Sugar Metabolism and Pineapple Flesh Translucency

Ching-Cheng Chen¹ and Robert E. Paull²

Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources, 3190 Maile Way, Honolulu, HI 96822

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ABSTRACT. Sugar accumulation and the activities of sugar metabolizing enzymes were related to the occurrence of pineapple [*Ananas comosus* (L.) Merr.] flesh translucency. During early fruit development, glucose and fructose were the predominant sugars. Sucrose began to accumulate 6 weeks before harvest at a higher rate in the fruitlet than in the interfruitlet tissue. Electrolyte leakage from pineapple flesh increased rapidly from 6 weeks before harvest and paralleled sucrose accumulation. Sucrose synthase activity was high in young fruit flesh and declined with fruit development, while the activity of sucrose phosphate synthase was relatively low and constant throughout fruit development. The activities of acid invertase, neutral invertase, and cell-wall invertase (CWI) were high in the young fruit flesh and declined to very low levels 6 weeks before harvest when sucrose started to accumulate. CWI activity increased again, more in the fruitlet than in the interfruitlet tissue, 4 weeks before harvest. Removal of 1/3 of the plant leaves 3 weeks before harvest significantly reduced fruit flesh total soluble solids, CWI activity, and translucency incidence at harvest. The activity of CWI in translucent fruit flesh was significantly higher than in opaque fruit flesh at harvest. CWI activities in the basal section of pineapple flesh and in the fruitlet, where translucency first occurred, were higher than those in the apical section and in the interfruitlet tissue, respectively. Results support the hypothesis that high CWI activity in pineapple flesh at the later stage of fruit development enhances sucrose unloading into the fruit flesh apoplast, leading to increased apoplastic solute concentration (decreased solute potential) and subsequent water movement into the apoplast. This, in turn, may reduce porosity and lead to increased fruit flesh translucency.

Pineapple (*Ananas comosus*) is one of the most economically important crops in tropical and subtropical areas. Pineapple is a perennial herbaceous monocotyledonous plant that produces a single syncarpous fruit at the apex of the stem by fusion of a number of berrylike fruitlets, called eyes, that develop from the flowers. In 'Smooth Cayenne', the most widely grown cultivar, there are 100 to 200 flowers per inflorescence, which are arranged in a compact spiral cluster. Flowering occurs in acropetal sequence with one to several flowers opening each day (Okimoto, 1948). Ovaries of adjacent flowers, developing into fruitlets, are separated by the noncarpellary parenchymatous tissue, consisting largely of sepal and bract bases (Okimoto, 1948), called 'interfruitlet tissue'. Flower initiation takes place at the terminal axis of the stem and is induced naturally by short days and cool nights, or can be forced with chemicals, such as ethephon (2-chloroethylphosphonic acid). Chemical forcing of flowering is done commercially at any time of year when the plants are of sufficient size. The days from forcing to harvest varies with solar radiation and temperature and takes from 6 to 7 months (3 to 4 months from the end of flowering to harvest) in Hawaii (Nakasone and Paull, 1998).

Translucency is a physiological disorder of pineapple flesh. The affected flesh has low porosity and a water soaked appearance, due to the intercellular free spaces being filled with liquid (Sideris and Krauss, 1933). Highly translucent fruit has flat and overripe off flavors, and significantly lower edible quality (Bowden, 1967). Translucent fruit are prone to preharvest sunburn (Keetch, 1977) and are extremely fragile (Py et al., 1987), making these fruit

susceptible to mechanical damage during harvest and postharvest handling. Postharvest peduncle leakage may lead to unsightly mold growth on the broken peduncle end and is correlated with translucency (Paull and Reyes, 1996). Pineapple translucency starts to occur 3 to 4 weeks before harvest in Hawaii and the incidence and severity increase with development. The more mature basal flesh usually is the first to show translucency (Paull and Reyes, 1996), suggesting that translucency is related to maturity and possibly to sugar accumulation, as basal tissue has 3% to 4% higher total soluble solids than the top (Sideris and Krauss, 1933), and the ratio of total soluble solids to acidity of pineapple juice increases with increasing translucency (Bowden, 1969).

