JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Cultivar-Dependent Cell Wall Modification of Strawberry Fruit under NaCl Salinity Stress

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Strawberry cultivars differ in their sensitivity to NaCl; fruits of cv. Elsanta suffer from softening, whereas those of cv. Korona retain their firmness. The mean fruit fresh weight is reduced in cv. Elsanta up to 46% and in cv. Korona up to 26%. Cell walls of fruits grown under 0, 40, or 80 mmol/L NaCl were extracted and analyzed. In fruits of cv. Korona, the content of the alcohol-insoluble residue remained comparatively stable as salt levels increased but was reduced in cv. Elsanta. The water-soluble pectin fraction was not affected in cv. Korona, but the content of low methoxy pectinates increased significantly, indicative of the generation of calcium and magnesium bridges that stabilize pectin polysaccharides of cell walls. In cv. Elsanta, the content of water-soluble pectin rose, indicating pectin solubilization. For both cultivars, the significant negative correlation of fruit Cl^- contents with the contents of NaOH-soluble pectinates, when expressed per fruit fresh mass, indicated that covalently bound pectic substances were degraded. Especially the response of cv. Elsanta is in line with the general observation that severe osmotic stress results in slower cell expansion and weaker cell walls.

KEYWORDS: ***Fragaria* × *ananassa* Duch.; fruit softening; NaCl salinity; pectin; cell wall**

INTRODUCTION

Salinity impairs plants by osmotic effects, reducing water uptake and availability. Moreover, salinity-induced disorders are the result of excessive ion uptake (e.g., of Na^+ and Cl^-) as well as of nutrient imbalances, which are a consequence of a limited nutrient availability or competitive uptake, transport, or partitioning within the plant (*1*−*3*). Elevated salt concentrations in the tissue affect enzyme activities and in consequence impair plant metabolism (*4*). Processes related to salt stress also involve cell walls (*5*), which are very sensitive to modification of their water content, because about 75% of their fresh mass (FM) consists of a very dense aqueous gel (*6*, *7*). For instance, water reduces interactions between cell wall polymers and facilitates cell wall expansion (*8*). In consequence, modifications in cell wall hydration should influence cell wall mechanical properties and its porosity to low molecular weight species and enzymes. Especially pectic polysaccharides are implicated in intercellular adhesion in the middle lamella, in controlling the porosity of the cell wall, and in the cation-responsive structure controlling cell wall properties (*9*). The metabolic environment of the cell wall and middle lamella is the apoplast.

Modifications of fruit firmness due to salinity stress are closely related to cell wall composition. For example, firmer fruits have been reported, when tomato was grown under saline conditions (*10*, *11*). Information on the influence of salt stress on the cell walls of strawberry fruit is limited, although strawberry is characterized by a short postharvest life due to fruit softening and elevated salinity of nutrient solution has been proposed to enhance fruit quality and to reduce vegetative growth (*12*−*14*). A comparison of strawberry cultivars with different softening rates revealed that, by the end of fruit ripening, changes in cell wall pectins content occur, which support the hypothesis that softening is closely related to pectin solubilization and depolymerization (*15*). The small amount of cellulose found in strawberry fruit remained unchanged during ripening (*15*, *16*) and hemicellulose degradation represented only a minor component in fruit softening (*15*, *17*). Because the biochemical basis of cell wall degradation in strawberry fruit under NaCl salinity stress has not yet been clearly established, the biochemical changes in cell walls of fruit of strawberry cultivars that differ in their sensitivity to NaCl stress were studied. Modifications of cell wall content and their components (pectins, hemicelluloses) were compared in relation to modifications of firmness. For these experiments, varying NaCl concentrations of nutrient solutions were supplied to strawberry plants growing in a sandy medium, because this medium allowed control of the NaCl content in the root medium in an optimal way. On the one hand, Na^+ and Cl^-, when supplied with the NaCl solution, were easily accessible to the plants, but on the other, the sandy medium allowed leaching of the soil and reduction of the salt levels by the addition of demineralized water. Leaching the soil should quickly improve the water relations of the plant but not affect salt levels within the plant (*4*). Using this experimental approach enabled us to minimize the effects of water stress.

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10.1021/jf071216o CCC: $37.00
Published on Web 08/04/2007

