JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9181–9189 9181
DOI:10.1021/jf100688t

Influence of Boron on Carrot Cell Wall Structure and Its Resistance to Fracture

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Plant cell wall structure integrity and associated tissue mechanical properties is one of key determinants for the perceived texture of plant-based foods. Carrots (*Daucus carota*) were used to investigate the effect of mineral supply of boron (B) and/or calcium (Ca), during plant growth, on the plant cell wall structure and mechanical properties of matured root tissues. Five commercial cultivars of carrots, Kuroda (orange), Dragon Purple, Kuttiger White, Yellow, and Nutri-Red, were cultivated under controlled glasshouse conditions over two seasons. Significant increases in the accumulation of B and Ca were found for all cultivars of carrots when additional B and Ca were included in the nutrient feeding solutions throughout the plant growth period. Elevated levels of B in carrot root tissue reduced the uptake of Ca and other mineral nutrients and enhanced plant cell wall structural integrity, its resistance to fracture, and the weight and size (both diameter and length) of carrots. Although higher amounts of Ca were accumulated in the plant materials, the additional supply of Ca did not have a significant effect on the mechanical properties of mature plant tissues or on the uptake of B by the plant. The results suggest that B cross-linking of pectin (rhamnogalacturonan II) has a greater influence on mature tissue mechanical properties than Ca cross-linking of pectin (homogalacturonan) when supplied during plant growth.

KEYWORDS: Soil nutrients; boron; calcium; cell wall structure; mechanical properties; cell fracture; carrot (*Daucus carota*)

INTRODUCTION

Fruits and vegetables constitute an important component in our daily diet, for example, as major contributors to our dietary fiber needs. The texture of fruits and vegetables is an important quality attribute that determines consumer sensory appreciation and directly influences liking of plant-based foods, whether fresh or processed. The texture of fruits and vegetables is primarily determined by the mechanical properties of the plant cell wall, the major structural component of fruits and vegetables (*1*−*3*). Together with the internal pressure of the cells (i.e., the turgor pressure) and intercellular adhesion, the properties of the plant cell walls influence the way in which plant tissues undergo mechanical deformation and subsequently break up during either mastication or food processing (*2*−*5*).

The morphological structure and the molecular architecture of the plant cell wall have an important bearing on its mechanical properties. The plant cell wall is a heterogeneous and dynamic structure composed of a three-dimensional interwoven network of cellulose microfibrils embedded in a complex matrix of pectins, hemicelluloses, and structural proteins (*6*−*8*). How these polymers are arranged (e.g., as matrix polysaccharides or associated with cellulose microfibrils) and interact, both physically and chemically, will largely determine the mechanical properties of the cell wall (*9*, *10*). Cellulose is a linear polymer of D-glucose units connected by β-(1→4) linkages. The absence of side chains allows cellulose molecules to associate closely and form microfibril structures, providing the mechanical framework of the cell wall, its rigidity and resistance to osmotic pressures (*11*, *12*). Hemicelluloses (e.g., xylans, mannans, xyloglucans) are thought to form links between cellulose microfibrils, modulating the strength of the wall and influencing its extensibility.

Pectin is the third major biopolymer in the plant cell wall. It makes up about one-third of the dry matter of primary cell wall, is the dominant component in the middle lamella (*8*, *13*). Pectin is probably the most complex polysaccharide in nature (*14*). It is generally accepted that pectins form structural networks via covalent and ionic cross-links that are independent of but synergistic with the cellulose−hemicellulose networks. Therefore, they play an important role in providing plasticity and mechanical strength to the wall and in the adhesion between cells (*6*, *14*−*16*). However, due to the complexity and the heterogeneity of the natural pectin polysaccharides, the impact of either their interconnections or their interactions on the

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Published on Web 07/23/2010

Table 1. Concentration of Minerals Added to Tap Water for the Feeding Solutions for the Various Treatments[a]

treatment	positive control	minus Ca	minus B	negative control
boron	5.0 μM H_2BO_3	5.0 μM H_2BO_3		
calcium	3.0 mM $CaCl_2$		3.0 mM $CaCl_2$	
sources of N, P, K, S, and Mg	0.5 mM NH_4NO_3, 0.4 mM KH_2PO_4, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$			
other trace elements	5.0 μM FeEDTA, 1.0 μM $MnSO_4$, 0.1 μM $ZnSO_4$, 0.1 μM $CuSO_4$,0.5 μM Na_2MoO_4, 0.02 μM $Co(NO_3)_2$			

[a] The concentrations of various minerals used were according to nutritional requirements (*29*).

mechanical properties of cell walls and plant tissue adhesion is not fully understood.

The pectic polysaccharides are generally divided into four polymer domains: homogalacturonan (HGA), xylogalacturonan (XGA), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) (*14*, *16*, *17*). The backbone of pectin polymers consists of polygalacturonic acid: homopolymers of (1−4) α-D-galactosyluronic acid (HGA) with minor amounts of xylose substitution (XGA). RG-I consists of a backbone of repeated (1−2) α-L-rhamnosyl-(1−4) α-D-GalA disaccharide units with extensive side chains based on (1−4) β-D-galactan and (1−5) α-L-arabinan. RG-II has the richest diversity of sugars, and the linkage structures are well-known (*18*, *19*). Pectin is believed to consist of "smooth" regions of HGA/XGA with various degrees of methyl esterification and "hairy" regions made of RG-I and RG-II polymers (*17*).