Sugar content is an important component of pineapple quality (Py et al., 1987). Even though the importance of sugar content in pineapple quality is recognized, our knowledge of carbohydrate metabolism in pineapples is limited. Different pathways are used for sugar accumulation in various fruit species (Hubbard et al., 1991), with different enzymes being involved, including acid invertase, sucrose synthase, and sucrose-phosphate synthase (Yamaki, 1995).

In translucent pineapple flesh, the low porosity suggests that the intercellular free air spaces are filled with liquid due possibly to increased membrane permeability of fruit flesh cells or enhanced water movement into the apoplast caused by sugar-induced solute potential gradient between symplast and apoplast. Developmental changes in fruit sugar composition and the activities of sugar metabolizing enzymes in pineapples have not been reported previously. In addition, the relationship between sugar accumulation and translucency occurrence is unknown. The objectives of this study were to determine the changes in the sugar composition and the activities of sugar metabolizing enzymes during pineapple development, and to describe the relationship between sugar accumulation and the occurrence of flesh translucency in mature pineapples.

Materials and Methods

PLANT AND FRUIT MATERIALS. The translucent susceptible 'Smooth Cayenne' pineapple (clone 3621) from the Dole Food Co. plantation on the island of Oahu in Hawaii was used in all experiments. The

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¹PhD fellow.

²Professor, to whom reprint requests should be addressed; e-mail: paull@hawaii.edu.

final date of fruit harvest in this study coincided within 1 week of commercial harvest. In Hawaii, commercial harvest for fresh fruit occurs when fruit have attained full size and as the color change begins to occur on the basal skin (Paull, 1997), and total soluble solids (TSS) reaches 12% (Nakasone and Paull, 1998). Changes in sugar content and the activities of sugar metabolizing enzymes in pineapple flesh during development were monitored by harvesting 24 fruit every 2 weeks from 12 weeks (≈ 2 weeks after end of flowering) before harvest. Half of the fruit was used for whole fruit flesh tissue analysis and the other half for fruitlet and interfruitlet tissue analysis (fruitlets were separated from interfruitlet tissues by a surgical knife). Flesh tissues from four fruit were diced and pooled together as one of three replications at each sampling date. Tissues were frozen immediately in liquid nitrogen after sampling and stored at -20°C until use.

Mature fruit, based on commercial standards, were grouped into the following categories by the percentage of area showing translucent flesh on a longitudinal cut half fruit: opaque (0%), low (10% to 30%), semi (40% to 60%), and high ($>70\%$) translucency. Cell-wall invertase (CWI) activity for each category was determined for three replications, comprised of a pooled sample of two fruit from each group. To determine fruit distribution of CWI, apical, medial, and basal sections of four semitranslucent (40% to 60% severity) fruit were assayed for activity. For determination of electrolyte leakage, six replicate fruit were used.

CARBOHYDRATE ANALYSIS. Frozen fruit flesh tissue (2 g) was heated to 90°C in a microwave oven to inactivate enzymes, then homogenized in 18 mL 90% (v/v) ethanol to extract soluble sugars (Paull et al., 1984). After standing for at least 24 h at 1°C , a 5 mL aliquot of the clear supernatant was dried under nitrogen gas and redissolved in 5 mL deionized water. Individual sugars were quantified by injecting a 20- μL aliquot of sample, filtered through a 0.45- μm filter, into a high-performance liquid chromatograph (HPLC) equipped with an automatic pump (Beckman Instrument Corp., Fullerton, Calif.), an automatic injector (model 725; Micromeritics Instrument Corp., Norcross, Ga.), an analysis column (Fast Carbohydrate, 100×7.8 mm, Bio-Rad Laboratories, Hercules, Calif.), a differential refractometer (model R401; Waters Corp., Milford, Mass.), and a reporting integrator (model 3390A; Hewlett Packard, Palo Alto, Calif.). The mobile phase was degassed deionized water, at a flow rate of $0.6 \text{ mL} \cdot \text{min}^{-1}$ and 80°C . Peak height measurements were used to quantify the soluble sugars by comparing them to peak height of a standard solution.