MATERIALS AND METHODS

Plant Material and Growth Conditions. Experiments were conducted twice in 3 years during two successive vegetative periods in 2002/03 and 2003/04 in Göttingen, Germany, with the NaCl-sensitive strawberry (*Fragaria* × *ananassa* Duch.) cv. Elsanta and the less-sensitive cv. Korona (*18*). NaCl treatments lasted from the end of April to the middle of September in 2002, 2003, and 2004. The salinity levels applied resulted in moderate (40 mmol/L NaCl) and excessive (80 mmol/L NaCl) stress; the excessive stress level caused plant death in about 10% of plants of the more sensitive cv. Elsanta. Control plants did not receive additional NaCl (0 mmol/L NaCl). Commercial strawberry plantlets (Frigo) were purchased from the Kraege Beerenobst Spezialkulturen Co. (Telgte, Germany). For experiments, plantlets were selected for similar size and cultivated in 6 L metallic Mitscherlich containers filled with quartz sand (particle size 0.7−1.2 mm). Plants were located randomly (12 plants per m^2) with 10 replications per combination to ensure a statistical design and to exclude position effects. They were grown in a greenhouse to avoid a dilution of salt applied to the plants by rainfall, but temperature and humidity were as similar as possible to natural conditions, because the large windows of the greenhouse were opened. A modified Hoagland solution (*19*) was supplied twice a week (200 mL per plant). Three weeks after planting, salt treatment started and NaCl solutions were supplied four times a week to each plant. Once a week, 200 mL of demineralized water was added to reduce salt accumulation in the root medium. Surpluses of solutions were allowed to pass the containers to avoid anoxia by water logging. All of the plants received extra demineralized water when needed. Runners were removed immediately. Fruits were harvested at the optimum of fruit maturity, when about 90% of the fruit surface had reached a fully red color. The fruits were divided into sepals and fruit flesh. Only fruit flesh was used for further investigations. Immediately after harvest, fruits were weighed, frozen in liquid nitrogen, and stored at −30 °C until further analyses.

Between salt treatments, plants were kept in the greenhouse, which was not heated during the winter. Strawberry plants received Hoagland solution and demineralized water until November; thereafter, watering of plants was interrupted until the first week of April.

Fruit Firmness. Fruit firmness was determined by a sensory panel test, which involved 10 untrained panelists, who could select fruits freely from bulk samples of the treatment combinations encoded with randomly selected numbers. The fruits were picked about 1 h before the beginning of the test and washed with tap water. The panelists were requested to assess fruit firmness based on a scale from 0 (soft) to 2 (firm).

Determination of Fruit Na^+, Cl^-, and Relative Water Contents. The contents of Cl^- and Na^+ were determined after digestion of fruits with HNO_3 and H_2O_2 in a MLS-1200 microwave laboratory system (MLS GmbH, Leutkirch im Allgäu, Germany). Cl^- contents were evaluated photometrically (Hewlett-Packard Aglient 8453 UV/vis spectrophotometer with multicell sampler) according to the mercury thiocyanate method of Merck (Germany). Na^+ contents were determined using a flame photometer Elex 6361 (Eppendorf, Germany). The water contents of fruit were calculated by comparing fresh and dry masses.

Cell Wall Extraction, Pectin, and Hemicelluloses/Cellulose Analyses. For cell wall investigations, the cell wall polysaccharides were obtained as alcohol-insoluble residues (AIR) modified according to a method for melon (*20*). Dried AIR was used for the extraction of further cell wall fractions.

Different pectin fractions were obtained by stepwise extraction with respect to their differences in solubility (*20*). First, water-soluble pectins (WSP) were isolated with distilled water, and then, the low methoxy pectinates (LMP) of calcium and magnesium were extracted with 0.4% sodium hexametaphosphate, where the sodium hexametaphosphate complexes with divalent cations. To extract any remaining pectinates, the residue from the last step was extracted with aqueous NaOH (0.05 mol/L) solution (NaOH-soluble pectinates; NSP). The filtrates of the pectin fractions were quantified using the colorimetric determination of uronic acid by the so-called 3-hydroxydiphenyl method using a Hewlett-Packard Aglient 8453 UV/vis spectrophotometer with a multicell sampler. Galacturonic acid was used as a standard, and the results are presented in galacturonic acid equivalents (GAE), either expressed per fruit FM or as a percentage of AIR.

From the residues of the pectin extractions, hemicelluloses and cellulose were extracted as glucose after stirring with 100 mL of 4 M NaOH and complete hydrolysis with 66% (v/v) H_2SO_4 at 100 °C (*15*). The resulting glucose was estimated by HPLC [LiChrospher 100 NH2 (5 μm) 4 mm × 4 mm precolumn (no. 1.50966.0001, Merck KGaA, Darmstadt, Germany) in combination with a LiChrospher 100 NH2 (5 μm) 4 mm × 250 mm separation column (no. 1.834.0001, Merck KGaA)], and an acetonitrile:pure water solution (80:20 v/v) was used as the mobile phase (flow rate, 1.0 mL min^{-1}; column temperature, 20 °C). Glucose was detected with a Knauer differential refractometer 198.00 (Knauer, Berlin, Germany).

Statistical Analysis. The data were analyzed with the statistical programs SPSS for Windows, release 12.0 standard version (SPSS Inc, 1989−1999). All data sets were tested for normal distribution and variance homogeneity. When they met requirements, Duncan tests were performed; otherwise, Tamhane tests were applied. Correlation analyses were performed using Pearson correlation tests.

RESULTS

Fruit Characteristics. The application of 0, 40, and 80 mmol/L NaCl to the root medium increased Na^+ and Cl^- levels of fruits and reduced fruit size (**Table 1**). Cl^- levels of fruits of cvs. Korona and Elsanta were similar and did not depend on whether plants were exposed to NaCl salinity for one or two seasons. By contrast, Na^+ levels were in tendency higher in cv. Elsanta than in cv. Korona at 40 mmol NaCl/L, and Na^+ levels were distinctly higher when plants were exposed to NaCl salinity for 2 years. Fruit weight is generally considered an appropriate measure of fruit size. While fruits of control plants were generally larger in cv. Elsanta than in cv. Korona, the reduction in size due to NaCl salinity was more pronounced in cv. Elsanta than in cv. Korona. Means of fruit weight decrease in cv. Korona by about 7 and 25% and in cv. Elsanta by about 22 and 44% at 40 and 80 mmol/L NaCl, respectively. The fruit water content was not significantly affected by NaCl stress in the root medium of plants. It was slightly higher in cv. Elsanta (0.91 g g^{-1} FM) than in cv. Korona (0.89 g g^{-1} FM).