Mineral nutrients (macroelements P, K, Mg, S, Na, Ca, and N and microelements Cl, B, Fe, Mn, Zn, Cu, Ni, and Mo) play a crucial role in the growth, survival, and reproductive success of plants (*20*, *21*). Among these essential elements for higher plants, calcium (Ca) and boron (B) have been shown to play important roles in maintaining the integrity of plant cell walls (*7*, *15*, *22*) through their localization in cell walls and their ability to bind pectic polysaccharides. Calcium ions have the potential to cross-link pectic chains through ionic bonding to polygalacturonic acid regions. It is believed that blocks of more than 10 nonesterified galacturonic acid residues are required for effective Ca cross-linking (*23*). The abundance of such cross-linking regions at the average levels of methyl esterification of pectins found in most plant cell walls (50−70%) is, however, very low. The primary and possibly sole function of B is as a structural component of growing tissues (*24*). RG-II is the sole receptor for B in plant cell walls. B cross-links two RG-II side chains through B-diol ester bonds to form a dimeric RG-II complex (*25*−*27*). Thus, both Ca and B can cross-link different pectin components and have the potential to influence cell wall integrity and mechanical properties. As the interactions of these two elements with the plant cell wall are different, it is likely that they may affect the mechanical properties of plant cell walls in different ways.

In this study, using carrot (*Daucus carota*) as a model plant, combinations of B and/or Ca in nutrient feeding solutions were applied throughout the plant's growing period to investigate the impact of mineral nutrient availability on plant cell wall structural integrity and the mechanical properties of the cell wall.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Seeds of five commercial carrot cultivars: 'Purple Dragon', 'Nutri-Red', 'Kuttiger White', and 'Yellow', were supplied by Whatcom Seed Co. (Eugene, OR), and 'Kuroda' (orange) was supplied by Syngenta Seeds Pty Ltd., (North Ryde, Australia). Seeds were surface sterilized with 2% sodium hypochlorite (White King, Sara Lee Household and Body Care, Sydney, Australia) and washed several times with sterile water before sowing in Petri dishes that were incubated at 22 °C in a growth room for 16 h under cool white fluorescent illumination (55−65 μE m^{-2} s^{-1}) followed by an 8 h night break. After 10 days, seedlings were transferred to 30 L styrofoam boxes (Andpack Pty Ltd., Hallam, Australia) containing a 1:2 (v/v) mixture of sand (Building Products Supplies Pty Ltd.) to perlite (Grade E, Australian Vermiculite and Perlite Co. P/L), which was washed first with nitric acid (0.01% v/v) followed by water to remove any minerals present in the growth medium. Carrots were grown in a glasshouse maintained at 16−20 °C at Merbein, northwestern Victoria, Australia (34° 12′ 49.83″ S latitude, 142° 02′ 40.14″ E longitude). There were five seedlings of each cultivar per box and eight replicate boxes per cultivar, per sowing. Each replicate box was 57 cm × 32 cm × 38.5 cm in size, and seedling spacing was 10 cm.

Treatments. Two glasshouse experiments were conducted in 2007 and 2008, respectively. The chemicals for preparing nutrient solutions were purchased from BDH (Poole, U.K.). The concentrations of B and Ca in the nutrient feeding solutions were within the range of the feeding nutrients that are normally applied to healthy plants and much lower than the levels that would be toxic or adversely affect the growth of the plants (*28*, *29*). Four treatments were applied: positive control, in which both B (5 μM in the form of H_2BO_3) and Ca (3 mM in the form of $CaCl_2$) were added to the feeding solution; minus Ca, in which Ca was not added; minus B, in which B was not added; and negative control, neither B nor Ca was added. As tap water was used to make up feeding solutions, there were background levels of B = 45 μg/L (equivalent to 4 μM) and Ca = 10.8 mg/L (equivalent to 0.3 mM). Various other minerals were used in the feeding solutions to provide all other mineral requirements (*29*). The mineral compositions for these treatments are shown in **Table 1**.

The seedlings were watered with the desired mixture of mineral fertilizers (1.0 L) once a day and twice a day (0.8 L) when the seedlings were 2−3 weeks old to ensure steady growth and prevent root splitting.

Carrots were harvested 90−110 days after transplanting and washed three or four times in distilled water. Each carrot was measured for fresh weight, length, and diameter (largest) and stored at 4 °C until further analysis. All analyses on the fresh tissues were completed within 3 weeks of harvesting. The water content of the carrots was determined by differences in the fresh and dry weights of samples and was found to be within 87−90% for all genotypes and treatments.

Microscopy. The microstructure of the phloem tissue of the plant cells in the edible tissue was studied by confocal laser scanning microscopy (CLSM). Small pieces of carrot tissue were cut with a razorblade and stained with a drop of Congo red (Ajax Chemicals, Sydney, Australia) (0.1% w/w in distilled water) for about 5 min, then observed at room temperature under a HC PL APO 20× objective using a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany). The fluorescent dye was excited by an argon 488 nm laser, and the emitted light was collected at 544−663 nm.

Image Analysis. The confocal micrographs were analyzed by calculating the number of detached (totally separated middle lamella) and attached cell walls, respectively. Between 100 and 200 cell walls originating from four different areas per sample were added for each sample type. The results are presented as percentage of detached cell walls for each sample type.

Mechanical Testing. Whole carrots (diameter about 20 mm) were cut into 15 mm length pieces with a sharp knife. Each carrot sample was cored using a 15 mm (internal diameter) core borer. The carrot cylinders were then trimmed on a fixed scale cutting plate (10 mm per cell) to the final size of 15 mm × 10 mm cylinders. The carrot cylinder samples contained the core, cambium, and phloem parts of the carrots, but no periderm and skin parts. Some of the carrot cylinders were blanched at 80 °C for 10 min in water, cooled immediately on an ice bath for 10 min, and then kept at room temperature for 20 min prior to the texture measurements. Compression tests were performed using an Instron 5564 instrument (Instron Pty Ltd., Melbourne, Australia) connected with a 500 N load cell and a 25 mm aluminum cylinder at the speed of 60 mm/min

to 70% strain. The true stress (σ_e) and true strain (ε_e) were calculated using the equations

$$\sigma_e = \frac{FH}{A_0 H_0} \quad (1)$$

$$\varepsilon_e = \ln\left(\frac{H}{H_0}\right) \quad (2)$$

where F is the force measured during compression, A_0 is the initial cross-sectional area of the sample, H_0 is the initial sample height, and H is the actual height after deformation.

