ± 0.01
Mechanical stress	48	1.35 ± 0.12‡	0.42 ± 0.04‡

*Per cent uronic acid methylation = esterified methanol content ÷ uronic acid content × 100

†Data represent the mean ± s.e.m. of three experiments. Samples from each experiment was analyzed in triplicate.

Table 2. Neutral sugar composition of total cell wall carbohydrates from the mesocarp and carpel of control, non-stressed and mechanically stressed cucumber fruit

Tissue	Treatment	Storage time (hr)	Neutral sugar (mol/mg cell wall) Rha*	Ara	Xyl	Man	Gal	Glc
Mesocarp	Control	0	24 ± 1 (0.5)†	48 ± 10 (1.0)	72 ± 16 (1.6)	234 ± 30 (5.0)	602 ± 76 (13.0)	2714 ± 306 (58.6)
	No stress	8	28 ± 6 (0.5)	54 ± 20 (1.0)	80 ± 20 (1.5)	286 ± 72 (5.5)	772 ± 154 (14.8)	2810 ± 220 (53.9)
		48	34 ± 14 (0.6)	56 ± 26 (1.0)	108 ± 50 (2.0)	316 ± 66 (5.8)	642 ± 130 (11.8)	3000 ± 240 (55.3)
	Mechanical	8	22 ± 2 (0.4)	54 ± 10 (1.1)	60 ± 12 (1.2)	220 ± 18 (4.4)	662 ± 56 (13.1)	2910 ± 152 (57.6)
	stress	48	20 ± 4 (0.4)	42 ± 1 (0.8)	88 ± 10 (1.7)	72 ± 24 (1.4)‡	314 ± 26 (6.1)‡	3254 ± 290 (63.3)
		0	20 ± 4 (0.4)	56 ± 8 (1.3)	84 ± 6 (1.9)	98 ± 56 (2.2)	690 ± 130 (15.4)	2982 ± 496 (66.6)
Carpel	Control	8	30 ± 12 (0.6)	60 ± 15 (1.3)	99 ± 33 (2.1)	246 ± 108 (5.3)	771 ± 261 (16.5)	2883 ± 1056 (61.6)
	No stress	48	33 ± 15 (0.7)	62 ± 12 (1.3)	225 ± 39 (4.9)‡	252 ± 9 (5.5)‡	732 ± 138 (15.8)	2685 ± 390 (58.1)
		8	22 ± 4 (0.5)	74 ± 12 (1.8)	114 ± 10 (2.7)	180 ± 32 (4.3)	650 ± 98 (15.4)	2540 ± 240 (60.3)
	Mechanical stress	48	33 ± 2 (0.7)	75 ± 9 (1.7)	201 ± 9 (4.5)‡	258 ± 39 (5.8)‡	642 ± 92 (14.4)	2513 ± 138 (56.4)

*Neutral sugar abbreviations: Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.

†Data represent the mean ± s.e.m. of three experiments. Samples from each experiment were analysed in duplicate. Values in parenthesis indicate the mol % of each sugar based on the total carbohydrate content (neutral sugar + uronic acid) of the cell walls analysed.

‡Significantly different ($p = 0.1$) from the control using the Student's t-test.

cent cells to separate, resulting in cell wall degeneration and softening. Thus, increased uronic acid methylation could lead to the mechanical stress-induced carpel degeneration described previously [1].

The neutral sugar composition (by nmol/mg cell wall) of mesocarp and carpel cell walls from control cucumbers (Table 2) was similar to that described previously [4], except that xylose was lower and mannose was higher in the present study. This may be due to cultivar differences. Further, mechanical stress did not affect the neutral sugar composition of carpel cell walls (Table 2). However, storage did increase the xylose (48 hr only) and mannose (8 and 48 hr) levels by 2- and 2.5-fold, respectively.

The most notable changes of neutral sugar composition were observed in the mesocarp cell walls 48 hr after mechanical stress treatment (Table 2). The mannose level decreased to 23% of that found in cell walls from non-stressed fruit stored for the same period, while galactose decreased 49% in cell walls from mechanically stressed fruit. Following a chilling treatment, Gross and Wang [5] observed a rapid decline of mesocarp cell wall galactose levels, but reported no change in levels of other neutral sugars. They also reported a decrease of galactose levels during prolonged storage at a non-chilling temperature. Hence mechanical stress, like chilling, may accelerate cell wall changes similar to those associated with normal aging. Further, levels of rhamnose, arabinose and xylose decreased slightly in mesocarp cell walls from mechanically stressed fruit 48 hr after treatment; whereas, these sugars increased slightly in mesocarp cell walls from unstressed fruit stored for the same period (Table 2).

Since uronic acid and rhamnose form the primary backbone and arabinose and galactose the main side chains of pectic substances [7], changes of uronic acid/neutral sugar ratios could significantly affect the tertiary structure of pectins and their association with other polymers [3]. As shown (Table 3), mechanical stress led to significant increases of the uronic acid/neutral sugar

methylation of mesocarp and carpel cell walls from control, non-stressed stressed cucumber fruit

		Carpel	
% Uronic acid methylation*	Uronic acid (μmol/mg cell wall)	Esterified methanol (μmol/mg cell wall)	% Uronic acid methylation
31	0.55±0.14	0.16±0.02	29
30	0.59±0.03	0.18±0.00	31
29	0.66±0.07	0.18±0.02	27
29	0.63±0.04	0.18±0.01	29
31	0.73±0.06‡	0.26±0.05‡	36

‡ Significantly different ($p=0.1$) from the control, using the Student's *t*-test.