The characterization of freshly harvested strawberry fruits by untrained panelists revealed that firmness was similar in fruits of control plants of both cultivars. While an increasing NaCl level in the root medium did not affect fruit firmness in cv. Korona, it was reduced in cv. Elsanta (**Figure 1**). In 2003, panelists also tested for differences between fruits from plants exposed to NaCl salinity for one and two seasons. However, differences were not detected (data not shown).

AIR. The amount of AIR was similar in control fruits of cvs. Korona and Elsanta, and the response of both cultivars to NaCl salinity was reproducible, irrespective of the number of years that plants were exposed to salt stress in the root medium. NaCl in the nutrient solution did not affect the AIR content in cv. Korona (**Table 1**). By contrast, the fruit cell wall content of 1 year old plants of the salt-sensitive cv. Elsanta decreased progressively with increasing salt levels, being about 13 and 21% lower, when plants were supplied with 40 and 80 mmol/L NaCl, respectively.

Pectins. The sequential extraction of AIR resulted in WSP, which contained 8−11% methoxyl groups, LMP, which represent ionically or covalently bound calcium and magnesium pectates, and water-insoluble NSP. Total pectin contents of cvs. Elsanta and Korona, calculated as the sum of WSP, LMP, and NSP, varied between 0.29 and 0.38 g GAE 100 g^{-1} FM and were not significantly affected by salinity (**Table 1**).

With respect to the less salt-sensitive cv. Korona, the WSP fraction was not affected by NaCl in the root medium similar

Table 1. Influence of NaCl Salinity on Cell Wall Components of Strawberry Cvs. Elsanta and Korona[a]

variety and treatment	fruit weight (g)	AIR (g 100 g^{-1} FM)	total pectins (g GAE 100 g^{-1} FM)	hemicelluloses and cellulose (g glucose 100 g^{-1} FM)	mmol kg^{-1} FM Na^+	mmol kg^{-1} FM Cl^-
fruits of 1 year old plants (one season of salt stress)						
Korona (0 mmol NaCl/L)	5.95 ± 1.05 bc	2.70 ± 0.18 a	0.32 ± 0.08 ab	0.42 ± 0.09 a	0.51 ± 0.18 d	1.30 ± 0.20 c
Korona (40 mmol NaCl/L)	5.56 ± 1.19 cd	2.47 ± 0.46 abc	0.29 ± 0.06 b	0.48 ± 0.20 a	13.26 ± 1.89 c	4.40 ± 1.44 ab
Korona (80 mmol NaCl/L)	4.49 ± 0.61 d	2.75 ± 0.50 ab	0.32 ± 0.06 ab	0.46 ± 0.14 a	24.65 ± 4.72 ab	4.88 ± 1.02 a
Elsanta (0 mmol NaCl/L)	9.59 ± 2.60 a	2.50 ± 0.11 a	0.38 ± 0.07 a	0.70 ± 0.39 a	0.25 ± 0.06 e	1.29 ± 0.22 c
Elsanta (40 mmol NaCl/L)	7.45 ± 1.16 ab	2.15 ± 0.15 b	0.36 ± 0.04 ab	0.38 ± 0.11 a	20.06 ± 5.14 b	3.38 ± 0.73 b
Elsanta (80 mmol NaCl/L)	5.66 ± 1.18 cd	1.90 ± 0.20 c	0.38 ± 0.07 a	0.11 ± 0.04 b	28.98 ± 3.49 a	6.32 ± 2.38 a
fruits of 2 year old plants (two seasons of salt stress)						
Korona (0 mmol NaCl/L)	6.37 ± 1.13 b	2.60 ± 0.25 ab	0.31 ± 0.05 a	0.64 ± 0.15 ab	0.61 ± 0.14 c	0.81 ± 0.44 b
Korona (40 mmol NaCl/L)	5.86 ± 1.31 bc	2.56 ± 0.31 ab	0.31 ± 0.07 a	0.58 ± 0.24 ab	38.31 ± 3.18 b	5.78 ± 3.55 a
Korona (80 mmol NaCl/L)	4.69 ± 1.26 c	2.68 ± 0.75 a	0.32 ± 0.12 a	0.55 ± 0.11 ab	75.68 ± 6.65 a	7.36 ± 2.82 a
Elsanta (0 mmol NaCl/)	10.34 ± 2.50 a	2.40 ± 0.33 abc	0.38 ± 0.06 a	0.72 ± 0.13 a	0.64 ± 0.06 c	0.95 ± 0.06 b
Elsanta (40 mmol NaCl/L)	8.14 ± 1.36 ab	2.10 ± 0.14 bc	0.37 ± 0.06 a	0.44 ± 0.13 bc	67.08 ± 6.32 a	5.82 ± 3.37 a
Elsanta (80 mmol NaCl/L)	5.54 ± 1.28 bc	1.95 ± 0.35 c	0.38 ± 0.11 a	0.28 ± 0.05 c	72.94 ± 7.56 a	6.48 ± 1.76 a

[a] Results are expressed per fresh FM. Different letters indicate significant differences by Duncan or Tamhane tests for fruits harvested within the same growing season ($P < 5\%$).