Stress/strain plots were used to represent the typical mechanical behavior of carrot tissues, and average strengths (maximum stresses to fracture) were calculated. The apparent Young's modulus was calculated using the slope of the stress/strain curve.

Preparation of Alcohol-Insoluble Solids (AIS). AIS were isolated according to the method described by Sila et al. (*30*) with some modifications. The carrot pieces (approximately 5 mm) were freeze-dried and ground to powder with a ball mill grinder (Retsch, Haan, Germany). Approximately 5 g of freeze-dried powdered material was weighed and homogenized in 10 mL of 70% v/v ethanol using an Ultra-Turrax (Rose Scientific Ltd., Edmonton, AB, Canada) for 1 min. The mixture was then centrifuged at 3200*g* (Beckman Coulter, Brea, CA) for 10 min at 4 °C. The pellet was re-extracted with another 10 mL of 70% (v/v) ethanol. The pellet obtained was resuspended in 5 mL of acetone (BDH) and centrifuged at 1900*g* for 1 min. The pellet was then dried overnight at room temperature and further dried under vacuum at 37 °C in an incubator. At least three to five replicates for each treatment and each cultivar were prepared. The AIS samples were then ground to powder using the ball mill grinder and kept in a desiccator at room temperature until further analyses for mineral contents.

Mineral Analyses. The concentrations of P, K, Ca, Mg, S, Na, and B in the AIS were determined using a Spectraflame inductively coupled optical emission spectrometer (Spectroflame ICP, Spectro Analytical Instruments, Kleve, Germany) following hydrolysis in boiling nitric acid (BDH) and dilution to a standard volume with deionized water (*31*). Mineral values were recorded on a dry weight basis, calculated and expressed as milligrams per 100 g of fresh weight (FW) of carrot tissue.

Statistical Analyses. Analysis of variance (ANOVA, GenStat, 11th ed.) and the Unscrambler (Camo Software, Oslo, Norway) were used to analyze data. Sources of variation (genotype, treatment, and interactions between genotype and treatment) were identified by an F ratio with a probability of ≤0.05. Significant differences between means were identified using the least significant difference (LSD). Correlations were identified by the Pearson product−moment correlation coefficient (r) with a two-sided test ($p < 0.05$).

RESULTS AND DISCUSSION

Mineral Uptake and Yield Responses. Figure 1 shows the amounts of B and Ca in carrot roots of each cultivar for each treatment, as well as the effects of genotype and treatment on the accumulation of B and Ca. Low levels of B and Ca mineral elements were found in the samples from the negative feeding solutions that had no added B or Ca. This was due to background levels of minerals (B = 4 μM and Ca = 0.3 mM) present in the tap water used to prepare the feeding solutions. The "baseline" contents of B and Ca mineral ions in these carrots were about 0.3−0.4 and 50−70 mg/100 g of FW, respectively. The value for B is slightly lower than reported for normal field-grown carrots (*32*), whereas Ca is within the range, but at the high end (*33*−*36*). Some root split, a symptom of boron deficiency, was observed in some of the plants from the negative control and minus B. A reduction in carrot fresh weight, as well as its size in both length and diameter, was also seen from the negative control treatment in comparison to all other treatments (**Table 2**).

When additional B was supplied through the nutrient feeding solutions during plant growth, the B contents in carrots of all the cultivars increased to an average of around 0.6 mg/100 g of FW,

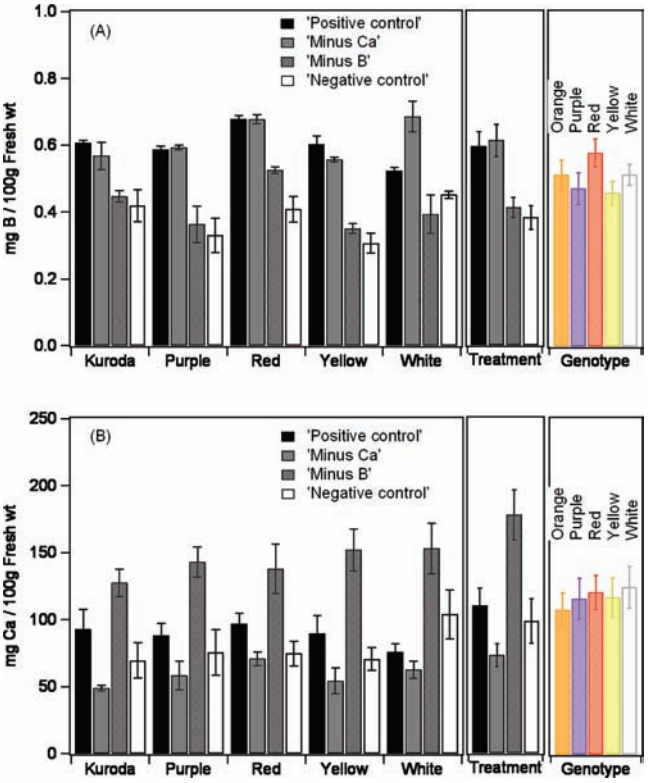

Figure 1. Amounts of B (A) and Ca (B) in the cell walls of carrot tissues, showing the effect of each treatment for each cultivar, the effect of each treatment for all five cultivars, and the effect of cultivar on B and Ca uptake. Bars represent standard error of difference.

