Anatomical Aspects of Juice Sacs of Satsuma Mandarin in Relation to Translocation

Naosuke Nii
College of Agriculture, Meijo University,
Tempaku, Nagoya 468

Bryan G. Coombe
Waite Agricultural Research Institute,
Glen Osmond, South Australia. 5064

Summary
Anatomical features of developing juice sac in citrus fruits, satsuma mandarin (Citrus unshiu Marc.), were studied by light and transmission electron microscopy, to seek an explanation for the translocation of solutes from the dorsal vascular elements in the albedo of the pericarp. The development of juice sacs commenced on the inner wall of the locules just before anthesis. The juice sac primordia developed primarily by anticlinal divisions in the epidermal cells of the endocarp and by anticlinal and periclinal divisions in the sub-dermal layers. Differentiation into stalk and sac body began when the juice sacs were about 0.7 mm in length. As the juice sac elongated, cell division occurred in the epidermal cells of the stalk region with walls in the stalk-axis direction. Accumulation of soluble solids into the juice sacs occurred after they had attained full size. There was no evidence of vascular or tracheid tissue in the stalk of the juice sac. Plasmodesmata were abundant in the thin cell walls of the parenchyma cells of the juice sac stalk.

Introduction
Juice sacs, which represent the edible portion of citrus fruit, arise as projections from the locular surface of the endocarp at flowering. More than two hundred sacs per segment are formed. There have been numerous studies of the developmental morphology and physiology of citrus fruits(1, 2, 3, 6, 7, 8, 13, 16, 17, 18, 20). These have shown the general structure of citrus fruit including that of juice sacs; however, only Shomer(18), Albrigo and Carter(1), and Koch et al. (11) have investigated the ultrastructural details of the juice sac and its stalk. Although several researchers have studied development in satsuma mandarin fruits(7, 8, 13, 14), there is little information on the structural development of their juice sacs.

Citrus juice sacs pose an interesting problem in solute translocation because of the long distances from the dorsal vascular bundles(9, 10, 11). Some sacs attain lengths of 30 mm or more, with half of the length as the stalk. Their only connection to the fruit appears to be the junction of the stalk with the endocarp; indeed, each sac has a cuticular wax covering(4, 5). How do solutes transverse the length of the sac?

Several workers(2, 3, 6, 11) have reported that juice sac primordia arise at a distance from the vascular bundles in the albedo and that vascular elements do not enter the juice sac stalk. However, Kordan(12) claimed that xylem elements with helical thickening were present in juice sacs of mature lemon fruits. Moreover, Schneider(16) and Shomer(18) suggested that tracheid-like cells were present in the juice sac stalks of shaddock (C. grandis (L.) Osbeck) and grapefruit (C. paradisi Macf.), although vascular bundles were lacking; the general appearance of these tracheid-like cells were described but not details of their structure. Such cells were not
found in mature Valencia orange fruits (the authors-unpublished). Recently, Koch et al. (11) reported that a portion of the influx through juice vesicle stalks in grapefruit could be theoretically maintained via plasmodesmata but suggested that transport by an apoplastic route was also possible.

In the present study we have investigated the formation of the juice sac in the fruit of satsuma mandarin, with particular emphasis on the development of possible translocatory elements in the juice sac stalk.

**Materials and Methods**

Fruits of satsuma mandarin (*Citrus unshiu* Marc.) growing on large trees of 14-year-old at Mei jo University Experimental Orchard were used for the experiment. Flower buds and fruit, ranging in development stage from one week before anthesis to mature yellow fruit with ovary or fruit diameters from 2.7 mm to 67 mm, were collected during the 1985 fruit growing season. Fruit diameter and the lengths of juice sac body and stalk were measured. The fruits taken from trees after the end of July were used for the determination of soluble solids by hand-refractometer and acid by titration with 0.1 N-NaOH in each fruit before section for the anatomy.

Small sections were cut from the fruit with a razor and fixed as follows. The tissue samples were placed immediately after cutting into 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) overnight at 4°C. After trimming into smaller pieces, samples were again fixed in fresh 3% glutaraldehyde buffer overnight. The samples were then prepared for study by light (LM) and transmission (TEM) electron microscopy.