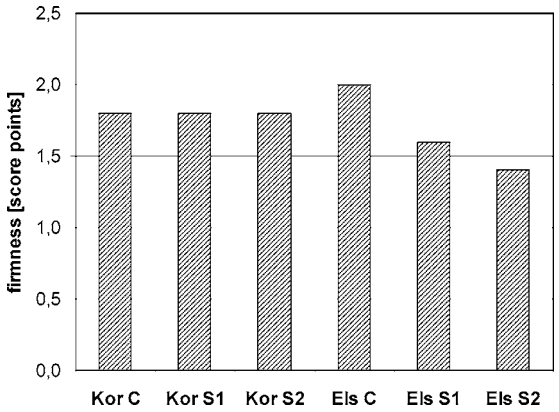

Figure 1. Fruit firmness of strawberry cvs. Korona (Kor) and Elsanta (Els) plants treated by 0 (C), 40 (S1), or 80 (S2) mmol/L NaCl; fruits of plants treated with NaCl during one and two seasons were lumped.

to total pectin content, but the content of LMP increased significantly, whereas that of NSP decreased. This response to salinity was independent of the expression of pectin fractions per fruit FM or per AIR, because AIR remained similar (**Figures 2** and **3**). The increase in LMP was approximately compensated by the decrease in NSP; however, the decrease of NSP was not significant on a per FM basis.

In contrast to cv. Korona, the contents of WSP rose in the salt-sensitive cv. Elsanta by about 17 and 47% at 40 and 80 mmol NaCl/L, respectively, whereas those of NSP were reduced in tendency by 11 and 21%, respectively, when expressed on a per fruit FM basis. The LMP content was less affected (**Figure 2**). Because the AIR content was significantly reduced in fruits of cv. Elsanta exposed to NaCl for one season and distinctly reduced in plants exposed to NaCl for two seasons, the relative changes of the pectin fractions, when expressed per AIR, differed from that expressed per fruit FM. The contents of WSP rose distinctly, that of LMP in tendency, while contents of NSP remained unchanged (**Figure 3**).

Hemicelluloses and Cellulose. The fruit hemicelluloses/cellulose content was not significantly changed in strawberry cv. Korona. Its amount was considerably reduced by 42 and 72%, when plants of cv. Elsanta were exposed to 40 and 80 mmol/L NaCl, respectively, in the nutrient solution (**Table 1**).

DISCUSSION

Strawberry is a fast growing nonclimacteric fruit, with a short postharvest life. During its development, cell division is detectable only until the seventh day after petal fall; thereafter, only cell enlargement occurs until the fruit reaches 25% red. In consequence, the fruit cell wall content measured as AIR is continuously reduced during fruit ripening (*15*, *21*).

In general, cell walls are complex and dynamic systems, composed of polysaccharides, phenolic compounds, and proteins. They are stabilized by ionic and covalent linkages with microfibrilar cellulose and hemicellulose embedded in a pectin network (*22*−*24*). Pectins are the major components of the primary cell wall and middle lamella of plant cells, and main components are acidic rhamnogalacturonans, homogalacturonan, as well as neutral arabinan, galactan, and arabinogalactan polymers (*25*). Hemicellulose represents a complex of xyloglucans, xylans, and mannans, which are bound tightly to the surface of cellulose.

The fruit of strawberry cv. Korona responded to NaCl stress by an increase of LMP and a decrease of NSP. The higher level of ionically bound pectinates in salt-stressed Korona fruit is reminiscent of the increase of ethylenediaminetetraacetic acid-soluble pectins found in strawberry fruit after heat treatment (*26*). This response was related to an increase in pectin methylesterase (PME) activity. PME catalyzes the elimination of methyl residues from esterified pectins, allowing further degradation by polygalacturonase (PG). Because the increase in LMP of salt-treated Korona fruit was compensated by the diminution of the NSP fraction, it is obvious that covalently bound pectic substances should have represented the substrate of PME. If these pectinates were not further degradated, for example, by PG and β-galactosidase (GAL), the first enzyme catalyzing the cleavage of pectin backbones and the second removing nonreducing terminal galactosyl residues from side chains of pectin polysaccharides (*26*), de-esterification of homogalacturonans should have generated calcium and magnesium bridges that stabilized the pectin polysaccharides of the cell walls by ionic and coordinate binding within polygalacturonic acid regions, thus reinforcing the cell wall structure (*27*, *28*). This response should also have resulted in a rigidification and a more sturdy structure of cell walls by impairing the swelling of pectinates (*8*, *9*) as observed, for example, in tomato

