Effects of Calcium Chloride Spray on Peroxidase Activity and Stone Cell Development in Pear Fruit (Pyrus pyrifolia ‘Niitaka’)

Sang-Hyun Lee1,2, Jin-Ho Choi2, Wol-Soo Kim3, Yong-Seo Park4 and Hiroshi Gemma1 *

1Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305–8572, Japan
2Pear Experiment Station, RDA, Naju Chonnam 523–820, Korea
3Department of Horticulture, Institute of Agriculture Science and Technology, Chonnam National University, Gwangju 500–757, Korea
4Department of Horticulture, Mokpo National University, Muan 534–729, Korea

The primary objective of this study was to characterize the effects of calcium chloride spray on the formation of stone cells and peroxidase activity in ‘Niitaka’ pear fruit. Calcium chloride (0, 0.3, 0.5, and 1.0%) was sprayed 4 times on selected 15-year-old ‘Niitaka’ pear trees at 10-day intervals with a handgun until runoff, beginning 20 days after full bloom (DAFB). The calcium content and peroxidase activity were determined in flesh extracted from harvested fruit. The distribution of stone cells in the fruit flesh was determined via light microscopy, using phloroglucinol dye. The calcium contents of the leaves and fruit increased significantly in the fruit sprayed with 0.5 and 1% CaCl2, as compared to the control, or the fruit sprayed with 0.3% CaCl2. The stone cell contents in the fruit flesh decreased significantly when the fruit were sprayed with 0.5 and 1.0% CaCl2. Fruit treated with calcium chloride showed an increase in the rate of small stone cell cluster formation (<200 µm2), whereas the rate at which medium (200–400 µm2) and large (400 µm2<) clusters formed was significantly reduced. Peroxidase activity in the fruit flesh increased substantially at 60 DAFB, and decreased with fruit development. Peroxidase activity was much higher in the non-treated fruit than in those treated with 0.5% CaCl2. The activities of bound and soluble peroxidase in the cell wall were lower in the fruit treated with 0.5% CaCl2 than in the non-treated fruit. In our experiment, the stone cell content and stone cell size decreased significantly in fruit treated with calcium chloride, as the result of a reduction in lignification due to low peroxidase activity (soluble and bound).

Key Words: calcium content, cell wall peroxidase, cytoplasm, phloroglucinol.

Introduction

The quantity of stone cells and their aggregation materially affects the edible qualities of the pear. Many pear variants are characterized by an abundant development of stone cells in the flesh, showing thick and lignified walls (Dibuz, 1998).

Stone cells in the pear are chiefly composed of lignocellulose, and their formation is the result of lignifications, which can be quantitatively measured by the determination of lignocellulose contents (Crist and Batjer, 1931). Smith (1935) determined that the total cell wall content, as well as lignin, increased gradually up to harvest, and postulated that sugars accumulated and were converted to lignified tissue following an inverse relationship between the alcohol-soluble fraction and the synthesis of cell wall materials. Ranadive and Haard (1973) concluded that stone cells are lignocellulosic, containing approximately 18% lignin and 82% of a material principally composed of carbohydrates. Despite the results of previous studies regarding the chemical nature of stone cells, only a small amount of research has thus far focused on a method for reducing the amount of stone cell formation in pear flesh.

Peroxidases (POD; EC 1.11.1.7) have been implicated in several primary and secondary metabolic functions, including the regulation of cell elongation, wound healing, pathogen defense, ethylene production, and fruit ripening (Gaspar et al., 1985). POD-type enzymes are normally directed to the vacuole and apoplast via the endoplasmic reticulum. Whereas the basic chemical reactions of such peroxidases, namely the reduction of H2O2 via a large array of hydrogen donors, have been well established, the functions of these isoenzymes and