Table 2. Effect of Cultivar and Boron and/or Calcium Supply on Fresh Weight (FW), Length, and Diameter of Storage Roots[a]

sample group	diameter (mm)	length (mm)	FW (g)
positive control	26.4	117.4	46.3
minus B	27.1	108.4	35.9
minus Ca	23.4	100.2	32.4
negative control	23.1	98.0	25.6
LSD $p < 0.001$	1.5	7.7	5.6
Kuroda	26.6	92.3	33.0
Dragon Purple	29.1	90.1	39.3
Nutri-Red	20.5	100.4	19.7
Yellow	22.2	109.2	30.9
Kuttiger White	23.4	163.2	44.8
LSD $p < 0.001$	1.7	8.8	6.7

[a] Values presented are means (n = 123−200). LSD, least-squares difference.

double the amount compared to carrots for which additional B was not included in the nutrient feeding solutions (**Figure 1A**). The uptake of B in the carrot roots was not affected by cultivar differences or by the copresence or absence of Ca in the feeding solutions. The absorption and translocation of B in the plant uptake is thought to be largely through passive diffusion (*37*, *38*). These results showed that its accumulation increased with the increase of B supplied through the feeding solutions.

Additional supply of Ca also increased the amount of Ca in the carrot roots to approximately 90−140 mg/100 g of FW. However, there was a significant difference in the Ca uptake depending on whether B was copresent (i.e., the positive control) or absent in the feeding solution (i.e., the minus B treatment). When additional B was omitted from the feeding solution, the Ca uptake by the carrot roots was much higher at 140−160 mg/100 g of FW, in

Table 3. Effect of Cultivar and Various Treatments on the Concentrations of Other Macronutrients in the Cell Walls of Carrot Root Tissues[a]

sample group	P (mg/100 g of FW)	K (mg/100 g of FW)	Mg (mg/100 g of FW)	S (mg/100 g of FW)	Na (mg/100 g of FW)
positive control	65.5	236.5	27.6	20.0	78.5
minus B	82.9	307.6	36.6	27.5	101.8
minus Ca	67.2	251.9	39.6	22.2	81.5
negative control	75.7	278.1	53.3	26.0	116.7
LSD $p < 0.001$	5.9	22.0	3.0	1.7	9.8
Kuroda Orange	72.7	270.8	42.3	28.6	99.3
Dragon Purple	69.6	257.5	34.2	23.7	93.8
Nutri-Red	87.6	344.4	40.8	25.3	63.6
Yellow	71.8	240.4	41.8	21.0	107.3
Kuttiger White	64.1	239.9	33.8	20.2	101.3
LSD $p < 0.001$	6.7	25.1	3.4	1.9	11.2

[a] Values presented are means ($n = 3-5$). LSD, least-squares difference.

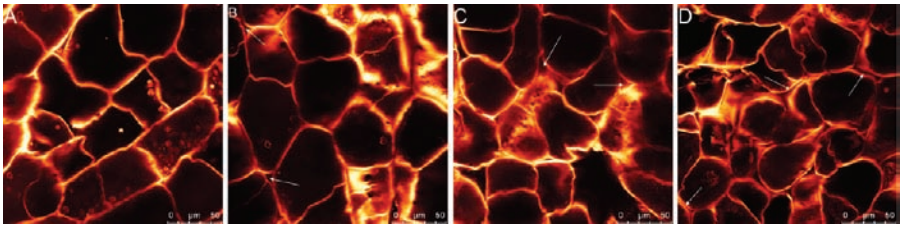

Figure 2. CLSM images of raw root tissues ('Yellow') showing cell wall structure differences from different mineral treatments: (**A**) positive control; (**B**) minus Ca; (**C**) minus B; (**D**) negative control. White arrows point to loose junctions (**B**, **C**) and separated cell walls (**D**) within the tissue.

comparison with the Ca uptake of 80−100 mg/100 g of FW when Ca was added to the feeding solution together with B, even though the Ca concentrations in these two nutrient solutions were the same.

The treatments also caused some changes in the accumulation of other mineral elements. Similar to the uptake of calcium, the accumulation of all other minerals tested (i.e., P, K, Mg, S, and Na) in carrots increased when boron was not supplied to the plants (i.e., the minus boron and negative treatments) (**Table 3**), whereas the presence or absence of calcium did not seem to affect the accumulation of the other minerals. 'Nutri-Red' had the lowest Na content and highest P and K contents.

Boron is important for the structural and functional integrity of plasma membranes (*39*, *40*). It is possible that the presence of boron in plant cell walls during plant growth may influence the physicochemical properties and structure (e.g., porosity) of cell walls, which, in turn, may affect apoplastic transport of other plant mineral ions (*41*).

