Anatomical Features on the Sieve Elements and Sorbitol Content in Various Organs of Rosaceae Fruit Trees

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Summary

The development of the nacreous cell wall in the sieve tube of the petiole, fruit stalk, and root in four Rosaceae fruit trees: loquat (Eriobotrya japonica Lindl.), peach (Prunus persica (L.) Batsch.), apple (Malus domestica L. Borkh.) and Japanese pear (Pyrus pyrifolia Nakai) was observed. In addition, the soluble carbohydrate contents in these organs and in the leaves and fruits were determined.

The organelles in the cytoplasm of the sieve tube elements in petioles of young, unexpanded peach leaves were observed. As the nacreous cell wall enlarged into the cytoplasm, the organelles began to degenerate and virtually disappeared when the cell wall of the sieve tube ceased to elongate. At the beginning of the nacreous cell wall formation in peach petioles, the p-protein body appeared in the cytoplasm of the sieve tube, then the p-protein body dispersed into cytoplasm during the cell wall thickening.

Ingrowth of the cell wall occurred in the sieve tubes of the petioles in all species examined. Its degree of development in the fruit stalk was species dependent; the ingrowth was thickest in the loquat, it was moderately thick in the peach and least thick in the apple and Japanese pear. The nacreous cell wall in sieve tubes of the loquat root was moderately thick; it was less thick in apple roots. Only a narrow nacreous cell wall in roots of the peach and Japanese pear was observed.

By examining the morphology of the phloem tissue, we hope to establish a relationship between the ingrowth degree of the nacreous cell wall in the sieve tubes of the petiole, fruit stalk, and root of Rosaceae fruit trees, which differed among species and organs, and the soluble carbohydrate transported therein.

Introduction

A mature leaf exports soluble carbohydrates, mainly sorbitol, into sink organs in the woody Rosaceae plants (Webb and Burley, 1962; Williams et al., 1967; Bieleski, 1969; Hansen, 1970; Reid and Bieleski, 1974; Williams and Raese, 1974). Sorbitol is subsequently converted to other sugars in the fruit; i.e. mature peach fruit is characterized by high sucrose content (Ishida et al., 1971; Kakiuchi et al., 1981; Moriguchi et al., 1990). The pathway of carbohydrate translocation is mainly via phloem tissue, particularly the sieve tube element. The mechanisms of carbohydrate translocation from the leaf involve both symplastic and apoplastic pathways. Many reports have addressed key structures, namely plasmodesmata and the cell wall protuberance of transfer cells (Gunning et al., 1968; Gunning and Pate, 1969; Pate and Gunning, 1972; Esau and Thorsch, 1985). Nii (1993) reported that the sieve tube in several Rosaceae fruit trees formed the nacreous cell wall which differed developmentally among species and organs, whereas, no such structure was found in the sieve elements of citrus, grape, and Japanese persimmon, whose translocated carbohydrate is mainly sucrose. Although the nacreous features of the sieve elements have been reported for Prunus and Pyrus (Esau and Cheadle, 1958), detail information on the vascular development in fruit trees still remains scarce. Presumably, sorbitol is translocated through this cell wall of the sieve elements in Rosaceae fruit trees, but no clear evidence has been uncovered which established the relationship between this structure and sorbitol content. Therefore, to test this hypothesis, we followed the

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development of the nacreous cell wall of a petiole during the leaf expansion and in other organs of four Rosaceae fruit tree species: loquat, peach, apple, and Japanese pear. Concurrently, the soluble carbohydrate contents of the organs were studied to determine if a relationship exists between the degree of cell wall ingrowth and sugar/sorbitol contents.

Materials and Methods

Cross sections of vascular bundles of the petiole, fruit stalk, and root in the following Rosaceae fruit trees: loquat (Eriobotrya japonica Lindl. cv. Nagasaki-wase), peach (Prunus persica (L.) Batsch. cv. Hakuto), apple (Malus domestica L. Borkh. cv. Oorei), and Japanese pear (Pyrus pyrifolia Nakai cv. Chojuro) were prepared and observed by light and transmission electron microscopes. In addition, the development and distribution of the nacreous cell wall in the vascular bundles of the petiole and loquat were observed during leaf development. The age of a leaf was distinguished by its surface area and chlorophyll content.