Received; April 10, 2006. Accepted; December 28, 2006.
* Corresponding author (E-mail: gemma@sakura.cc.tsukuba.ac.jp).
their differential regulation remain primarily unknown (Huystee, 1987). More specifically, in cases in which isoenzyme forms have been studied, cationic peroxidases have been implicated in auxin metabolism, whereas anionic peroxidases have been loosely implicated in lignification (Gaspar et al., 1985). POD expression levels have been demonstrably altered by several types of stresses, including wounding (Lagrimini, 1991). The basic peroxidase isoenzyme has been implicated in lignification in the endocarp of peach fruit (Abeles and Biles, 1991), similarly to that which occurs during tracheid differentiation in plant cell cultures (Church and Galston, 1988). In strawberries, this basic peroxidase isoenzyme, which is similar to its homologous isoform, contributes to the lignification of vascular tissues during fruit development (Lopez-Serrano and Ros Barcelo, 1995). Therefore, we have hypothesized that the activity of peroxidase, which participates in the lignification of plant tissue, may exert some effect on stone cell formation.

The Ca content of fruit has been known to exert desirable effects on physiological disorders, storage life, softening, and overall quality in fruit. Once Ca is incorporated into the sap, it is directed preferentially to the parts of the plant that exhibit the highest degree of transpiration. Therefore, when transpiration is intense and Ca uptake is insufficient, the organs with the least degree of transpiration tend to exhibit a Ca deficiency, as Ca is transported only minimally by the phloem (Hill, 1980). Faust (1989) asserted that Ca in the apoplast exerts a binding effect in the complex of polysaccharides and proteins comprising the cell wall, and that cytoplasmic Ca may regulate several enzyme activities. Ca that is covalently bound to the pectin fractions of the cell walls controls the cell separation characteristics of a given cell, and helps to maintain the firmness of fruit tissues (Siddiqui and Bangerth, 1995). On the other hand, Lipetz and Garro (1965), who worked with crown gall tissues (Siddiqui and Bangerth, 1995). Therefore, we have hypothesized that the activity of peroxidase, which participates in the lignification of plant tissue, may exert some effect on stone cell formation.

The Ca content of fruit has been known to exert desirable effects on physiological disorders, storage life, softening, and overall quality in fruit. Once Ca is incorporated into the sap, it is directed preferentially to the parts of the plant that exhibit the highest degree of transpiration. Therefore, when transpiration is intense and Ca uptake is insufficient, the organs with the least degree of transpiration tend to exhibit a Ca deficiency, as Ca is transported only minimally by the phloem (Hill, 1980). Faust (1989) asserted that Ca in the apoplast exerts a binding effect in the complex of polysaccharides and proteins comprising the cell wall, and that cytoplasmic Ca may regulate several enzyme activities. Ca that is covalently bound to the pectin fractions of the cell walls controls the cell separation characteristics of a given cell, and helps to maintain the firmness of fruit tissues (Siddiqui and Bangerth, 1995). On the other hand, Lipetz and Garro (1965), who worked with crown gall tissue cultures, observed that the presence of Ca induced the release of wall-bound peroxidase, and exerted a consequent reduction in lignin deposition.

In addition, calcium has been the most frequently studied foliar-applied nutrient, due to its demonstrated ability to enhance acclimation against some stresses. Anionic peroxidases have been loosely implicated in lignification (Gaspar et al., 1985). POD expression levels have been demonstrably altered by several types of stresses, including wounding (Lagrimini, 1991). The basic peroxidase isoenzyme has been implicated in lignification in the endocarp of peach fruit (Abeles and Biles, 1991), similarly to that which occurs during tracheid differentiation in plant cell cultures (Church and Galston, 1988). In strawberries, this basic peroxidase isoenzyme, which is similar to its homologous isoform, contributes to the lignification of vascular tissues during fruit development (Lopez-Serrano and Ros Barcelo, 1995). Therefore, we have hypothesized that the activity of peroxidase, which participates in the lignification of plant tissue, may exert some effect on stone cell formation.