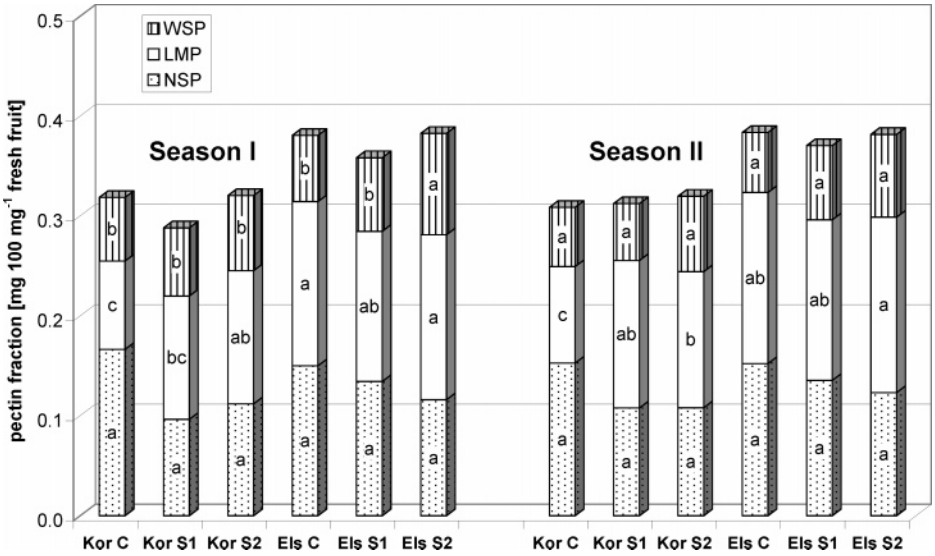

Figure 2. Quantitative composition of the pectin fractions WSP, LMP, and NSP expressed per fresh FM of fruits grown on plants treated by 0 (C), 40 (S1), or 80 (S2) mmol/L NaCl. Season I, one season of salt stress; season II, two seasons of salt stress. For each fraction, different letters indicate significant differences for fruits harvested in the same growing season by Tamhane or Duncan tests at $P < 5\%$.

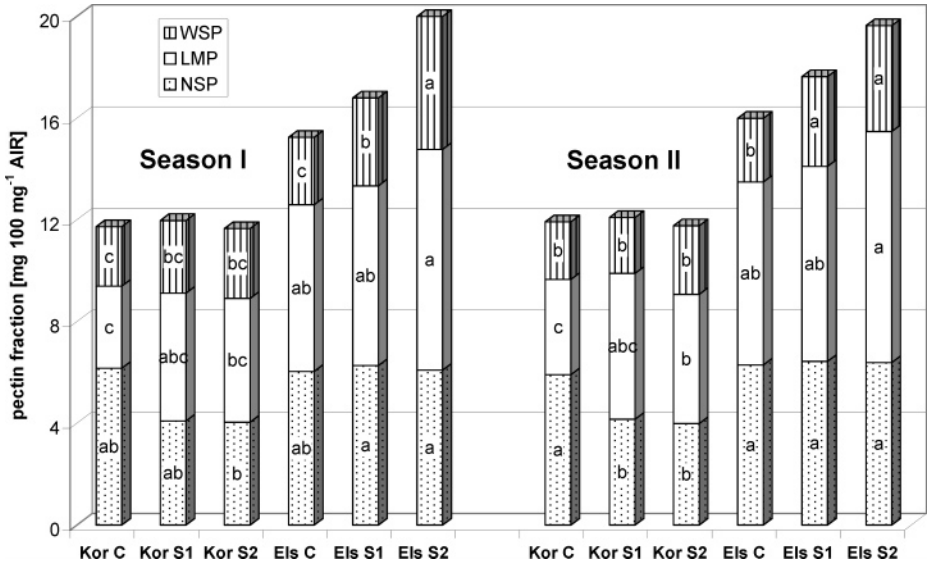

Figure 3. Quanititative composition of the pectin fractions WSP, LMP, and NSP expressed per 100 mg AIR of fruits grown on plants treated by 0 (C), 40 (S1), or 80 (S2) mmol/L NaCl. Season I, one season of salt stress; season II, two seasons of salt stress. For each fraction, different letters indicate significant differences for fruits harvested in the same growing season by Tamhane or Duncan tests at $P < 5\%$.

fruit grown under elevated salinity (*10*, *29*). These explanations are well in line with the result that fruit firmness was not impaired in cv. Korona (**Figure 1**). On the basis of these results, the hypothesis may be put forward that the response of Korona fruit to salt stress could be explained by modifications in the activities of PME, PG, and GAL.

The response of the more salt-sensitive cv. Elsanta to NaCl in the root medium was more complex than that of cv. Korona. The AIR, which reflects the contents of cell walls, was significantly reduced in NaCl-stressed fruits of cv. Elsanta. For strawberry fruit, it has been documented (*15*) that the content of AIR is not correlated with strawberry fruit firmness, when different genotypes are compared, but a relationship between fruit softening and AIR content within cultivars was evident, as was the case in cv. Elsanta (**Figure 1**). Also, in cells of tobacco grown under severe osmotic stress, weaker cell walls were accompanied by a lower proportion of the total amount of cell wall and an increase of soluble material within the pectic substances (*30*).

