Anatomical Features of the Sieve Elements in the Vascular Bundles of *Rosaceae* Fruit Trees

Naosuke Nii

College of Agriculture, Meijo University, Tempaku, Nagoya 468

Summary

The anatomy of the sieve elements in the vascular bundle in a variety of *Rosaceae* fruit trees—peach (*Prunus persica* (L.) Batsch.), plum (*Prunus salicina* Lindl.), Japanese apricot (*Prunus mume* Sieb. et Zucc.), apple (*Malus domestica* L. Borkh.), Japanese pear (*Pyrus pyrifolia* Nakai), and loquat (*Eriobotrya japonica* Lindl.)—was observed by both optical and transmission electron microscopy. As a comparison, sieve elements of satsuma mandarin (*Citrus unshiu* Marc.), Japanese persimmon (*Diospyros kaki* L. f.), and grape (*Vitis vinifera* L.) were examined. A distinguishing feature of the thickening portion of the sieve elements in the vascular bundles of the midrib, petiole, fruit stalk, and pericarp in *Rosaceae* fruit species appeared to be structural ingrowth of the cell wall, but only as a trace in the roots. From the anatomical observations, the ingrowth thickening in the sieve elements, which is referred to as nacreous wall formation, was completely different from the apparatus of the transfer cells. This sieve-element structure is a characteristic feature of *Rosaceae* fruit trees, in which sorbitol is the main photosynthetic product. In contrast, the sieve elements of fruit stalks of the other species, whose principal translocatory carbohydrate is sucrose, do not appear similar.

Introduction

Since Abbe and Crafts (1939) have reported the presence of thick, "secondary" walls in the sieve elements of *Pinaceae*, many researchers observed similar thickened walls (nacreous walls) in the sieve elements of terrestrial vascular plants (Esau and Cheadle, 1958; Srivastava, 1969; Neuberger and Evert, 1974; Evert and Eichhorn, 1976; Kuo, 1983; Behnke, 1989). Detailed ultrastructural studies have been made of these walls. The recent proposal by Chafe and Dooham (1972) that microfibrils lie flat in the plane of the cell wall is consistent with a generally accepted view. Although the nacreous features of the sieve elements have been reported in fruit trees, such as *Prunus* and *Pyrus* (Esau and Cheadle, 1958), detailed information on the vascular development in fruit trees remains scarce. During a study of the vascular bundles in many fruit trees, I found how the structure of the sieve element wall develops in many *Rosaceae* fruit trees. General morphology of these

walls was similar to that of the nacreous walls of the sieve elements of other plants. In contrast, no such structure was found in the sieve elements of citrus, grape, and Japanese persimmon.

It is well-established that sorbitol is the major photosynthetic product in mature leaves of many woody *Rosaceae* plants. Furthermore, sorbitol plays a major role in the carbohydrate economy of these plants, even though the concentration of sorbitol changes with the development of the leaf (Webb and Burley, 1962; Bielecki and Redgwell, 1985). In addition, the mature leaf exports carbohydrates, mainly in the form of sorbitol, into the sink organs of these plants (Webb and Burley, 1962; Williams et al., 1967; Bielecki, 1969, 1982; Williams and Raese, 1974). The principal pathway of the carbohydrates is phloem tissue, particularly the sieve elements. The mechanisms of carbohydrate translocation from the leaf involve both symplastic and apoplastic pathways. Many reports have addressed the plasmodesmata and the cell wall protuberance of transfer cells. The role of the nacreous-like cell structure of the sieve element of *Rosaceae* fruit trees is of interest with regard to

the mechanism of translocation of carbohydrate products.

To obtain information about the translocatory cell structure of *Rosaceae* fruit trees, the anatomy of the sieve elements in the vascular bundles was studied.

Materials and Methods

The vascular bundles of the *Rosaceae* fruit stalks were observed by optical and transmission electron microscopy during the various stages of fruit development of the following; peach (*Prunus persica* (L.) Batsch cv. Hakuto), plum (*Prunus salicina* Lindl. cv. Sordum), Japanese apricot (*Prunus mume* Sieb. et. Zucc. cv. Shirokaga), apple (*Malus domestica* L. Borkh. cv. Fuji), Japanese pear (*Pyrus pyrifolia* Nakai cv. Kosui), and loquat (*Eriobotrya japonica* Lindl. cv. Tanaka). For comparison, the fruit stalks of satsuma mandarin (*Citrus unshiu* Marc. cv. Okitsuwase), Japanese persimmon (*Diospyros kaki* L.f. cv. Fuyu), and grape (*Vitis vinifera* L. cv. Muscat of Alexandria) were examined. In addition, the development of sieve elements in the vascular bundles of the midrib, petiole, pericarp, and root of *Rosaceae* was observed.