The fruit were cut in half and dyed with phloroglucinol-HCl added sulfuric acid, in order to confirm the existence of stone cell clusters in the flesh. The distribution of stone cell clusters was then assessed and the development and formation of stone cells in the flesh of the pear. That is to say, if Ca can effect a reduction of peroxidase activity in the cell wall, stone cell formation might be controlled by Ca spray. Therefore, Ca spraying appears to be a good method by which plant tissues can be enriched with Ca when found to be deficient in this essential nutrient. CaCl2-based materials have been shown to be the most effective for spraying, and most advantageous with regard to the improvement of fruit quality in the pear (Raese and Drake, 2000). The primary objective of this study was to characterize the influence of Ca spray on peroxidase activity and on stone cell formation in the pear tree.

Materials and Methods

Tree materials and treatments

A uniform block of 15-year-old ‘Niitaka’ pear (Pyrus pyrifolia Nakai) trees was selected for this study. The trees were grafted onto Pyrus betulaefolia seedlings and spaced at 7.0 × 6.0 m intervals, with a grass cover crop growing underneath. This orchard was located in Naju, Chonnam province, Korea. The properties of the soil were determined at a depth of 0–20 cm at 20 days after full bloom (DAFB) using the A.O.A.C. (1990) method (Table 1). Calcium chloride (34% Ca) was sprayed using a handgun until runoff, at Ca concentrations of 0, 0.3, 0.5, and 1.0%. These Ca treatments were applied 4 times at 10-day intervals, beginning at 20 DAFB. Tap water was also sprayed as a control, for comparison with the Ca treatments. Each of the treatments was conducted with five single-tree replications.

Isolation and distribution of stone cells in flesh

At 90 DAFB, 10 fruits were acquired from five trees from each treatment group. Each of the fruits was peeled, cored, and diced into cubes. Twenty g pear flesh samples were then homogenized for 5 min in a warming blender with 500 mL distilled water. The homogenate was diluted with 1 L of 0.1 M NaCl. The suspension was incubated for 30 min at 20–22°C, and the supernatant was decanted. The sediment was incubated for 30 min with 500 mL of 0.5 N NaOH and decanted. Finally, the sediment was suspended for 30 min in 500 mL of 0.5 N HCl, decanted, and washed with distilled water. The above washing operations were then repeated several times, until the stone cells were free of extraneous cell debris (Ranadive and Haard, 1973).

The fruit were cut in half and dyed with phloroglucinol-HCl added sulfuric acid, in order to confirm the existence of stone cell clusters in the flesh. The distribution of stone cell clusters was then assessed

Table 1. Soil properties of the ‘Niitaka’ pear orchard used in the experiment.

| Items          | pH (H2O) (1 : 5) | Organic matter (%) | P2O5 (mg·kg⁻¹) | Ca (c mol·kg⁻¹) | K (c mol·kg⁻¹) | Mg (c mol·kg⁻¹) |
|----------------|-----------------|---------------------|----------------|----------------|----------------|----------------|
| Value          | 6.5 ± 0.33a     | 1.8 ± 0.21          | 785 ± 117      | 5.0 ± 0.57      | 1.8 ± 0.23      | 2.5 ± 0.79     |

a Each value means ± SD (n = 3).
Calcium contents

Young fully-expanded leaves five and six nodes from the top were picked at 90 DAFB and thoroughly washed with de-ionized and distilled water, in order to remove any contaminants on the leaf surface prior to extraction and drying in an oven at 70°C for 72 h. After this step, the leaves were ground prior to determination of the Ca content. For fruit Ca analyses, 50 fruits per treatment (ten samples from each of tree 1–5) were harvested at 90 DAFB. Individual fruits were peeled and sliced, dried in an oven at 70°C for 72 h, and subsequently ground. Each fruit was then washed thoroughly with de-ionized and distilled water prior to grinding, in order to remove any external contamination. The Ca contents of the leaves and fruit were analyzed using an atomic absorption spectrophotometer (AAS 5300, Hitachi, Japan) after being subjected to acid digestion.