For anatomical studies, tissues were cut with a razor blade and fixed in 3% glutaraldehyde (0.1 M cacodylate buffer, pH 7.2) or 3% glutaraldehyde and 1% osmic acid. The fixed specimens were dehydrated through a graded ethanol-acetone series and finally embedded in Spurr resin (Spurr, 1969). Transverse sections (1.5 μm) were stained with methylene blue for light microscopy. For electron microscopy, the ultrathin sections were stained with uranyl acetate followed by lead citrate (Reynolds, 1963) and photographed with a JEOL 100CX electron microscope.

The soluble carbohydrate contents in the lamina, petiole, fruit, fruit stalk, and root were determined by HPLC. A 1.0 g (fresh wt.) sample of an organ was macerated with distilled water at 4°C. Several drops of 2% sulfosalicylic acid were added to the homogenate which was filtered through a 45 μm filter and the filtrate made to volume. A 10-μl aliquot of the filtrate was injected into a HPLC equipped with a Shodex CH-801 column. The column was eluted with water.

Results and Discussion

1. Ultrastructural appearances of nacreous cell wall in sieve tube of petiole

Fig. 1 shows the nacreous cell wall development during the leaf area enlargement in peach. In the petiole of young, unfolded peach leaves of 5.1 cm², the nacreous cell wall was already present and continued to develop rapidly until the leaf area became 9.8 cm². Subsequently, the number of cells containing nacreous cell wall in the phloem proliferated gradually until leaf expansion ceased.

The cytoplasm of the sieve tube element underwent drastic alterations with respect to the organelles and membrane systems during the thickening of the nacreous cell wall (Fig. 2). However, the exact temporal sequence between cytoplasmic changes and cell wall thickening is not clear; many changes may occur simultaneously. For example, the endoplasmic reticulum, ribosomes, and dictyosomes became less prominent and finally disappeared from the cytoplasm. Mitochondria were also present in very young sieve tube cells but their substructure was somewhat disorganized. During nacreous wall formation, the vesicles and cell organelles in the cytoplasm even-
Finally collapsed and degenerated. These changes seem to be accompanied by the loss of the vacuolar membranes. Nucleoli were not detected at any developmental stage. Several researchers described that cell organelles in the sieve element protoplast remain quite apparent during most of the period when the nacreous wall material is being deposited (Srivastava and O'Brien, 1966; Evert and Eichhorn, 1974; Neuberger and Evert, 1974; Evert and Eichhorn, 1976; Kuo, 1983). Neuberger and Evert (1974) reported that the mature, plasmalemma-lined sieve tube contains a degenerated nucleus and mitochondria, intact plastids, but no vesicular membranes. Kuo (1983) has also reported that the appearance of the nacreous cell wall differs according to the type, age and growing conditions of organs. In this study, the organelles in mature sieve tube elements of peach leaves were absent.

In photomicrographs obtained with the TEM reveal that the wall of many sieve elements consists of two distinct layers, a comparatively thin inner layer and a thick outer layer (Fig. 3A and 3B). Ultrastructurally, the wall contains parallel micro-

![Fig. 2. Transmission electron micrographs of the sieve tube elements in petioles of (A) a young, unexpanded peach leaf and (B) a mature leaves. The organelles are: mitochondrion (m), dictyosome (d), ribosome (large arrow), and ER (small arrow). Scale bar: 1 μm.](image-url)
fibrils or loose fibrils embedded in an amorphous matrix. Other researchers have made similar observations (Srivastava, 1969; Stevenson, 1977). We observed no plasmodesmata in any developmental stage of the sieve tube, whereas Neuberger and Evert (1974), Stevenson (1974), and Kuo (1983) reported that open pores were present in sieve plates along with branching plasmodesmata. In the petiole of young, unfolded peach leaves (5.1 cm²/leaf area), a feature of p-protein body occurred in the central part of the cytoplasm of the sieve tube (Fig. 4). This relatively large body, containing hexagonally arranged p-protein tubules was dispersed into the cytoplasm during leaf expansion, as reported by Stevenson (1974) and Behnke (1989). These particles disappeared from the cytoplasm of sieve tubes of mature leaves. In the loquat, the area of the phloem increased and the sieve tube, having cell wall ingrowth, also increased during leaf area enlargement (Fig. 5). Nacreous cell wall occurred in the petiole of very young loquat leaves (12.3 cm²/leaf area). The nacreous cell wall in sieve tubes of mature leaves nearly occupied the entire cell (Fig. 3C and 3D), so that the cell organelles were absent in the cytoplasm.