For LM, tissues were usually dehydrated in an ethanol series and embedded in an epoxy resin(19). For LM, sections were cut, usually 1.5 μm thick, mounted on glass slides and stained with methylene blue. For TEM of samples on May 31 (2 weeks after anthesis) and October 22, when skin color began to change to yellow, after fixation in 3% glutaraldehyde, the tissue samples were rinsed several times (10 to 20 min each time) in the same buffer, then post-fixed for two days in 2% OsO₄ fixation in 0.1 M cacodylate buffer (pH 6.9) at 4°C. A long period of OsO₄ fixation (two days) was found desirable, presumably because of the slow reaction with juice sacs. The tissues were dehydrated and embedded as for LM. Ultra-thin sections were mounted on formvar-coated grids, then were stained with uranyl acetate and lead citrate(15).

**Results and Discussion**

Fruit diameter increased in a regular single-sigmoid curve, from anthesis until matu-

![Fig. 1. Cumulative growth curve and changes in growth rate of satsuma mandarin fruit in terms of fruit diameter between bloom and harvest. Bars through data points indicate standard deviation.](image-url)
The maximum rate of increase in fruit weight occurred at about six weeks after anthesis when the fruits were one third of their final diameter (Fig. 1). These results agree with those of Kuraoka and Kikuchi (13). Juice sacs from the equatorial region began growth just before anthesis (Fig. 4) and grew rapidly during the first half of the fruit growth curve, reaching their final length about 18 weeks after anthesis (Fig. 2). Coincidental with the cessation of sac elongation, compositional changes began in the juice with an increase in soluble solids content and a decrease in acid (Fig. 3), also in agreement with Kuraoka and Kikuchi (13).

Juice sacs were initiated as bulges on the locular side of the endocarp surface just before and at anthesis (Fig. 4). Sac primordia were found to be positioned in the vicinity of dorsal vascular budles within the albedo, as

---

**Fig. 2.** Juice sac lengths of satsuma mandarin fruits throughout the fruit growing season. Bars through data points indicate standard deviation.

---

**Fig. 3.** Changes in soluble solids and acid in the juice of satsuma mandarin (F. wt. basis).

---

**Fig. 4.** Light microscope sections of juice sac primordia of satsuma mandarin fruits. Sac primordia initials (arrowed) in the first and second layers of the endocarp in an ovary at anthesis (A) and one week before anthesis (B). VB; vascular bundle.
shown in Figs. 4 and 5; numerous sections were examined, but no vascular connections were found between the endocarp bundles and the sacs. Sac primordia were initiated by anticlinal divisions in a small group of adjacent cells and by periclinal divisions in the neighbouring sub-epidermal layers (Fig. 4 B). Further development was by anticlinal divisions in the epidermal cells of the endocarp and by anticlinal and periclinal divisions in the sub-dermal layers. This pattern is similar to that described for lemon(6).

A week after anthesis, when the fruits were 4 mm in diameter, the sac primordia commenced rapid growth and became cylindrical in shape (Fig. 5). Growth was by repeated transverse divisions in epidermal cells and by cell division along many axes at the tip (Fig. 5 B). Epidermal cells were distinguished by their thick outer walls and large rounded nuclei (Fig. 5 B). The cells beneath the epidermis were smaller and divided repeatedly. By three weeks after anthesis, when fruit were 12 mm in diameter and sac primordia were approaching 0.7 mm in length, the form of the mature sac was initiated by the swelling of the distal sac body and the delineation of the proximal stalk as shown in the longitudinal sections in Fig. 6 A. Initially, the juice sac stalks represented more than half the total sac length, but from four weeks after anthesis the stalks maintained the same proportion of the total length, viz., 33 to 40 per cent (Fig. 2). Stalk development was characterized by anticlinal divisions