Peroxidase activity

POD activities were investigated 5 times over a 5-month period at 30-day intervals, beginning at 30 DAFB. Fifty fruits per treatment (ten samples from each of five trees) were harvested. Each of the fruits was peeled, cored, and diced into cubes.

Frozen fresh samples were homogenized with 50 mM phosphate buffer (pH 5.8) using a pestle and mortar, and then centrifuged for 20 min at 10000×g at 4°C (Lee and Lin, 1995). The supernatants were combined and used as soluble fractions. The pellets were collected and employed as cell wall fractions. The peroxidase (POD) in cell walls was extracted by 1 M NaCl solution at 30°C for 2 h, followed by 20 min of centrifugation at 20000×g at 4°C. Peroxidase activity was assayed in accordance with the method of Chance and Maehly (1955). The reaction mixture consisted of 50 µL of 20 mM guaiacol, 2.8 mL of 10 mM phosphate buffer (pH 7.0), and 0.1 mL of enzyme extract solution. The reaction was initiated via the addition of 20 µL of 40 mM H₂O₂. Increases in absorbance at 470 nm were recorded for 1 min with a UV-visible spectrophotometer (UV-1601, Shimadzu, Japan). POD activity was calculated using an absorption coefficient for tetraguaiacol, 26.6 mM·cm⁻¹ at 470 nm. One unit of enzyme activity was defined as the amount of enzyme required for the formation of 1 µmol of tetraguaiacol per min.

Microscopic analysis

Small cubes of tissue were cut from the pear flesh at 60 DAFB, and fixed for 90 min in 2.5% (v/v) glutaraldehyde at 4°C. The samples were then washed 5 times with 0.1 M phosphate buffer (pH 7.2) and post-fixed for 90 min in 1% tetroxide at 4°C. After fixing, the samples were washed an additional 5 times with 0.1 M phosphate buffer (pH 7.2). Following dehydration in an ethanol series (40%, 60%, 90%, 95%, and 100%), the tissues were transferred to a mixed solution of ethanol and propylene (1:1, v/v), infiltrated, and then embedded in epon.

For light microscopic examination, the samples were cut to a thickness of 1.5 µm using a microtome (Ultracut R, Leica Co, Austria) and stained with Schiff’s reagent and 1% sodium bisulfite. The sections were examined and photographed under light microscopy (Axioskop 2. Karl Zeiss Co., Germany).

Results

The foliar applications of calcium chloride (CaCl₂) induced an elevation in the Ca content in the leaves and fruit (peel and flesh) of the pear trees as compared to controls (Table 2). The effects of CaCl₂ spray on calcium in the leaf, peel, and flesh and stone cell contents in ‘Niitaka’ pear fruits at 90 DAFB.

Table 2. Effects of calcium chloride spray on calcium in the leaf, peel, and flesh and stone cell contents in ‘Niitaka’ pear fruits at 90 DAFB.

| Treatment | Calcium contents (mol·g⁻¹ DW) | Stone cell content (mg·g⁻¹ FW) |
|-----------|-------------------------------|-------------------------------|
|           | Leaf                         | Peel                          | Flesh                         |                                 |
| Control   | 0.336 c                      | 0.039 c                       | 0.010 b                       | 25.6 a                         |
| 0.3% CaCl₂| 0.378 b                      | 0.042 b                       | 0.011 b                       | 24.9 a                         |
| 0.5% CaCl₂| 0.423 a                      | 0.047 a                       | 0.012 a                       | 17.3 b                         |
| 1.0% CaCl₂| 0.421 a                      | 0.048 a                       | 0.013 a                       | 20.8 ab                        |

*CaCl₂ was sprayed in 10-day intervals 4 times from 20 to 60 DAFB.