ENZYME EXTRACTION. The soluble enzymes, sucrose synthase (SS), sucrose phosphate synthase (SPS), acid invertase (AI) and neutral invertase (NI), were extracted as described by Hubbard et al. (1991), with some modifications. Frozen fruit flesh tissue was ground to a powder in liquid nitrogen, using a chilled mortar and pestle with a 1:4 tissue-to-buffer ratio. The buffer contained 100 mM 3-[N-morpholino] propanesulfonic acid (MOPS) (pH 7.5, adjusted with 5 M NaOH), 5 mM magnesium chloride, 1 mM ethylenediaminetetraacetic acid (EDTA), 2.5 mM DL-dithiothreitol (DTT), 0.05% (v/v) Triton X-100, 10 mM ascorbic acid, 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 μM trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butane (E-64), 1 $\text{mg} \cdot \text{mL}^{-1}$ bovine serum albumin (BSA), and 10 $\text{mg} \cdot \text{mL}^{-1}$ polyvinylpyrrolidone (PVPP). The homogenate was filtered through eight layers of cheesecloth and centrifuged at $20,000 g_n$ for 20 min. The supernatant was desalted (Amicon, CF25 membrane cone) with a buffer containing 100 mM MOPS (pH 7.5), 5 mM magnesium chloride, and 2.5 mM DTT. For cell-wall invertase (CWI) extraction, the homogenate, without filtering through cheesecloth, was centrifuged at $20,000 g_n$

for 30 min. After centrifugation, the supernatant was discarded, and the pellet washed with deionized water and centrifuged again at $20,000 g_n$ for 30 min. The pellet was then suspended in 10 mL 100 mM citrate-phosphate buffer (pH 4.5) containing 2.5 mM DTT, 1 M sodium chloride, and 1 $\text{mg} \cdot \text{mL}^{-1}$ BSA. The suspension was kept at 1°C for 24 h. The extract was then centrifuged at $20,000 g_n$ for 30 min. The supernatant was desalted as described above with a buffer containing 100 mM citrate-phosphate (pH 4.5), 5 mM magnesium chloride, and 2.5 mM DTT.

ENZYME ASSAY. SPS activity was assayed as described by Hubbard et al. (1989), with some modifications. Reaction mixtures (100 μL) contained 100 mM MOPS (pH 7.5), 15 mM magnesium chloride, 10 mM fructose-6-P, 50 mM glucose-6-P, 10 mM uridine 5'-diphosphoglucose (UDPG), and 50 μL desalted extract. Reaction mixtures were incubated at 35°C and terminated at 0 and 30 min with 100 μL of 30% (w/v) potassium hydroxide, followed by placing the tubes in boiling water for 10 min to destroy any unreacted reducing sugars. After cooling, 2 mL of a mixture of 0.15% (w/v) anthrone in 13.8 M sulfuric acid was added and incubated at 40°C

Fig. 1. Changes in sugar contents and the activities of sugar metabolizing enzymes in developing pineapple flesh. (A) Sucrose (Suc), glucose (Glu), and fructose (Fru) contents. (B) Activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS). (C) Activities of acid invertase (AI), neutral invertase (NI), and cell-wall invertase (CWI). Fruit were harvested every 2 weeks from 12 weeks before harvest. Symbols represent means $\pm$ SD of three replications.

Fig. 2. Changes in electrolyte leakage in pineapple flesh cells during fruit development. Fruit were harvested every 2 weeks from 10 weeks before harvest. Symbols represent means $\pm$ SD of six replications.

Table 1. Differences in electrolyte leakage between highly translucent and opaque pineapple flesh at harvest.

Translucency severity	Electrolyte leakage (%) ^z
High (>70%)	72.7 a ^y
Opaque (0%)	66.7 b

^zMean of six replications.