2. Nacreous cell wall in sieve tube and sorbitol content in various organs

Figs. 6, 7, and 8 show the area occupied by the nacreous cell wall and the number of cells containing nacreous cell wall per phloem in various organ of different species. The distinctive cell wall ingrowth typically appeared in the sieve tube of

![Fig. 3. Transmission electron micrographs of the nacreous cell wall of the sieve tube elements in the petioles of mature peach (A, B) and loquat (C, D) leaves.](image-url)
petiole in the Rosaceae plants. The ingrowth of the cell wall is of uneven thickness and seemingly filled the entire lumen of some cells. The cell wall development in the sieve tubes of the fruit stalk differed among the species, but they all exhibited the nacreous cell wall. Among the species, the relative degree of ingrowth thickening in the sieve elements were: (1) loquat had the thickest cell wall; (2) that in the peach was intermediate; whereas, (3) it was not noticeable in the apple and Japanese pear. These results agree with the previous findings of Nii (1993).

Nacreous cell walls were barely detectable in the phloem of apple, peach, and Japanese pear roots, whereas it was easily detectable in sieve

Fig. 4. Transmission electron micrographs of the p-protein body in the sieve tube in the petiole of an unfolded leaf (A) and the dispersed p-protein body in that of a mature peach leaf (B). In the inset a, the p-protein body in A is enlarged.

Fig. 5. The number of sieve tubes containing the nacreous cell wall estimated per hundred cells in phloem in the petiole of loquat during the leaf area expansion. Bars through data points indicate standard deviations.
tubes of loquat roots. Kuo (1983) previously reported that the behavior and development of the nacreous layer were quite variable and were dependent upon the type of organs, the age of the sieve elements, and the growing conditions. He observed nacreous cell walls in leaf blade, leaf sheaths, rhizomes, and erect stems but not in roots tissues.

Analyses of extracts from mature leaves, petioles, fruit stalks, fruit, and large roots revealed that sorbitol was the predominant component in the lamina and petiole of all species (Fig. 9). Similar results were reported earlier (Williams et al., 1967; Bieleski, 1969; Yamaki et al., 1979; Hirai, 1983). In the fruit stalk, sorbitol content differed among species. Sugar composition in ripe fruit also varied within and without the species but sorbitol level usually remained low. The sorbitol content in roots was uniformly lower in all species, except that in loquat contained sorbitol at moderate levels. It is not possible to ascertain the roles of the nacreous cell wall in sieve elements with respect to sorbitol transport because the sorbitol content in the sap of individual sieve tubes was not determined.

**Literature Cited**


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Fig. 7. Photomicrographs of sections of petiole (A, B), fruit stalk (C, D), and root (E, F) of loquat (left) and peach (right). Arrows show the nacreous cell wall. Loquat-petiole, Oct. 1; fruit stalk and root, June 12; peach-petiole, fruit stalk, and root, July 10. Scale bar: 20 μm.
Fig. 8. Photomicrographs of sections of petiole (A, B) and fruit stalk (C, D) of apple (left) and Japanese pear (right). Arrows show the nacreous cell wall. Sampling date: July 8. Scale bar: 20 μm.

Fig. 9. Soluble carbohydrate concentrations in lamina, petiole, fruit stalk, fruit, and root of loquat, peach, apple, and Japanese pear. Sampling dates are the same as in Fig. 6.

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バラ科の果樹類の種々の器官における師管の細胞構造とソルビトール含量

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摘要

バラ科の果樹類のうちピーブ、モモ、リンゴ、ニホンナシを用いて、葉柄、果柄、根の維管束の師管細胞における真珠層（nacreous cell wall）の発達状況とそれらの器官と果実の可溶性炭水化物含量を調査した。
モモの果実、末摘果実の葉柄の師管の細胞質には細胞小器官が観察されたが、真珠層細胞壁が細胞質中に拡張するとともに、細胞小器官は変化し始め、師管の細胞壁の拡大が終わる時期には、ほとんど消失した。
モモの果実における真珠層の形成初期には、師管の細胞質に p-protein body が観察でき、細胞壁の拡張時に分散していった。
調査したすべての果樹における葉柄の師管の細胞壁の内部成長は顕著であった。果柄における師管の細胞壁の拡大程度は種によって相違し、ピーブの細胞壁の内部成長が最も著しかった。ついでモモの拡大程度が大きく、リンゴ、ニホンナシでは小さかった。ピーブの根には真珠層の拡大がある程度観察されたが、リンゴの根では少なく、モモ、ニホンナシの根ではその発達はあるほどみられず、わずかな薄い細胞壁がみられる程度であった。
バラ科の果樹類において、種や器官によって相違するものの、葉柄と果柄、根の師管の真珠層の発達程度とそれらに転流する可溶性炭水化物との関係を検討することは重要であると考えられる。