The carrot root FW was significantly increased with the addition of B and/or Ca supply during growth. In the absence of B and/or Ca there was a significant reduction ($p < 0.001$) in the root diameter, length, and FW for all of the cultivars (**Table 2**). There were also some differences among cultivars. Kuttiger White produced significantly ($p < 0.001$) longer carrots with highest fresh weight, whereas Nutri-Red had the lowest diameter and fresh weight (**Table 2**). In addition, significant interaction ($p < 0.001$) between cultivars and mineral treatments suggested that carrots of all the cultivars had maximum length, diameter, and FW provided that levels of minerals, Ca and B, were adequate. It has been proposed that extreme ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+}, and Cl^-/NO_3^- may affect crop production and quality (*29*, *42*, *43*). Our results show that the presence of B in particular had a significant influence on the accumulation of other minerals (**Tables 3**). This altered the mineral balance, which could result in carrots becoming susceptible to osmotic and specific-ion injury as well as to nutritional disorders that induced the reduced yield and quality. For example, in Kuroda cultivar, due to low Ca concentration from the minus Ca and negative treatments, the Na^+/Ca^{2+} ratio was significantly higher ($p < 0.001$), 7.45 and 6.88, respectively, than in the carrots from the positive control (3.7). The Na^+/Ca^{2+} ratio was also lower in the carrots from the minus B treatment and hence had no influence on the diameter and length of the carrots produced. Furthermore, in all of the cultivars the K^+/Na^+ ratio was 1.6 in controls and lowest for the negative control at ~1.2. High Na^+/Ca^{2+} ratio and K deficiency have been linked to poor quality of Chinese cabbage and artichokes (*44*, *45*) as well as growth and yield reductions of various crops, including tomato, spinach, fennel, and maize (*42*, *43*).

Effect of B and Ca on Plant Cell Wall Structure. Figure 2 shows typical examples of the plant cell wall structure of raw carrots ('Yellow') grown under the positive control, minus B, minus Ca, and negative control treatments, respectively. All genotypes displayed cells with diameters between 20 and 50 μm. The size of the cells varied within each specimen depending on its location within the phloem tissue. No obvious structural differences were observed by CLSM between the five genotypes of carrots. However, differences in the plant cell wall structure were observed for all five cultivars with the different mineral treatments. Further imaging analysis was carried out to examine the number of detached cell walls and junction zones, and the results are summarized in **Table 4**.

The cell walls of carrot tissues from the positive control displayed common features of defined shape and rigid cell wall structures with tight junctions between the cells and low levels of cell wall detachment (3−19%, **Table 4**). By contrast, the carrots grown under the minus B, minus Ca, and negative control treatments all had detectable abnormalities of cell wall structure. A small degree of cell deformation was seen in carrots grown under the minus B or minus Ca treatment. The degree of cell deformation was more pronounced for the negative control treatment in which no additional B or Ca was available to the plant. More cell separation and loose junctions were also found in the carrots from the negative control treatment (26−40%, **Table 4**)

than in those from minus B or minus Ca treatments, except for Kuttiger White.

The effect of low levels of B and/or Ca supply (i.e., only the amounts from the tap water) on the cell wall structure defect was enhanced after the carrot tissues were blanched at 80 °C for 10 min. **Figure 3** shows typical examples of blanched carrots (Dragon Purple) from all four treatments. After blanching, the percentage of detached cell walls increased respectively for all growing conditions, except the negative control for Dragon Purple and the minus Ca treatment for Kuttiger White (**Table 4**). This is largely due to the loss of turgor pressure. However, for all five cultivars, carrots grown without additional B and/or Ca supplied had a significantly higher percentage of detached cell walls than the carrots from the positive control treatment (**Table 4**), demonstrating that both B and Ca are required for plant cell wall structure integrity.

Intracellular localization of B has been extensively examined, and it is found mainly in the plant cell walls. Almost all of the cell wall-bound B is in the form of a B−dimeric RG-II complex, in which B cross-links two RG-II chains through a B−diol ester bonding (*46*). Ishii et al. found that in B-deficient cells, the cell walls swelled and a high proportion of monomeric RG-II was detected (*47*). Addition of boric acid to B-deficient pumpkin plants resulted in the rapid formation of the borate ester cross-linked RG-II dimer (dRG-II−B) and a decrease in wall thickness (*47*). Fleischer and co-workers also demonstrated that the addition of boric acid to growing B-deficient cells resulted in B binding to the cell wall, the formation of dRG-II−B, and a rapid reduction in wall pore size (*48*). This may also explain why, when a high amount of B was supplied and accumulated in cell walls of carrots, there was reduced accumulation (diffusion) of other minerals including Ca as a result of the increased tightness of the cell wall matrix.

Table 4. Percentage of Detached Cell Walls of Raw and Blanched Carrots Grown under Different Mineral Treatments, from Analysis of CLSM Images for Carrots from All Five Cultivars

	detached cell walls[a] (%)				
treatment	Kuroda Orange	Dragon Purple	Nutri-Red	Yellow	Kuttiger White
		Raw Carrots			
positive control	5	19	6	3	10
minus Ca	4	14	7	21	45
minus B	10	16	11	19	40
negative control	29	39	38	26	31
		Blanched (80 °C, 10 min) Carrots[b]			
positive control		23		11	19
minus Ca		54		39	30
minus B		33		25	48
negative control		31		62	40

[a] The total numbers of cell walls counted for each sample were between 100 and 200 originating from four different micrographs. [b] Heat treatment was not carried out on Kuroda and Nutri-Red samples due to limited sample availability.

The primary structural role of Ca in plant cell walls is to cross-link pectin nonesterified galacturonic acid backbone residues through ionic bonding. Most Ca ions are found in the middle lamellae and junctions, the regions rich in pectin homogalacturonan stretches (*13*, *49*). Thus, Ca is known to play an important role in cell−cell adhesion. It is also believed that Ca within the cell wall is associated with the RG-II region, as a native constituent of the B−RG-II complex, and works to strengthen the B−diol ester bonding (*22*, *50*).

Our results showed that both B and Ca are important in maintaining the integrity of the plant cell wall structure. Carrots grown under the low Ca treatments (i.e., the minus Ca and the negative control) had more cell separation than the positive control, indicating that Ca is more important for cell adhesion, probably through enhanced pectin cross-links in the middle lamellae.