The tissues were cut with a razor blade and fixed in 3% glutaraldehyde (0.1 M phosphate buffer, pH 7.2) at 4°C until they were sectioned. The fixed specimens were dehydrated through a graded ethanol-acetone series, embedded in Spurr resin (Spurr, 1969), thin-sectioned (1.5 μ m), and stained with methylene blue for optical microscopy. For electron microscopy, the tissues were fixed in 3% glutaraldehyde and 2% osmic acid. After specimens were dehydrated and embedded in Spurr resin, the ultrathin sections were stained with uranyl acetate and then with lead citrate (Reynolds, 1963). They were examined by a JEOL model 100CX electron microscope.

Results and Discussion

The distinctive ingrowth in the sieve elements of the fruit stalks was observed in all the *Rosaceae* plants investigated (Figs. 1 and 2). In contrast, no such structure was found in the sieve elements of the other 3 species (Fig. 2D). Specifically, the *Rosaceae* plants demonstrated the following degrees of ingrowth-like thickening in their sieve elements: (1) that in the loquat was markedly developed; (2) that in the peach, plum, and Japanese apricot was

intermediate; (3) that in the apple and Japanese pear was either not noticeable or barely visible. The ingrowth-like development of the sieve elements in the vascular bundles occurs in the midrib (Fig. 1C) and pericarp (Fig. 2B) in all the *Rosaceae* plants; whereas, it was not as evident in roots (Fig. 1D). The ingrowth-like structure in the sieve elements extended longitudinally along the cell wall (Fig. 1B). A typical ultrastructural features of these elements in the midrib of a peach leaf is the nacreous wall formation of the sieve elements (Fig. 3). These protrusions apparently gave rise to the vesicular structures which accumulated outside the plasmalemma, as reported by Evert and Eichhorn (1976). During nacreous wall formation, the vesicles and other organelles in the cytoplasm eventually collapsed and degenerated (Fig. 3C and D).

The appearance of this ingrowth structure was completely different from that of the transfer cell reported previously (Pate and Briarty, 1968; Gunning and Pate, 1969; Pate and Gunning, 1972). The distinct morphological feature of that ingrowth structure developed smoothly and became thick enough to be clearly visible by means of optical microscopy. On the other hand, the cell wall protuberance (ingrowth) of the transfer cell develops in a highly irregular structure. The transfer cell has its nucleus, usually central; its cytoplasm has vacuoles, chloroplasts with grana and starch grains, and numerous mitochondria with well-developed cristae. Also observed are dictyosomes and a rough endoplasmic reticulum, as well as numerous polyribosomes and plasmodesmata. It is possible that the capacity for loading and translocating nutrients from the source organs is promoted by an increase in the transfer cell's inner surface area (Pate and Briarty, 1968; Gunning and Pate, 1969; Pate and Gunning, 1972).

The structural ingrowth in the sieve elements of woody *Rosaceae* plants occurs at the expense of the cytoplasm; consequently, the cell organelles in the cytoplasm almost disappear. Evert and Eichhorn (1976) and Kuo (1983) reported that the sieve element protoplast retains a good deal of the cell organelles during most of the period when the nacreous wall material is being deposited. Neuberger and Evert (1974) reported that the mature, plasmalemma-lined sieve tube cell contains a degenerated nucleus, mitochondria, and intact plas-

Fig. 1. Optical micrographs of sections of fruit stalks (A; transversal section, B; longitudinal section), midrib (C), and root (D) of peach. a is a detail of the boxed area in A. Arrows indicate the ingrowth structure of the sieve element. ca: cambium layer, x: xylem. Scale bar: 20 μ m (A, C, D), 10 μ m (B, a).