^yMean separation by LSD at $P = 0.05$.

in a water bath for 20 min. After cooling, color development was measured at 620 nm. SS was assayed in the sucrose synthesis direction and the procedure was identical to that of SPS except the reaction mixtures contained 20 mM fructose, without fructose-6-P or glucose-6-P. Reaction mixtures (100 μ L) to determine NI activity contained 100 mM MOPS (pH 7.5), 100 mM sucrose, and 50 μ L desalted extract. The mixtures were incubated at 35 °C and terminated at 0 and 30 min by placing the tubes into boiling water bath for 3 min. Hexose sugar concentration was determined as described by Honda et al. (1982) with slight modifications. After the reaction was terminated, 1.0 mL of cold 100 mM borate buffer (pH 9.0) was added, followed by 0.1 mL of 1% (w/v) 2-cyanoacetamide. Tubes

were then placed in a boiling water bath for 10 min. After cooling, the absorbance at 276 nm was determined. Measurements of reducing sugars were expressed relative to standard solutions containing equal concentrations of glucose and fructose. The procedures for AI and CWI assays were identical to that of NI except the reaction buffer was 100 mM citrate-phosphate (pH 4.5) and the reaction was terminated by adding 1.0 mL of cold 100 mM borate buffer (pH 9.0) instead of heating to prevent acid breakdown of sucrose.

ELECTROLYTE LEAKAGE AND TSS DETERMINATIONS. For determination of electrolyte leakage, four longitudinal flesh plugs from the middle portion of individual fruit were removed with a cork borer (10 mm diameter) and sliced into $\approx$ 2 mm thick disks. The disks from each fruit were pooled and two sets of six disks ($\approx$ 6 g total) each were randomly selected and washed three times with deionized water to remove lysed cell material. The disks were then shaken gently and incubated in 60 mL of 0.3 M mannitol solution for 2 h. The conductivity of this solution was then determined by a conductivity meter (model CDM83; Radiometer America Inc., Westlake, Ohio). The sample was then boiled for 2 h to release all electrolytes and total conductivity determined after adjusting the volume lost during boiling with deionized water. Electrolyte leakage was represented as the percentage of the total conductivity. TSS of fruit flesh juice taken from the middle portion of the fruit was determined by refractometry.

LEAF DEFOLIATION. Field-grown plants were defoliated 3 weeks before harvest. Total number of leaves were counted and none, one-third, two-thirds, or all leaves were removed, starting from the top youngest leaf and moving downward. Each treatment had four replications with eight plants each. Fruit fresh weight, TSS, and translucency incidence (percentage of translucent fruit) were determined at harvest. For determination of electrolyte leakage, each treatment had six replicate fruit, and three replicate fruit for CWI activity assay.

STATISTICAL ANALYSIS. Data were subjected to analyses of variance and least significant difference (LSD) procedures were used for mean separation when the F test was significant at $P = 0.05$ (SAS Institute, Inc., Cary, N.C.).

Results

Total soluble sugar content was low and composed mainly of glucose and fructose, with glucose at a slightly higher concentration than fructose in the pineapple flesh from 12 to 8 weeks before

Table 2. Effects of defoliation 3 weeks before harvest on pineapple characteristics at harvest. Leaves were removed from the top youngest downward.

Defoliation level	TSS (%)	Translucency incidence (%) ^z	CWI activity (μ mol·h ⁻¹ ·g ⁻¹ FW)	Fruit FW (g)	Electrolyte leakage (%)
All leaves removed	8.4 d ^y	6.3 c	1.7 d	1416	60.6
Two thirds removed	9.3 c	12.5 bc	2.9 c	1443	61.4
One third removed	10.2 b	34.4 b	4.7 b	1463	63.9
None (control)	11.2 a	71.9 a	10.5 a	1495	67.0
Linear regression analysis ^x					
R^2	0.91	0.71	0.87		
$P > F$	1.09×10^{-8}	4.08×10^{-5}	1.13×10^{-5}		

^zAnalysis of variance for the means of translucency incidence was based on the angular transformed data. Data presented are the original untransformed means.

^yMean separation within columns by LSD at $P = 0.05$. Values of TSS, translucency incidence, and fruit FW are the means of four replications. Values of CWI activity and electrolyte leakage are the means of three and six replications, respectively.

^xLinear regression analysis was used on defoliation level over fruit characteristics.

Table 3. Differences in pineapple cell-wall invertase activity from flesh tissues at harvest having different levels of translucency severity.

Translucency severity	Activity ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ fresh wt) ^z
High (>70%)	8.4 a ^y
Semi (40%–60%)	6.1 ab
Low (10%–30%)	4.3 bc
Opaque (0%)	1.5 c

^zMean of three replications.