Mechanical Properties. Figure 4 shows the stress/strain curves of raw and blanched carrot pieces from Kuroda (**A** and **C**) and Dragon purple (**B** and **D**) to represent the general behavior of carrots from the four mineral nutrient treatments. The results show that the mechanical properties of carrot tissues were clearly affected by the different mineral feeding solutions that were applied. The carrots grown with no additional supply of B or with lower B accumulation in the root tissues, that is, those from the minus B and the negative control treatments, exhibited lower stresses to tissue failure than those grown with a supply of additional B or with higher amounts of B accumulated in the root tissues, that is, those from the minus Ca and positive control treatments (**Figure 4A**,**B**). The carrots grown under the minus Ca condition showed mechanical properties, that is, stress/strain profiles, similar to those of the carrots grown under the positive control treatment (high Ca content), whereas the carrots grown under the minus B treatment, in which the highest concentration of Ca was accumulated in the root tissues, had mechanical behavior similar to that of the carrots grown under the negative control treatment, for example, low Ca content in the mature roots. The different behaviors in their mechanical properties between carrots from the different treatments were still distinguishable after the carrots were blanched (**Figure 4C**,**D**). Although all of the carrot samples displayed weakened mechanical properties and slightly delayed failure in comparison to their respective carrot root tissues in the raw state, the carrots with lower B content from minus B and negative control treatments failed at slightly smaller strains and at much lower stresses in comparison to those with higher B content grown under the minus Ca and positive control treatments (**Figure 4A**,**B**).

The apparent Young's modulus was calculated using the linear region of the stress/strain curve for each of the treatments from texture analyses of raw as well as blanched tissues, and the values are summarized in **Table 5**. There was a general reduction of the

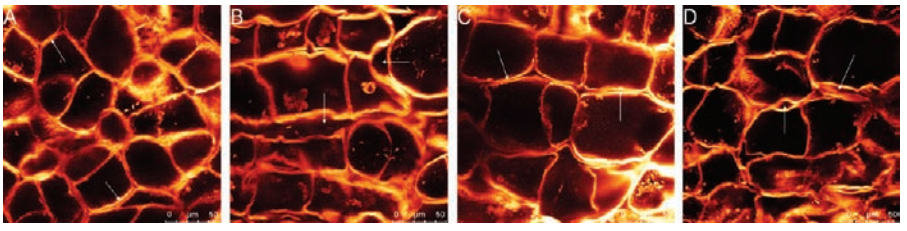

Figure 3. CLSM images of blanched (80 °C for 10 min) root tissues (Dragon Purple) from different mineral treatmentsL (**A**) positive control; (**B**) minus Ca; (**C**) minus B; (**D**) negative control. White arrows point to areas where the middle lamella has split to separate the cells within the tissue.

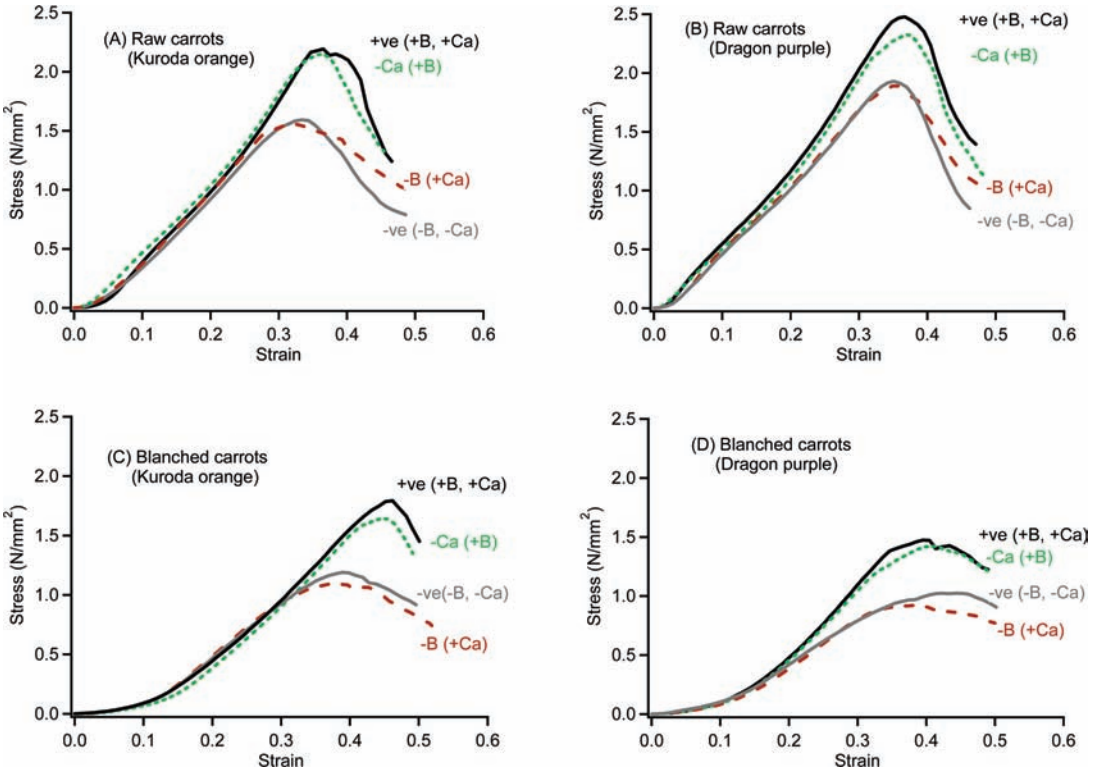

Figure 4. Mechanical behavior of raw (**A**, **B**) and blanched (80 °C for 10 min) (**C**, **D**) carrots: typical stress/strain profiles chosen from Kuroda orange (**A**, **C**) and Dragon Purple (**B**, **D**) to represent general behavior of carrot pieces from different mineral treatments; positive control (+ve) where both B and Ca were added to the feeding solutions, minus Ca (−Ca), minus B (−B) and negative control (−ve) where neither B nor Ca was added to the feeding solutions.

