Sugar Accumulation by *in vitro* Cultured Juice Vesicles of Satsuma Mandarin

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Summary

Juice vesicle explants including some mesocarp tissue excised from young satsuma mandarin (*Citrus unshiu* Marc. cv. Miyagawa wase) fruit, were cultured *in vitro* on MS solid medium supplemented with 1 mg/L BA and 5 or 10% sucrose under dark conditions at 25 °C. The juice vesicles grew expansively and turned orange. The juice vesicles accumulated sugar when cultured on the medium supplemented with 10% sucrose and incubated for two months. Sugar composition in the cultured juice vesicles differed from that of fruit grown in the field. The *in vitro* culture of juice vesicles may aid in elucidating the mechanism of sugar accumulation and other compounds in *Citrus* fruit.

Key Words: *Citrus* fruit, *in vitro* culture, juice sac, sugar content.

Introduction

During the growth and maturation of *Citrus* fruit, many compounds, including sugars, acids, and flavonoids, which determine fruit quality, accumulate in juice vesicles. Unlike the mesocarp tissue of other fruits, the *Citrus* juice vesicles are easy to be isolated without wounding and cultured on a nutrient medium. Thus, there are several reports on *in vitro* culture of juice vesicles. Earlier attempts to culture juice vesicles *in vitro* resulted in callus formation instead of organized vesicle enlargement (Kordan, 1963, Unger and Feng 1978). Altman *et al.* (1982) reported the enlargement of juice vesicles *in vitro* without callus formation and found that either BA, GA or IAA enhances vesicle enlargement. However, they investigated the growth and the physiological changes of cultured juice vesicles for an experimental period of 16 days so that sugar accumulation in the juice vesicles did not occur.

In our study, we found that sugar accumulation could be induced when juice vesicles are incubated for a long period on a medium containing, a high concentration of sugar. In this report, the method for studying sugar accumulation in juice vesicles *in vitro* is described.

Materials and Methods

Young fruit of 'Miyagawa wase' satsuma mandarin were harvested on June 25 (50 days after full bloom) and on July 30 (85 days after full bloom). The fruit were surface-sterilized by a 10-min soak in 70% ethanol, a 30-min soak in 1% (w/v) NaOCl, and rinsed in sterile water. Juice vesicles, including the mesocarp (albedo), which were excised from the equatorial region of the fruit, were placed on 10 ml of agar medium in culture

tubes (18 X 150 mm) and incubated in the dark at 25 °C. The explants were placed with the endocarp side up, so that the juice vesicles were not in contact with the Murashige and Skoog (MS) medium (1962) supplemented with 1 mg/L BA, 5% or 10% sucrose and 1% (w/v) agar. The pH of the MS medium had been adjusted to 5.6–5.8 and autoclaved for 15 min at 121 °C. A minimum of 25 vesicle explants were used for each medium.

The explants were taken out of their tubes and their growth and sugar concentration determined monthly. The juice vesicles were separated from the mesocarp, squashed, and boiled in 80% ethanol. The extracts were filtered and the filtrate evaporated to dryness. After the residue was re-dissolved, the sugar content was determined with a high-performance liquid chromatograph (HPLC) equipped with a refractive index detector.

For comparison, the sugar content in juice vesicles of orchard-grown fruit was similarly determined. The weight of the vesicle juice was determined by crushing the vesicles on a pre-weighed filter paper, removing the debris, and quickly weighing the moist filter paper. The juice weight was calculated by subtracting the initial weight from the total weight. The growth and sugar contents of the juice vesicle were determined by calculating the mean of eight replicate vesicle explants in each treatment. Mean comparisons were made by using t test.

Results and Discussion

The juice vesicles grew, becoming enlarged, rounder, and more compact than those in intact fruit. Guardiola *et al.* (1993) reported that the vesicle explants excised from young fruit 5 to 50 days after full bloom developed vigorous callus irrespective of the hormone added to the medium. However, those excised 60 or more days after

Fig. 1. Photographs of juice vesicles grown *in vitro*.

Top: Section of satsuma mandarin fruit on June 25. The part enclosed with a dashed line was excised and cultured MS medium supplement with 1mg/LBA, 10% sucrose, and 1% agar. Bar=2 mm.

Middle: The juice vesicles after one month in the culture.