Means within columns separated by Tukey’s Studentized Range Test, P<0.05.
Ca contents in the leaves and fruit increased along with increasing Ca concentrations, and were detected at maximal levels in fruit treated with 1.0% CaCl₂ (Table 2). However, some physiological injury was observed, in which the leaf margin had turned brown and fruit lenticels had darkened. The stone cell contents in the flesh declined as a result of Ca treatment and were determined to be significantly lower in fruit sprayed with 0.5% and 1.0% CaCl₂ than in control fruit or fruit sprayed with 0.5% CaCl₂.

With regard to the rates of stone cell distribution according to their sizes (Figs. 1 and 2), small clusters (less than 200 µm²) were much more sparse in the non-treated fruit than in the treated fruit. Ca spraying, however, resulted in a decrease in medium (200–400 µm) and large (more than 400 µm) stone cell clusters, as compared to the controls. Therefore, Ca spraying resulted in a reduction in the size of stone cell clusters in pear fruit, regardless of the Ca concentration.

Peroxidase activity in flesh spiked abruptly at 60 DAFB and decreased considerably until 90 DAFB, and its activity was much lower in plants treated with 0.5% CaCl₂ than the control at the initial stages of fruit development. We detected no differences in peroxidase activity between 90 and 150 DAFB (Fig. 3). At 60 DAFB, the activities of cell wall bound and soluble peroxidase were lower in fruit treated with 0.5% CaCl₂ than in the control (Fig. 4).
Discussion

The majority of postharvest disorders may prove to be related to preharvest factors in a variety of fruit, including Asian pears. Ca has been considered to be one of the preharvest factors that significantly influence the development of disorders. A low level of Ca during fruit development has been shown to induce some alterations in metabolism, indicating low mobility within trees. Ca absorption is limited in the roots, and so foliar spraying is crucial during the growing season, in order to enhance the Ca content of fruit (Ford and Quinlan, 1979).

Foliar applications of 0.5% Ca during the early stages of fruit development resulted in an augmentation of the Ca content of leaves and fruit (peel and flesh). This finding is consistent with results suggesting that the spraying of calcium chloride increased the Ca content of fruit and reduced the incidence of cork spots in ‘Anjou’ pears (Pyrus communis L.) during four seasons (Raese and Drake, 1995). In this study, it was reported that five kinds of calcium chloride spray at 3-week intervals from late May or early June to middle or late August constituted a viable approach for the augmentation of Ca and the control of cork spots in fruit. Yoon et al. (2000) reported that the Ca content of leaves and fruit increased as the result of foliar applications of 0.3% calcium chloride to ‘Niitaka’ pears, at 50 DAFB.

However, physiological injuries were also apparent with the application of 1.0% calcium chloride. This phenomenon may be attributed to Ca toxicity. Raese and Drake (1995) demonstrated that Ca spraying inflicted some degree of damage on leaves and fruits. They also reported that fruits treated with low concentrations of Ca were injured to the lowest degree, and these injuries tended to be less severe early in the season (June to July) than late in the season (July to August) in leaves and fruit of ‘Anjou’ pears. Consequently, the high Ca content in leaves and fruit at 90 DAFB may be attributable to the ready absorption of Ca in foliar applications.

A reduction of peroxidase activity in fruit flesh has been observed following the application of 0.5% calcium chloride. This tendency can be observed in the peroxidase activity of the cell wall and cytoplasm of fruit sprayed with calcium chloride. Peroxidase activity in these organs may be associated with the Ca content. Ranadive and Haard (1972) identified a correlation between peroxidase activity and lignification in cell walls of pear fruit, and demonstrated differences in peroxidase activity at different Ca concentrations. Penel and Greppin (1994) showed that isoperoxidases exhibited an affinity for pectins in their Ca$^{2+}$-induced conformations, via a variety of in vivo binding trials. Ca$^{2+}$ appears to be necessary because it induces the cross-linking of polygalacturonan chains into a structure that can be recognized by its isoperoxidase (Penel et al., 1999). The above results indicated that Ca within a cell wall can be affected by peroxidase activity.