In NaCl-stressed Elsanta fruit, not only the contents of cell walls decreased but also the amount of WSP rose. The observed increase in WSP, expressed not only per AIR but also per fruit FM, came from pectin degradation rather than de novo synthesis. The latter can be excluded at late ripening stages of strawberry fruit (*28*). The increase WSP indicates that covalently bound pectic substances were released and became soluble in salt-stressed fruit of cv. Elsanta (*28*, *31*, *32*). This represents a typical feature of fruit softening during ripening (*15*).

The amount of hemicelluloses and cellulose per fruit FM was significantly reduced in cv. Elsanta (**Table 1**), which is also in line with fruit ripening (*15*, *33*). The enzymes endo-1,4-β-D-glucanase (EG) and β-xylosidase (XYL), the first hydrolyzing internal linkages of 1,4-β-D-glucan and the second decomposing xylans (*26*), possibly in cooperation with cell wall-modifying proteins such as expansins, could have been involved in the progressive destabilization of fruit cell walls (*17*, *26*). Because of its high activity in ripe strawberry fruit (*34*, *35*), EG might have contributed to the hydrolyzation of noncrystalline regions

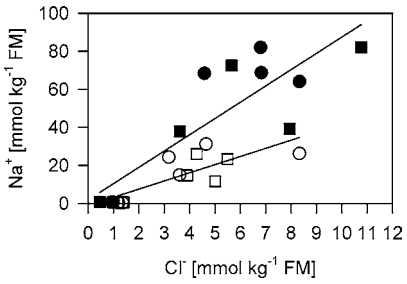

Figure 4. Relationship of fruit Na^+ and Cl^- contents as affected by cultivar (Elsanta: ○, ●; Korona: ■, □) and growing season (plants exposed for 1 year to NaCl: ○, □; plants exposed for 2 years to NaCl: ●, ■). The regression lines indicate the relationship between Na^+ and Cl^- incorporation irrespective of cultivar to show the effect of plant age on Na^+ uptake.

of cellulose, hemicelluloses, xyloglucans, and glucomannans (*26*), whereas xylans and some pectates of the fruit cell wall have been suggested as the main target of XYL. β-Xylose may account for 5−10% of hemicellulose composition (*26*); hence, this enzyme could have degraded this cell wall compound in the fruit of cv. Elsanta, although its activity has been reported to decline during the late stage of fruit ripening (*36*).

The response of the more salt-sensitive cv. Elsanta to NaCl in the root medium is reminiscent of the fruit ripening processes, that is, the extensive decrease of fruit cell wall content measured as AIR, the increase in WSP, and the diminution of hemicelluloses and cellulose (*15*). Pectin solubilization and hemicelluloses/cellulose degradation contribute significantly to softer fruits in cv. Elsanta. The entire response is best explained by an acceleration of fruit ripening under NaCl salinity stress in this genotype.

Worthy of note is the smaller mean fruit size, especially in cv. Elsanta, but also in cv. Korona (**Table 1**) grown under NaCl salinity. Because it is not related to differences in fruit water content, it is reminiscent of the smaller cell expansion rate and smaller volume of expanded cells of tobacco growing in culture medium under osmotic stress (*30*). Tobacco cells decreased their ability of the cell wall to expand despite osmotic adjustment and diversion of carbon away from the synthesis of cell wall polysaccharides. Also, in other species, plant organs fail to enlarge despite complete osmotic adjustment and reestablishment of adequate turgor (*37*). Observations on NaCl-stressed cells growing in culture medium suggest that changes in extensibility or other mechanical properties of the cell wall are responsible for the failure to expand (*38*). The loss of tensile strength was correlated with a substantial decrease in the proportion of crystalline cellulose in the primary cell wall, indicating a reduction in the mass of the cellulose−extensin network (*30*). However, the significance of this observation in strawberry is open to discussion, because the small amount of cellulose found in strawberry fruit remained unchanged during ripening (*15*, *16*). Moreover, substantial alterations in the chemical composition and organization of the pectic substances in cell walls have been observed (*39*), which may be involved in reducing the ability of the cell wall to extend. These extracellular polymers are composed mainly of arabinogalactan proteins, uronic acid-rich material, smaller amounts of protein and small polysaccharides, and oligosaccharide fragments with chemical compositions resembling hemicellulosic polysaccharides found in the primary cell wall (*38*). At least part of these polymers, for example, arabinogalactan proteins, are involved in membrane stabilization (*40*). Thus, in cv. Elsanta, decreasing fruit firmness seems best reflected by the increase in WSP and is physiologically coupled with the reduced size of fruits grown under NaCl salinity.