^yMean separation by LSD at $P = 0.05$.

Table 4. Differences in the cell-wall invertase activity in various sections of semitranslucent (40% to 60% severity) pineapple flesh at harvest, cut transversely into three sections, apical, medial, and basal.

Fruit sections	Activity ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ fresh wt) ^z
Apical	5.9 b ^y
Medial	8.2 ab
Basal	11.8 a

^zMean of four replications.

^yMean separation by LSD at $P = 0.05$.

harvest (Fig. 1A). Sucrose began to accumulate rapidly 6 weeks before harvest and ultimately exceeded the glucose and fructose concentration. Though glucose and fructose remained relatively constant throughout development, there was a slight increase in fructose during the last 2 weeks of fruit development. The SS activity was high in young fruit and declined to low level 6 weeks before harvest, while SPS activity was relatively low and constant throughout fruit development (Fig. 1B). The activities of all three invertases (Fig. 1C) were $\approx 2.5 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ fresh weight (FW) 12 weeks before harvest and declined to less than $1 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ FW 6 weeks before harvest. The CWI activity increased again 4 weeks before harvest, while activities of NI and AI remained low.

Electrolyte leakage from pineapple flesh tissue increased rapidly from 6 weeks before harvest (Fig. 2). Electrolyte leakage from highly translucent (>70%) fruit flesh at harvest was 6% higher than that in the opaque fruit flesh (Table 1).

Defoliation conducted 3 weeks before harvest did not significantly affect fruit weight and electrolyte leakage at harvest, while fruit flesh TSS, translucency incidence, and CWI activity were significantly reduced (Table 2). The CWI activity increased with increased translucency severity (Table 3). The CWI activity in the basal section of semitranslucent pineapple flesh was also higher than that in the apical section (Table 4). The fruitlet showed translucency symptoms and accumulated sucrose earlier than the interfruitlet tissue (Fig. 3A). In addition, the CWI activity in the fruitlet also increased 2 weeks earlier than the interfruitlet tissue (Fig. 3B).

Discussion

The activities of sucrose hydrolyzing enzymes (SS and invertases) were high in young pineapples and declined while sucrose began to accumulate rapidly at the later stage of fruit development. Similar results were also observed in muskmelon [*Cucumis melo* L. (Reticulatus Group)] (Chrost and Schmitz, 1997; Hubbard et al., 1989; Lingle and Dunlap, 1987), tomato (*Lycopersicon peruvianum* L.) (Stommel, 1992), and peach [*Prunus persica* (L.) Batsch (Peach Group)] (Vizzotto et al., 1996). In muskmelon fruit, SPS activity increases with sucrose accumulation (Hubbard et al., 1989), however, in pineapples, the SPS activity (Fig. 1B) did not increase during

the period of sucrose accumulation, suggesting that activities of sucrose hydrolyzing enzymes (SS, AI, and NI) were sufficiently low to allow sucrose accumulation during this period.

Permeability of plasma and tonoplast membranes to sugars in apple flesh tissues [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] increases with fruit maturation (Yamaki and Ino, 1992). A breakdown of apoplast/symplast compartmentation, caused by declining cell membrane integrity, also occurs at the onset of grape berry [*Vitis vinifera* (L.) 'Riesling'] ripening as the rate of phloem translocation to the fruit suddenly increases (Lang and Düring, 1991). Pineapple translucency severity increases with development. In the present study, the average translucency severity of fruit used for sugar and enzyme analysis was 6% 2 weeks before harvest and 18% at harvest, and 4% 2 weeks before harvest and 19% at harvest from fruit used in the electrolyte leakage analysis (data not presented). Electrolyte leakage from pineapple flesh increased rapidly from 6 weeks before harvest (Fig. 2), paralleled the accumulation of sucrose, and was followed by the occurrence of translucency. However, electrolyte leakage from highly translucent (>70%) fruit flesh at harvest was only 6% higher than that in the opaque fruit flesh (Table 1). Similarly, Soler (1994) reported that the conductance of translucent pineapple flesh was only $\approx 4\%$ higher than that of normal fruit flesh. The small difference in electrolyte leakage between opaque and translucent fruit flesh led us to conclude that

Fig. 3. Differences in (A) sucrose content and (B) cell-wall invertase activity between fruitlet and interfruitlet tissues during pineapple development. Fruit were harvested every 2 weeks from 12 weeks before harvest. Symbols represent means $\pm$ SD of three replications.

membrane permeability may be related to, but was not a major factor in the occurrence of fruit translucency. This conclusion was supported by the fact that electrolyte leakage was not significantly affected by defoliation while translucency was significantly reduced (Table 2).