However, the isoenzyme pattern of peroxidase also appears to be very dependent on the developmental stage of the plant. Much of the peroxidase activity is present in the cell wall in both young tissues and lignified parts (De Jong, 1967). Ryugo (1969) has reported that a close correlation exists between the lignin synthesis rate and the activities of catalase and peroxidase. Lopez-Serrano and Ros Barcelo (1995) insisted that this basic peroxidase isoenzyme is involved in the lignification of vascular tissues during fruit development in the strawberry. Another important approach to clarify the role of peroxidases in lignification is related to the obtaining of transgenic plants with modified peroxidase activity. Lagrimini (1991) obtained several transgenic lines of tobacco with significant over-expression or down-regulation of a specific peroxidase supposed to be involved in lignification. Although it was suggested to be a function of indole acetic acid (IAA), the plants with a lower POD activity showed less lignin deposition in the cell wall (Sitbon et al., 1999).

In our experiment, lignification was shown to be attenuated in the cell walls of flesh, due to low peroxidase activity, when fruit were sprayed with calcium chloride in a range of 0.5–1.0%.

A decrease in the stone cell content and stone cell cluster size was apparent in fruit that had been sprayed with calcium chloride. Under light microscopy, the stone cells in fruit were small and uniform. This may be attributable to differences in peroxidase activity, and this phenomenon appears to be associated with lignification of the cell wall (Ranadive and Haard, 1972). Moon et al. (2002) reported that the middle lamella of the cell wall exhibited an elevated degree of integrity in fruit treated with liquid Ca, as compared to non-treated plants, on transmission electron microscopic (TEM) studies. In this experiment, the stone cell content declined in fruit sprayed with both 0.5 and 1.0% CaCl$_2$, whereas leaf and fruit injuries occurred as the result of 1.0% CaCl$_2$ treatment. Ca, when applied as a foliar treatment, is absorbed into the fruit and influences peroxidase activity in the cell wall. Low peroxidase activity results in a decrease in stone cell formation in the flesh, via a reduction of cell wall lignification. Also, stone cell contents were responsible for the overall texture of fruit, and may also be directly related with sucrose contents in the flesh of fruit (Lee and Kim, 2001).

In conclusion, the stone cell content and stone cell cluster size in ‘Niitaka’ pear were reduced by the spraying of calcium chloride onto fruit in a Ca concentration range of 0.5%, during the early stages of development.

Literature Cited

A.O.A.C. 1990. Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington, D.C.

Abeles, F. B. and C. L. Biles. 1991. Characterization of peroxidases in lignifying peach fruit endocarp. Plant Physiol. 95: 269–273.
Chance, B. and A. C. Maehly. 1955. Assay of catalases and peroxidases. Methods Enzymol. 2: 764–775.

Church, D. L. and A. W. Galston. 1988. Coenzyme a ligase isoperoxidase expression in Zinnia mesophyll cells induced to differentiate into tracheary elements. Plant Physiol. 88: 679–684.

Crist, J. W. and L. P. Batjer. 1931. The stone cells of pear fruits, especially the kieffer pear. Mic. Agric. Exp. Stn. Tech. Bull. 113: 1–55.

De Jong, D. W. 1967. An investigation of the role of plant peroxidase in cell wall development by the histochemical method. J. Histochem. Cytochem. 15: 335–346.

Dibuz, E. 1998. Sclereid formation in the flowers and of pears. Acta Hort. 475: 317–325.

Faust, M. 1989. Utilization of major nutrients by fruit trees. p. 83–95. In: M. Faust (ed). Physiology of temperate zone fruit trees. John Wiley & Sons, New York.

Ford, E. M. and J. D. Quinlan. 1979. The distribution of 45Ca in apple fruits when supplied to the roots at three times during the season. J. Hort. Sci. 54: 181–188.

Gaspar, T., C. Penel, F. J. Castillo and H. Greppin. 1985. A two-step control of basic and acidic peroxidases and its significance for growth and development. Physiol. Plant. 64: 418–423.