Bottom: The juice vesicles after two months in the culture.

full bloom formed little or no callus. In our study, no callus formed even on the explant excised 50 days after full bloom. The addition of a high concentration of sugar to the medium seemingly inhibited callus formation. Two months after the start of the culture, the juice vesicles grown on the medium containing 10% sucrose

became orange and juicy; they were easy to crush by hand, whereas those on the 5% sucrose medium were translucent and hard to crush (Fig. 1). Table 1 shows the fresh weight, juice weight, and sugar content of the cultured juice vesicles two months after the start of the culture (The explants were excised 85 days after full bloom). The fresh weights of each vesicles ranged from 26 to 32 mg. Sucrose concentration in the medium did not seem to influence the fresh weight of juice vesicles. However, the juice weight was greater in those cultured on the 10% rather than on 5% sucrose (Table 1). The sucrose content in vesicles cultured on 10% sucrose gradually increased until two months after the start of the culture, then increased rapidly. This pattern of sugar accumulation was similar to that of juice vesicles in the field-grown fruit. However, sugar composition differed between *in vitro* and *in vivo* (in the field) grown vesicles, in that sucrose ratio (sucrose content/total sugar content) in the cultured juice vesicles was higher than that in the intact fruit (Table 2). The vesicles accumulated sugars on the 10% sucrose medium, whereas those on the 5% medium lost sugar during their culture (Table 1).

Altman *et al.* (1982) found that sugar concentration in cultured juice vesicles did not change during a 16-day culture, even though the total volume of the explant increased. The authors gave no sugar concentrations in the medium. Our results indicate that to induce sugar

Table 2. The changes in total sugar content and sucrose ratio in the juice vesicles cultured *in vitro* and *in vivo*.

	June 25	July 31	Aug. 24	Sep. 25
Total sugar content (%)				
<i>In vitro</i>	—	3.53	4.01	6.95
<i>In vivo</i>	2.08	3.36	3.91	6.66
Significance	—	N.S.	N.S.	N.S.
Sucrose ratio (%) ²				
<i>In vitro</i>	—	65.7	65.8	63.1
<i>In vivo</i>	62.5	46.0	38.2	45.6
Significance	—	**	**	**

**Significant ($p < 0.05$).

² Sucrose content/ total sugar content x 100.

Table 1. Effects of sucrose concentration in the medium on fresh weight, juice weight and sugar content of juice vesicles cultured *in vitro*.

Sucrose (%)	Juice vesicle weight (mg)	Juice weight (mg)	Total sugar (%)	Sucrose (%)	Glucose (%)	Fructose (%)
5	26.7	15.7	2.09	0.94	0.51	0.64
10	31.9	22.7	6.92	5.39	0.70	0.83
Significance	N.S.	**	**	**	N.S.	N.S.

Data were determined after two months of the culture.

The total sugar, sucrose, glucose, and fructose content (%) in the vesicles at the start of the culture (July 30) were 3.36, 1.55, 0.90, and 0.92 (%) respectively.

**Significant ($p < 0.05$).

accumulation in cultured juice vesicles, it is necessary to culture the vesicle explants on a medium containing at least 10% or more sucrose for more than a month. In *Citrus*, sugar is transported to the juice vesicles through phloem in the form of sucrose. In our study, the sugar was translocated via phloem of the albedo attached to the explant. Although the sugar concentration in phloem effluents of *Citrus* grown in the field has not been determined, the data reported for other plant species indicate that it is maintained at concentrations as high as 200 to 800 mM (Gifford et al., 1981). We assume that sugar translocated to the juice vesicles *in vitro* could be induced as the sugar concentration in the phloem increases to a threshold level due to the high concentration of sucrose in the medium. Therefore, the sugar accumulation by *in vitro* cultured juice vesicles enables us to study the mechanism of sugar accumulation in *Citrus* juice vesicles in a simpler, more controlled fashion than in the field.

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In vitro 培養におけるウンシュウミカン 砂じょうの生長と糖集積

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摘 要

ウンシュウミカン幼果の砂じょうを MS 基本寒天培地に植え付け、砂じょうの発達、ジュースの集積および糖含量について調査した。培養 2 カ月後の 8 月下旬には砂じょうがオレンジ色に着色し、ジュースの蓄積や糖含量の上昇が認められた。培地中のスクロース含量が 10% の場合は、5% に比べて着色、ジュース量および糖含量の点で優れた。また、糖含量の経時の変化は、インタクトな果実と比べて、糖組成の点では異なるが全糖含量はほぼ同じであった。今後、このような砂じょう培養系が果実の糖や他の化学物質の集積の機構を明らかにすることに利用できることが示唆された。