Na^+ and Cl^- contents of fruits correlated significantly with each other (Figure 4). The comparison of the Na^+/Cl^- ratios, calculated for each year of experiment, showed that the Na^+ levels were higher in fruits of plants exposed for 2 years to NaCl than in fruits of plants grown for only 1 year under salinity stress. The Na^+-buffering capacities of root and crown were limited during the second year of experiment as a consequence of the salinity load during the first year, resulting in higher Na^+ translocation rates to fruits during the second year. This observation may be of significance in order to decide whether Na^+ or Cl^- is the causative agent and responsible of certain NaCl salinity-related symptoms. In the present study, none of the measured concentrations of AIR, WSP, LMP, NSP, as well as hemicelluloses and cellulose were significantly correlated with Na^+ contents of fruits ($r^2 < 0.26$), implying that Cl^- is the causative agent initiating the signaling cascade that induces the changes in cell wall metabolism in general and pectin in particular. For example, the Cl^- content was significantly and negatively correlated with NSP content when expressed per fruit FM in both cultivars ($r = -0.916$ and $= -0.852$ for Elsanta and Korona, respectively), indicating that covalently bound pectic substances were degraded. It may be added that due to the observed differences in Na^+ and Cl^- transport between years, it may be necessary to conduct at least 2 year studies to investigate the response of strawberry cultivars to NaCl salinity, at least with respect to fruits.

ABBREVIATIONS USED

EG, endo-1,4-β-D-glucanase; FM, fresh mass; GAL, β-galactosidase; LMP, low methoxy pectinates; NSP, NaOH-soluble pectinates; PG, polygalacturonase; PME, pectin methyl-esterase; WSP, water-soluble pectins; XYL, β-xylosidase.

ACKNOWLEDGMENT

We thank the co-workers of the Section of Quality of Plant Products for technical support: Gunda Jansen and Antje Wulkow for cultivation, harvest, and sample preparation.

LITERATURE CITED

(1) Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press: London, Great Britain, 1995; 889 pp.
(2) Cheeseman, J. M. Mechanisms of salinity tolerance in plants. *Plant Physiol.* **1988**, *87*, 547−550.
(3) Greenway, H.; Munns, R. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.* **1980**, *31*, 149−190.
(4) Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.* **2002**, *25*, 239−250.
(5) Kinraide, T. B. Possible influence of cell walls upon ion concentrations at plasma membrane surfaces. Toward a comprehensive view of cell-surface electrical effects upon ion uptake, intoxication, and amelioration. *Plant Physiol.* **2004**, *136*, 3804−3813.
(6) Chazen, O.; Neumann, P. M. Hydraulic signals from the roots and rapid cell-wall hardening in growing maize (*Zea mays* L.) leaves are primary responses to polyethylene glycol-induced water deficits. *Plant Physiol.* **1994**, *104*, 1385−1392.
(7) Passioura, J. B. The physical chemistry of the primary cell wall: Implications for the control of expansion rate. *J. Exp. Bot.* **1994**, *45*, 1675−1682.
(8) Cosgrove, D. J. Relaxation in a high-stress environment: The molecular bases of extensible cell walls and cell enlargement. *Plant Cell* **1997**, *9*, 1031−1041.