Table 3. Uronic acid/pectic sugar molar ratios of mesocarp and carpel cell walls from control, non-stressed and mechanically stressed cucumber fruit

			Uronic acid/pectic sugar ratio*		
Tissue	Treatment	Storage time (hr)	Rha	Ara	Gal
Mesocarp	Control	0	79.2	39.6	3.2
	No stress	48	74.7	45.4	4.0
	Mechanical stress	48	135.0	64.3	8.6
Carpel	Control	0	55.0	19.6	1.6
	No stress	48	60.0	31.4	2.7
	Mechanical stress	48	66.4	29.2	3.4

* Ratios calculated from the mean uronic acid and sugar contents, respectively.

ratios of mesocarp cell walls. Mesocarp cell walls from non-stressed fruit stored for the same period had ratios similar to control fruit. Carpel cell walls on the other hand, exhibited increased uronic acid/neutral sugar ratios for only arabinose and galactose in response to storage, but not mechanical stress. An increased uronic acid/rhamnose ratio in mesocarp cell walls following mechanical stress could cause pectin molecules to be more rod-shaped [8]. The increased uronic acid/arabinose and galactose ratios suggests that pectins may have fewer and/or shorter side chains per unit length in mesocarp cell walls after mechanical stress and carpel cell walls after storage. The significance of these changes to cell wall structure, integrity, and tissue degeneration needs to be clarified.

Miller *et al.* [1] reported that mechanical stress increased the activity of several cell wall-degrading enzymes in both mesocarp and carpel tissues of cucumber. The data presented here show that significant changes of carbohydrate composition occur in cucumber cell walls as well, following this treatment. However, the enzyme activity changes previously reported do not account for the cell wall composition changes. For example, increased pectin methylesterase activity [1] should demethylate existing uronic acid residues, but the percent uronic acid methylation of carpel cell walls increased following mechanical stress, while there was no change in mesocarp cell walls (Table 1). Further, increased polygalacturonase and xylanase activities [1] did not lead to reduced amounts of uronic acid and xylose, respectively, in cell walls from either tissue (Table 2). Several factors could account for these contradictory results. (i) Cell wall composition at any point in time is a function of the relative rates of synthesis and degradation [9], and enzymes may have been degrading polymers while synthesis and deposition of the same or similar components was occurring concurrently. (ii) The first step of the cell wall purification scheme was to homogenize the tissue in 100% methanol, which would precipitate poly- and oligosaccharides. Thus, if the enzymes had endo-hydrolase activity, their reaction products as well as the undegraded polysaccharides would have been precipitated and they would be included in the carbohydrate analysis. Future studies should take advantage of the differential solubilities of the cell wall components to fractionate and analyse potential oligosaccharide degradation products. (iii) As yet undetermined enzymes including pectin methyltransferase, β-galactosidase and mannosidase may play roles during cucumber development and stress responses. Tomatoes [10] and apples [11] do contain β-galactosidase activity.

In summary, cucumber mesocarp and carpel cell walls undergo significant modification following mechanical stress treatment. Mesocarp cell wall changes were characterized by the loss of specific neutral sugars and increased uronic acid/neutral sugar ratios, whereas carpel cell walls exhibited increased uronic acid methylation. Thus, cell walls from the two tissues appear to be modified by different mechanisms. Studies are in progress to further characterize cell wall composition changes and possibly identify other enzymes involved in the mechanical stress response.

EXPERIMENTAL

Plant material and treatment. Cucumber (*Cucumis sativus* L. cv. "Heinz 3534") plants were grown at the Ohio Agricultural Research and Development Center. Wooster. Female flowers were tagged at anthesis. For each experiment, 15 14-day-old fruit (45–50 mm in diameter) were harvested, washed with cool tap H_2O, and divided into treatment groups. One group was divided into three subgroups of three cucumbers and stored. Another group was mechanically stressed by rolling and dropping as previously described [1], then divided into two sub-groups of

three cucumbers and stored. Relative humidity and temperature were maintained at 100% and 25°, respectively, throughout storage. At 0, 8 and 48 hr after treatment, three cucumbers from each stress-regime were removed for tissue preparation. The peel was discarded, then the mesocarp and carpel tissues were separated with a knife, lyophilized, and stored at −40° for subsequent cell wall purification and analysis.

Cell wall purification and analysis. Cell walls were purified by homogenizing tissues in 100% MeOH, then sequentially extracting with organic solvents as previously described [12]. The uronic acid and methylester contents of purified cell walls were determined in triplicate according to [13]. Purified cell walls were hydrolysed with H_2SO_4 and neutral sugars were determined in triplicate after preparation of alditol acetate derivatives [14], which were separated by packed column GC using myo-inositol as int. standard [12]. Injections were done in duplicate.

The experiment was repeated ×3 and the data presented represents the mean of all experiments.

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