![Fig. 5. Light microscope sections of developing juice sacs of satsuma mandarin fruits. Dome-shaped primordia on the locular surface and a vascular-trace in longitudinal section at one week after anthesis. B is a detail from the boxed part of A. VB; vascular bundle.](image)

![Fig. 6. Light microscope longitudinal sections of juice sacs of satsuma mandarin fruits showing differentiation and developmental changes in the stalk. A; 4 weeks after anthesis, B; 5 weeks after anthesis.](image)

![Fig. 7. Light microscope longitudinal sections of the sac stalk of satsuma mandarin fruits. Longitudinal section of a group of epidermal cells showing pit fields (arrows in C). A; Oct. 1, 19 weeks after anthesis, B and C, detail from the boxed part of A.](image)
in epidermal cells at the base near the endocarp (Fig. 6 B) followed subsequently by elongation along the axis of the stalk (Fig. 7). During the first part of this process internal cells continued to divide and maintained their isodiametric shape as the epidermal cells elongated (Figs. 6 and 7). After epidermal cell division had ceased, growth was by extensive cell elongation leading to a thread-like stalk surmounted by a bulbous sac. Longitudinal sections of the stalk showed a core of parenchymatous cells, elongated along the long axis of the stalk (Figs. 7 and 8). The core cells were surrounded by multiples of narrow, spindle-shaped cells up to 300 μm long (Fig. 8). These cells had unusual features. When newly-formed, their walls were thin and “undulating” in appearance (Figs. 7 and 8). Transmission electron microscopy showed that abundant plasmodesmata were present in these cells in both young (+2 wk) and mature (+22 wk) fruits (Fig. 9). In mature fruits the internal cells of the stalk were large and those in the central part of the sac body were also large and thin-walled.

Koch (9) studied the non-vascular translocation of 14C-labelled carbohydrates through the stalk into the vesicle body and showed that considerable movement occurred up to the end of ripening. In further experiments, Koch et al. (11) related the properties of the stalk cells to translocation rates and calculated that the density of plasmodesmata may have been sufficient to maintain a symplastic transport of sucrose at demonstrated rates. At the same time, in other experiments involving translocation through stalks after localized freeze-killing of cells, they demonstrated that an apoplastic transfer could also be possible. Our data do not help resolve this problem, but we confirm the view that the stalks of juice sacs, in our case of satsuma mandarin, contain no vascular elements as had previously been suggested by Kordan (12), Schneider (16) and Shomer (18). We have shown that, instead, juice sac stalks contain a complex of spindle-shaped cells with many plasmodesmata which could be construed as aiding a symplastic route for solute transport into the juice sac.

Acknowledgements

We thank Messrs. T. Goto and G. Ishimaru for their invaluable assistance.

Literature Cited

1. ALBRIGO, L. C. and R. D. CARTER. 1977. Struc-


ANATOMICAL ASPECTS OF JUICE SACS OF SATSUMA MANDARIN

Bryan G. Coombe
アデレード大学, オーストラリア

摘 要

ウンシュウミカン果実の砂しょうの発達及び
転流に関する解剖学的研究

新 居 直 祐
名城大学農学部 468 名古屋市天白区

ウンシュウミカン果実の砂しょうの発達過程を光学顕
微鏡並びに透過型電子顕微鏡観察によって解剖学的に調
べ, アルベド組織中の外周糸管束からの溶質の転流機構
について検討した。砂しょうは開花直前に心室内壁より
突起した。砂しょう突起において, 内果皮の表皮細胞で
は主として垂直分裂がみられ, 下皮層では垂直分裂や
並層分裂がみられた。砂しょうの長さが約 0.7 mm に達
した段階で砂しょうの袋状の部分と柄の分化が認められ
るようになった。また, 砂しょうが伸長するにつれて,柄の表皮細胞で柄の軸方向への細胞分裂がみられた。砂
しょう中への可溶性固形物の蓄積は砂しょうの大きさが
最大に達したころに開始した。砂しょう柄中には維管束
や仮導管組織はみられなかったが, 砂しょう柄の柔組織
細胞の薄い細胞壁には数多くの原形質連絡が認められ
た。