Hill, J. 1980. The remobilization of nutrients from leaves. J. Plant Nutr. 2: 407–444.

Huystee, R. B. 1987. Some molecular aspects of plant peroxidase biosynthetic studies. Ann. Rev. Plant Physiol. 37: 165–186.

Lagrimini, L. M. 1991. Wound-induced deposition of polyphenols in transgenic plants over expressing peroxidase. Plant Physiol. 96: 577–583.

Lipetz, J. and A. J. Garro. 1965. Iionic effects on lignification and peroxidase in tissue culture. J. Cell Biol. 25: 109–113.

Lee, J. E. and W. S. Kim. 2001. Morphological characters of stone cells and their effect on fruit quality of pears. J. Kor. Soc. Hort. Sci. 42: 449–452 (In Korean).

Lee, T. M. and Y. H. Lin. 1995. Changes in soluble and cell wall-bound peroxidase activities with growth in anoxia-treated rice (Oryza sativa L.) coleoptiles and roots. Plant Sci. 106: 1–7.

Lopez-Serrano, M. and A. Ros Barcelo. 1995. Peroxidase in unripe and processing-ripe strawberries. Food Chem. 52: 157–160.

Moon, B. W., W. L. Lu, J. S. Choi and H. L. Zheng. 2002. Effects of tree-spray of liquid calcium compounds on the calcium contents, quality, and cell wall structure change of ‘Jingfen’ pear fruits. J. Kor. Soc. Hort. Sci. 43: 51–53 (In Korean).

Penel, C. and H. Greppin. 1994. Binding of plant isoperoxidases to pectin in the presence of calcium. FEBS Lett. 343: 51–55.

Penel, C., P. Cutsem and H. Greppin. 1999. Interactions of a plant peroxidase with oligogalacturonides in the presence of calcium ions. Phytochemistry 51: 193–198.

Raese, J. T. and S. R. Drake. 1995. Calcium sprays and timing affect fruit calcium concentrations, yield, fruit weight, and cork spot of Anjou pears. HortScience 30: 1037–1039.

Raese, J. T. and S. R. Drake. 2000. Effect of calcium sprays, lime of harvest, cold storage, and ripeness on fruit quality of ‘Anjou’ pears. J. Plant Nutr. 23: 843–853.

Ranadive, A. S. and N. F. Haard. 1972. Peroxidase localization and lignin formation in developing pear fruit. J. Food Sci. 37: 381–383.

Ranadive, A. S. and N. F. Haard. 1973. Chemical nature of stone cells from pear fruit. J. Food Sci. 38: 331–333.

Ryugo, K. 1969. Seasonal trends of titrable acidity, tannins and polyphenolic compounds and cell wall constituents in oriental pear fruit (P. serotina, Rehd). J. Agr. Food Chem. 17: 43–47.

Smith, W. W. 1935. The course of stone cell formation in pear fruit. Plant Physiol. 10: 587–611.

Shear, C. B. and M. Faust. 1970. Calcium transport in apple trees. Plant Physiol. 45: 670–674.

Siddiqui, S. and F. Bangerth. 1995. Effect of preharvest application of calcium on flesh firmness and cell wall composition of apples influence on fruit size. J. Hort. Sci. 70: 949–954.

Stibon, F., S. Hennion, C. H. A. Little and B. Sundberg. 1999. Enhanced ethylene production and peroxidase activity in IAA-overproducing transgenic tobacco plants is associated with increased lignin content and altered lignin composition. Plant Sci. 141: 165–173.

Youn, C. K., S. K. Kim, S. C. Lim, H. Y. Kim, Y. H. Kim, C. H. Lee and K. S. Choi. 2000. Effects of GA paste and calcium chloride on tree growth, fruit quality and storability of Niiitaka pears. J. Kor. Soc. Hort. Sci. 41: 517–522.