Table 5. Apparent Young's Modulus (Mean Values ± Standard Deviation) Calculated Using the Linear Slope of Strain/Stress Curve

	apparent Young's modulus (N/mm^2)				
treatment	Kuroda Orange	Dragon Purple	Nutri-Red	Yellow	Kuttiger White
			Raw Carrots		
positive control	6.8 ± 0.4	6.6 ± 0.4	6.9 ± 0.7	7.4 ± 0.6	6.4 ± 0.7
minus Ca	6.8 ± 0.4	6.3 ± 0.7	6.4 ± 0.6	7.5 ± 0.4	6.9 ± 0.4
minus B	6.3 ± 0.6	5.4 ± 0.5	5.5 ± 0.8	6.7 ± 0.3	6.5 ± 0.2
negative control	6.4 ± 0.6	5.7 ± 0.4	5.5 ± 0.5	6.5 ± 0.2	6.5 ± 0.5
			Blanched (80 °C, 10 min) Carrots		
positive control	5.9 ± 0.4	5.6 ± 1.0	5.4 ± 0.6	5.0 ± 0.4	5.5 ± 0.4
minus Ca	5.5 ± 0.7	5.6 ± 0.2	5.2 ± 0.7	4.9 ± 0.5	6.0 ± 0.6
minus B	4.8 ± 0.7	4.0 ± 0.3	5.2 ± 0.8	4.9 ± 1.0	4.5 ± 0.6
negative control	5.1 ± 0.5	4.2 ± 0.2	3.9 ± 0.2	4.2 ± 0.7	4.5 ± 0.8

apparent Young's modulus from raw to blanched tissues for each treatment due to the weakened mechanical properties of carrot tissues caused by blanching. Although there was a trend for the Young's modulus of the positive control and minus Ca treatments, that is, carrot tissues with high B content, to be higher than that of the minus B and negative treatments, in which lower amounts of B were accumulated in carrot roots. In the case of both raw and blanched carrots, the differences were small; the greater measurement variance excludes those values from being statistically significant. The major difference between the treatments seems to be in susceptibility to failure of the root tissues rather than cell wall strengthening.

The variations in the strength of the tissues (i.e., maximum stress to failure) for all five genotypes from each mineral treatment are shown in **Figure 5**. Due to the limited material available, for each treatment, tests were performed using five samples selected from close approximate areas from each carrot per genotype. The variations in the five measurements were < 10%, except Dragon Purple (both raw and blanched) roots of minus B and blanched yellow/white carrot roots of negative control, in which the variations were between 10 and 13%. All genotypes showed a similar trend. The strengths of the carrot roots containing higher amounts of B, that is, those from the positive control and minus Ca treatments, were greater than the carrots containing lower amounts of B, that is, those from the minus B and negative control. There were no significant differences between the carrots with higher or lower amounts of Ca, that is, positive control compared to minus Ca or minus B compared to negative control treatment.

Both cell turgor pressure and the integrity of cell walls are important in determining the rigidity or firmness of plant materials. Ormerod et al. (*51*) proposed a generic model linking mechanics and structural failure of plant tissue using Chinese water chestnut and carrot as model examples. They demonstrated that plant tissues with strong intercellular adhesion and low porosity fail by cell rupture, which causes the loss of turgor pressure and internal fluid, whereas plant materials that have weaker intercellular adhesion fail by cell separation, a typical feature of plant materials which have been heat treated. It is known that the mechanical failure of raw carrots is primarily caused by cell rupture, indicated by the brittle fracture through cells (*51*) and as shown in the CLSM images of broken surfaces after the compression testing (**Figure 6**). In contrast, the mechanical failure of blanched carrots is caused by a combination of cell rupture and cell separation due to the weak adhesion between the cells.

Boron had a profound impact on the fracture properties of cell wall materials. The additional B accumulated in the carrot root cell walls is likely to be associated with the borate ester cross-linked RG-II dimer. Although direct mechanical measurements

on plant materials have not been reported as far as we are aware, evidence that additional B during plant growth and subsequent formation of B−RG-II complex reduces the wall thickness, porosity, and wall rupture has been reported (*47*, *48*, *52*). Although the location of RG-II within the cell wall could be critical, it is plausible that a few additional cross-links, via B, could form additional tight polymer bundles and thus strengthen the mechanical properties of the cell wall matrix.

Our results also showed that although higher amounts of Ca were accumulated in the mature plant materials when the additional supply of Ca in the nutrient feeding solutions was applied, there was no significant effect on the mechanical properties of these carrots in comparison to carrot tissues containing low amounts of Ca. The primary structural role of Ca in the plant cell walls is to cross-link pectins in the middle lamellae and junctions, thus playing an important role in determining the degree of cell adhesion/separation. In the raw state, cell adhesion is strong; therefore, the mechanical failure of the cell wall is dominated by the physical fracture of the cell wall. Thus, the degree of Ca−pectin cross-links, particularly in the middle lamellae, did not have a significant effect on the mechanical failure of carrot tissues. Another explanation would be that the low level of Ca accumulated in the carrots is adequate for the available pectin to cross-link. It has been suggested that a block of 7−10 nonesterified galacturonic acids would be required to form an effective Ca cross-link of pectin molecules (*49*). As natural pectins are highly branched and esterified, the numbers of available carboxyl groups in a continuous block for Ca to form effective cross-links may be limited. Therefore, under the nutrient feeding conditions designed in this study, the high level of Ca in the tissue may not have been utilized to form additional cross-links with pectin molecules, and therefore the Ca may have made no further contribution to the mechanical properties of the cell wall.