tids, but that dictyosomes, ribosomes, and vesicular membranes are lacking. Furthermore, open pores that occur in sieve plates and branching plasmodesmata are present in enlarged sieve areas (Kuo, 1983). However, in the present observation the plasmodesmata was not detected even by transmission electron microscopy. In mature peach leaves the sieve elements of the midrib contained no organelles in the cytoplasm. The behavior and development of the nacreous layer are also quite variable and are dependent upon the type of organs, the age of the sieve elements, and the growing conditions (Kuo, 1983). In some sieve elements the nacreous layer may or may not be reduced in thickness as the cell ages (Esau and Cheadle, 1958; Evert and Eichhorn, 1976). In the *Rosaceae* phloem, the volume of cell wall increased apparently by reduction in cytoplasmic volume. An investigation of the above-mentioned *Rosaceae*

plants reveals that the ingrowth-like development of the cell wall in the sieve elements is a very widespread component of the vascular bundle in the various tissues. Such a development, not only in the fruit stalk but also in other tissues, could be related to the translocation mechanism.

In many woody *Rosaceae* plants the translocatory product from the leaf mesophyll is sorbitol (Webb and Burley, 1962; Williams et al., 1967; Williams and Raese, 1974; Bielecki, 1969, 1982). Sorbitol is readily accumulated or loaded by the phloem-loading mechanism (Webb and Burley, 1962; Bielecki, 1969; Bielecki and Redgewell, 1985). Furthermore, the absolute concentration of sorbitol is particularly high in the phloem sap; values of approximately 15 to 25% have been recorded (Zimmermann and Ziegler, 1975). Sorbitol has also been found in xylem sap, in concentrations ranging from 0.02% to as high as 0.8%

Fig. 2. Optical micrographs of sections of fruit stalk (A) and pericarp (B) of loquat, and fruit stalk of Japanese pear (C) and satsuma mandarin (D). a and b are details of the boxed areas in A and B, respectively. Arrows indicate the ingrowth structure of the sieve element. ca: cambium layer, x: xylem. Scale bar: 40 μm (A), 20 μm (B, C, D), 10 μm (a, b).

at certain times of the year (Williams and Raese, 1974; Hansen and Grauslund, 1978; Raese et al., 1978). The movement of sorbitol in the xylem is likely to be an important process, particularly in the early spring (Webb and Burley, 1962). However, sorbitol has always been the major carbohydrate found in the phloem of woody *Rosaceae* plants.

In the majority of these plants in which the nacreous walls of sieve elements occur, sorbitol is not the main carbohydrate to be translocated. According to Kuo (1983), whether there is any physiological significance in the reduction in the size of the lumen of sieve elements with nacreous wall in relation to the translocation rate in seagrasses re-

mains to be determined. Recently, Behnke (1989) also reported that the function of the nacreous walls was not yet known. However, the ingrowth structure in the sieve element in the vascular bundles of the various tissues of the *Rosaceae* plants may be connected specifically with sorbitol translocation or loading, given that it was absent in the other species, e.g., citrus, Japanese persimmon, and grape, whose translocatable carbohydrate is mainly sucrose.

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Fig. 3a. Details are given in Fig. 3b.

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Fig. 3b Transmission electron micrographs of transversal sections of the midrib in peach leaves. B and D are details of the boxed area in A and C, respectively. Arrows indicate the ingrowth structure of the sieve element. Scale bar: 2 μm (C), 1 μm (A, D), 0.5 μm (B).

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バラ科の果樹類における師管の細胞壁の内部生長

新居直祐

名城大学農学部 468 名古屋市天白区

摘 要

数種のバラ科の果樹類（モモ、スモモ、ウメ、リンゴ、ニホンナシ、ビワ）について、師管の細胞構造を光学顕微鏡ならびに透過型電子顕微鏡を使って観察した。また、カンキツ、カキ、ブドウを対照樹として用いた。その結果、バラ科の果樹類の中肋、葉柄、果柄、果肉中の維管束の師管細胞において、細胞壁が肥厚し、内部生長した特徴的な構造がみられた。いっぽう、根

においては、このような構造の発達は少なかった。細胞構造の観察から師管が内部生長した細胞壁は nacreous wall とみなされ、転送細胞とは異なったものであった。ソルビトールが主要な転流糖であるバラ科の果樹類において、師管の細胞壁の内部生長が観察されたが、スクロースの形態で主として転流する対照樹ではみられなかった。