(9) Ryden, P.; MacDougall, A. J.; Tibbits, C. W.; Ring, S. G. Hydration of pectic polysaccharides. *Biopolymers* **2000**, *54*, 398−405.
(10) Sato, S.; Sakaguchi, S.; Furukawa, H.; Ikeda, H. Effects of NaCl application to hydroponic nutrient solution on fruit characteristic of tomato (*Lycopersicon esculentum* Mill.). *Sci. Hortic*. **2006**, *109*, 248−253.
(11) Serio, F.; De Gara, L.; Caretto, S.; Leo, L.; Santamaria, P. Influence of an increased NaCl concentration on yield and quality of cherry tomato grown in posidonia (*Posidonia oceanica* (L.) Delile). *J. Sci. Food Agric*. **2004**, *84*, 1885−1890.
(12) Awang, Y. B.; Atherton, J. G. Growth and fruiting responses of strawberry plants grown on rockwool to shading and salinity. *Sci. Hortic*. **1995**, *62*, 25−31.
(13) Awang, Y. B.; Atherton, J. G.; Taylor, A. J. Salinity effects on strawberry plants grown in rockwool. II. Fruit quality. *J. Hortic. Sci*. **1993**, *68*, 791−795.
(14) Galletta, G. J.; Bringhurst, P. J. Strawberry management. In *Small Fruit Crop Management*; Galletta, G. J., Himelrick, D. G., Eds.; Prentice Hall: New Jersey, 1990; pp 83−156.
(15) Rosli, H. G.; Civello, P. M.; Martinez, G. A. Changes in cell wall composition of three *Fragaria* × *ananassa* cultivars with different softening rate during ripening. *Plant Physiol. Biochem*. **2004**, *42*, 823−831.
(16) Koh, T. H.; Melton, L. D.; Newman, R. H. Solid state ^{13}C NMR characterization of cell walls of ripening strawberries. *Can. J. Bot*. **1997**, *75*, 1957−1964.
(17) Woolley, L. C.; James, D. J.; Manning, K. Purification and properties of an endo-β-1,4-glucanase from strawberry and down-regulation of the corresponding gene, *cel*. *Planta* **2001**, *214*, 11−21.
(18) Keutgen, A. J.; Keutgen, N. Influence of NaCl salinity stress on fruit quality in strawberry. *Acta Hortic*. **2003**, *609*, 155−157.
(19) Keutgen, A. J.; Pawelzik, E. Modifications of strawberry fruit antioxidant pools and fruit quality under NaCl stress. *J. Agric. Food Chem*. **2007**, *55*, 4066−4072.
(20) Mackey, A. C.; Hard, M. M.; Zaehringer, M. V. *Measuring of Textural Characteristic of Fresh Fruits and Vegetables—Apples, Carrots, and Cantaloupes. A Manual of Selected Procedures*; Technical Bulletin; Agricultural Experiment Station, Oregon State University: Corvallis, OR, 2004; Vol. 123 (update), pp 1−26.
(21) Manning, K. Soft fruit. In *Biochemistry of Fruit Ripening*; Seymour, G. B., Taylor, J. E., Tucker, G. A., Eds.; Chapman & Hall: London, Great Britain, 1993; pp 347−377.
(22) Shiga, T. M.; Lajolo, F. M.; Filisetti, T. M. C. C. Changes in the cell wall polysaccharides during storage and hardening of beans. *Food Chem*. **2004**, *84*, 53−64.
(23) Yakushiji, H.; Sakurai, N.; Morinaga, K. Changes in cell wall polysaccharides from the mesocarp of grape berries during veraison. *Physiol. Plant* **2001**, *11*, 188−195.
(24) Carpita, N. C.; Gibeaut, D. M. Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *Plant J*. **199**3, *3*, 1−30.
(25) Cosgrove, D. J. Growth of the plant cell wall. *Nat. Rev./Mol. Cell Biol*. **2005**, *6*, 850−861.
(26) Vicente, A. R.; Costa, M. L.; Martinez, G. A.; Chaves, A. R.; Civello, P. M. Effect of heat treatment on cell wall degradation and softening in strawberry fruit. *Postharvest Biol. Technol*. **2005**, *38*, 213−222.
(27) Barnavon, L.; Doco, T.; Terrier, N.; Ageorges, A.; Romieu, C.; Pellerin, P. Involvement of pectin methyl-esterase during the ripening of grape berries: Partial cDNA isolation, transcript expression and changes in the degree of methyl-esterification of cell wall pectins. *Phytochemistry* **2001**, *58*, 693−701.
(28) Knee, M.; Sargent, J. A.; Osborne, D. J. Cell wall metabolism in developing strawberry fruits. *J. Exp. Physiol*. **1977**, *28*, 377−396.
(29) Petersen, K. K.; Willumsen, J.; Kaach, K. Composition and taste of tomato as affected by increased salinity and different salinity sources. *J. Hortic. Sci. Biotechnol*. **1998**, *73*, 205−215.
(30) Iraki, N. M.; Bressan, R. A.; Hasegawa, P. M.; Carpita, N. C. Alteration of the physical and chemical structure of the primary cell wall of growth-limited plant cells adapted to osmotic stress. *Plant Physiol*. **1989a**, *91*, 39−47.
(31) Redgwell, R. J.; MacRae, E.; Hallett, I.; Fisher, M.; Perry, J.; Harker, R. *In vitro* and *in vivo* swelling of cell walls during fruit ripening. *Planta* **1997**, *203*, 162−173.
(32) Huber, D. J. Strawberry fruit softening: The potential role of polyuronides and hemicelluloses. *J. Food Sci*. **1984**, *49*, 1310−1315.
(33) Koh, T. H.; Melton, L. D. Ripening-related changes in cell wall polysaccharides of strawberry cortical and pith tissues. *Postharvest Biol. Technol*. **2002**, *26*, 23−33.
(34) Harpster, M. H.; Brummell, D. A.; Dunsmuir, P. Expression analysis of a ripening specific, auxin repressed endo-1,4-β-glucanase gene in strawberry fruit. *Plant Physiol*. **1998**, *118*, 1316−1317.
(35) Trainotti, L.; Spolaore, S.; Pavanello, A.; Baldan, B.; Casadoro, G. A novel E type endo-β-1,4-glucanase with a putative cellulose-binding domain is highly expressed in ripening strawberry fruits. *Plant Mol. Biol*. **1999**, *40*, 323−332.
(36) Martinez, G. A.; Chaves, A. R.; Civello, P. M. β-xylosidase activity and expression of a β-xylosidase gene during strawberry fruit ripening. *Plant Physiol. Biochem*. **2004**, *42*, 89−96.
(37) Termaat, A.; Passioura, J. B.; Munns, R. Shoot turgor does not limit shoot growth of NaCl-affected wheat and barley. *Plant Physiol*. **1985**, *77*, 869−872.
(38) Iraki, N. M.; Bressan, R. A.; Carpita, N. C. Extracellular polysaccharides and proteins of tobacco cell cultures and changes in composition associated with growth-limiting adaptation to water and saline stress. *Plant Physiol*. **1989c**, *91*, 54−61.
(39) Iraki, N. M.; Singh, N.; Bressan, R. A.; Carpita, N. C. Cell walls of tobacco cells and changes in composition associated with reduced growth upon adaptation to water and saline stress. *Plant Physiol*. **1989**, *91*, 48−53.
(40) Lamport, D. T. A.; Kieliszewksi, M. J.; Showalter, A. M. Salt-stress upregulates periplasmic arabinogalactan-proteins: Using salt-stress to analyse AGP function. *New Phytol*. **2006**, *169*, 479−492.

Received for review April 25, 2007. Revised manuscript received June 25, 2007. Accepted June 28, 2007.

JF071216O