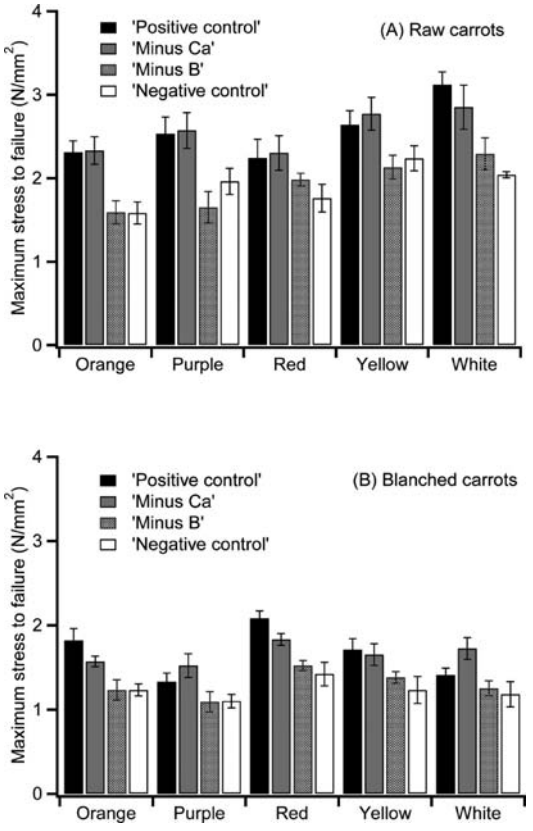

Figure 5. Variations in tissue strength (taken from the maximum stress at failure) of (**A**) raw and (**B**) blanched (80 °C for 10 min) carrot root samples from different mineral treatments for all five cultivars.

It has been shown that blanching at low temperature and long duration (e.g., 60 °C for 40 min) could improve the texture of carrots by activating pectinmethylesterase (PME) and deactivating endogenous polygalacturonase (PG), thereby allowing the Ca to form cross-links with the de-esterified pectin, particularly after the plant materials have been soaked in $CaCl_2$ solution (*53*). It remains to be seen whether the abundant levels of Ca in the plant can be utilized to provide enhanced texture properties in combination with specific enzyme treatment without the use of postharvest soaking.

In conclusion, additional mineral nutrients (B and/or Ca) supplied to the carrot plants doubled their accumulation in the edible carrot root tissue. Structural examination by CLSM and imaging analysis showed that the additional B and/or Ca played a role in enhancing plant cell wall structure integrity. The compression results showed that the resistance to fracture of the plant materials was enhanced by the elevated levels of B in the plant materials. Further analysis of the formation of B−dRG-II and its effect on cell wall porosity, thickness, and tissue mechanical properties may provide insight into the role of B on pectin structural assembly and how this affects cell wall and tissue properties.

Although higher amounts of Ca were accumulated in the carrot roots, the additional supply of Ca did not have a significant effect on the mechanical properties of the mature root tissues. It remains to be investigated whether the presence of high levels of Ca would provide better mechanical properties and eating texture of these

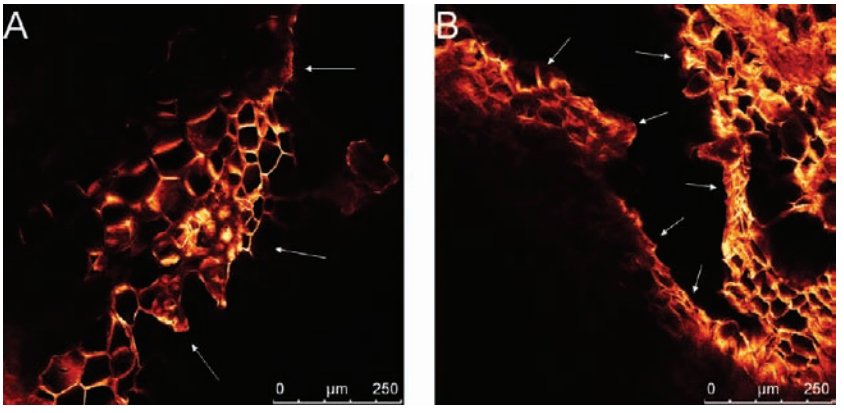

Figure 6. CLSM images of compression-fractured Kuroda carrots: (**A**) raw positive control; (**B**) blanched (80 °C for 10 min) minus Ca. White arrows point toward the fractured surfaces.

plant materials if specific processing conditions were applied to de-esterify pectin molecules and form additional cross-links with available Ca.

To our knowledge this is the first study to report the effect of B accumulation in plant materials during growth on their subsequent mechanical properties. Further investigation of the molecular structure assembly of natural pectins, in particular, the cross-linking mechanism of B and/or Ca with pectins, could further enhance our understanding of their impact on the mechanical properties of plant cell walls.

ABBREVIATIONS USED

g, acceleration due to gravity; AIS, alcohol-insoluble solids; ANOVA, analysis of variance; CLSM, confocal laser scanning microscopy; FW, fresh weight; GalA, galactosyluronic acid; HGA, homogalacturonan; LSD, least significant difference; micro E, μE m^{-2} s^{-1}; PME, pectinmethylesterase; PG, polygalacturonase; RG, rhamnogalacturonan; STD, standard deviation.

ACKNOWLEDGMENT

We thank Gail Di Gregorio in assisting the harvest of carrot materials and texture analysis.

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Received for review February 19, 2010. Revised manuscript received April 24, 2010. Accepted July 6, 2010. This research was partly funded by Victoria Government Department of Primary Industry.