It has been suggested that the long-distance transport of carbohydrates, mostly sucrose, between source and sink is driven by the pressure gradient generated by loading of photoassimilate at the source and unloading at the sink organs (van Bel, 1992). The sink tissue apoplast can accumulate sugars; 13% of the total sugars in strawberry fruit (*Fragaria × ananassa* Duchesne) (John and Yamaki, 1994) and 20% of the sucrose in mature sugarcane tissue (*Saccharum* L. sp. 'H65-7052') (Welbaum and Meinzer, 1990) are held in the apoplast. An increase in the apoplastic solute level in the fruit flesh could promote movement of water from the phloem into the apoplast due to a decrease in the osmotic potential (Lang and Thorpe, 1986). The decreased apoplastic osmotic potential and the subsequent increase in water movement into the apoplast would favor the occurrence of pineapple translucency. Reduced photoassimilate supply caused by leaf defoliation during the last stage of pineapple development significantly reduced fruit flesh TSS and the translucency incidence at harvest (Table 2), suggesting that the occurrence of translucency may relate to sugar accumulation. Fruit FW was not significantly affected by defoliation and therefore fruit growth was not a confounding factor.

Since the removal of sucrose is believed to maintain the pressure gradient and the flow toward sinks (van Bel, 1992), enzymes involved in sucrose metabolism are important both for phloem unloading and for the import of sucrose into sink organs. The plant invertases, AI, NI, and CWI, may play important biological functions in source–sink relationships, phloem loading and unloading, and growth and development (Tymowska-Lalanne and Kreis, 1998). The role of CWI in apoplastic phloem unloading has been recognized (Roitsch et al., 1995). A high CWI activity in the sink tissue would favor maintenance of a sucrose gradient between the phloem end and the sink tissue apoplast, by cleaving unloaded sucrose into glucose and fructose. In pineapple flesh, the positive correlation between CWI activity and translucency suggests a close relationship between them. Since the physiological age of the fruitlet in the bottom of fruit is ≈3 weeks older than that of the top fruitlet (Okimoto, 1948) and the basal section has higher TSS than the apical section (Sideris and Krauss, 1933), the higher CWI activity in the basal section could be therefore related to maturity. In addition, the fruitlets, showing translucency symptoms earlier than the interfruitlet tissues, had higher CWI activity and sucrose content than the interfruitlet tissue during the last 4 weeks of fruit development (Fig. 3A and B). Therefore, the higher CWI activity at the later stage of pineapple development may be responsible for maintaining a sucrose gradient between the phloem and the flesh tissue apoplast. Moreover, a high CWI activity may increase the solute concentration, decreasing the osmotic potential in the apoplast and thereby enhance water movement into the apoplast. This, in turn, could reduce porosity and lead to flesh translucency. However, we still lack direct evidence to prove that sucrose unloading and subsequent cleavage by CWI in pineapple flesh apoplast increases the occurrence of translucency. Evidence showing that sucrose unloading and subsequent cleavage in the pineapple flesh apoplast occur at the later stage of fruit development will be supportive.

In summary, sucrose began to rapidly accumulate in pineapple flesh ≈6 weeks before harvest, paralleling an increase in membrane permeability and decreases in activities of sucrose hydrolyzing enzymes (SS, AI, and NI). The activity of CWI in pineapple flesh

increased rapidly 4 weeks before harvest and was followed by the first symptom of translucency. Pineapple translucency, which occurred at the later stage of fruit development, was correlated with sugar accumulation and high CWI activity, and was possibly caused by a decrease in the solute potential and subsequent increase in water volume in the apoplast. It is also possible that similar translucency symptoms may be caused by other factors, such as high field temperature, or cultivar differences (Py et al., 1987).

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