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Sugar Accumulation and Partitioning in Satsuma Mandarin Tree Tissues and Fruit in Response to Drought Stress

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Abstract. **Mechanisms of sugar accumulation in response to drought stress in Satsuma mandarin (*Citrus unshiu* Marc.) fruit were investigated. Predawn leaf water potentials averaged –0.35MPa for well-watered, –0.60 MPa for moderately drought-stressed, and –1.00 MPa for severely drought-stressed glasshouse-grown 3-year-old trees. Fruit peel turgor and fruit growth of the moderately drought-stressed trees recovered to a similar value to that of the well-watered trees. Photosynthetic rates and stomatal conductance of both moderately and severely drought-stressed trees were significantly lower than those of the well-watered plants. However, the total sugar content per fruit of moderately drought-stressed trees was the highest among the drought treatments. A ^{13}C-labeling experiment showed that ^{13}C distribution in fruit grown under the moderately drought-stressed condition was the highest. These findings indicate that sugar accumulation in fruit was caused by an increase in translocation of photosynthates into fruit, especially into the juice sacs, under drought stress.**

It is well known that drought stress affects many physiological processes of most plants including citrus trees (Kramer and Boyer, 1995). Citrus fruit growth (Elfving and Kaufmann., 1972), photosynthesis (Vu and Yelenosky, 1989), stomatal closure (Syvertsen, 1982), abscisic acid content (Norman et al., 1990), and sugar accumulation (Kadoya, 1973; Yakushiji et al., 1996) are all affected. High quality Satsuma mandarin fruit in Japan typically are 6.1 to 6.7 cm in diameter, having deep-orange colored peel, >12% of soluble solids concentration (SSC) and <1.0% acidity in the juice. These fruit, especially those having higher SSC, are commercially attractive, yet they can be seldom produced under well-watered conditions. Although drought stress causes reduced fruit size and lowered photosynthates, it is well known that Satsuma mandarin trees can produce fruit with >12% of SSC under water deficit conditions during summer and/or autumn (Maotani and Machida, 1980; Sugai and Torikata, 1976). However, the physiological mechanisms of drought stress on growth and sugar accumulation in Satsuma mandarin fruit have not been studied critically.

When many plants acclimate to water deficits, the maintenance of cell turgor by osmotic adjustment is an important physiological mechanism which can minimize the detrimental effects of drought stress (Morgan, 1984). The drought tolerance of many plants is largely dependent on their capacity for osmotic adjustment and maintenance of cell turgor through the accumulation of solutes. Inorganic cations, organic acids, amino acids, and sugars are known as primary osmotica that accumulate after internal synthesis or uptake from external media (Kramer and Boyer, 1995). Drought-stressed cherry trees (Ranney et al., 1991) and apple trees (Wang et al., 1995) accumulate more sugars than unstressed trees. However, most studies have reported on osmotic adjustment and carbohydrate metabolism in response to drought stress in leaves, stems, or roots but not in fruit. Thus, little is known about the relationships between sugar accumulation of fruit and osmotic adjustment in Satsuma mandarins.

Sugar accumulation in Satsuma mandarin fruit may be induced by dehydration after transpiration from stomata of fruit peel surfaces during drought stress conditions. If this were case, concentrations of sugars in fruit would be increased due to passive loss of water from cells in fruit rather than significant increases in amount of sugar content per fruit (Sugai and Torikata, 1976). Results of Kadoya (1973), however, have shown that the total sugar content per fruit of drought-stressed trees was significantly higher than those of well-watered trees. We reported similar results in Satsuma mandarin fruit when osmotic adjustment was observed in fine roots, peels and juice sacs (Yakushiji et al., 1996).

In soybeans, sugars and amino acids were accumulated in cells in the zone of elongation when growth was inhibited at low water potentials (Meyer and Boyer, 1981). Sugar accumulation originated from materials of cell walls and membranes translocated from cotyledons (Meyer and Boyer, 1981). The sugar accumulation in Satsuma mandarin fruit may be different under drought stress, because fruit always accumulate photosynthates during the process of growth and ripening (Sawamura et al., 1975). If osmotic adjustment is taking place actively in Satsuma mandarin fruit during drought stress, is it possible that drought-stressed fruit contain more sugars than well-watered fruit?

Thus, we tested the possibility that photosynthates may actively accumulate in Satsuma mandarin fruit during drought stress by labeling photosynthates with $^{13}CO_2$ and evaluating translocation. Specifically, we investigated the relationship between osmotic adjustment and the sugar accumulation in fruit by measuring the water status of tissues and assimilate partitioning while Satsuma mandarin were subjected to different levels of drought stress.

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Materials and Methods

Plant materials. The plants used in this study were 3-year-old Satsuma mandarins (*Citrus unshiu* Marc. 'Okitsu-Wase') grafted on *Poncirus trifoliata*, and planted in 20-L pots filled with a mixture of 1 soil : 1 humus (by volume) in an unheated glasshouse under natural sunlight. Fifteen pots were prepared, and plants were watered once daily so that the soil was saturated. Nutrients were supplied at 2- to 3-week intervals with 5 g compound fertilizer containing 16N–10P–14K and micronutrients throughout the growing season except in winter. This variety flowers in mid-May and is harvested in mid-December in this glasshouse. Before controlling drought stress, all trees were fruit-thinned to one fruit per 25 to 30 leaves in early August, when average fruit size varied from 20 to 30 g. The water status of soil in pots was controlled from 1 to 16 Sept. The field capacity of soils was 53.1% ± 1.7% (w/w) (n = 3). During the treatment, maximum photosynthetically active radiation (PAR) was 2100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Temperature ranged from 26 to 33 °C between 1000 and 1500 HR and from 20 to 23 °C between 2200 and 0400 HR. Relative humidity (RH) was 60% to 70% during the day and 70% to 80% during the night. The maximum temperature was 32.9 °C, and the minimum temperature was 19.6 °C during the treatment.

All trees were watered equally before being treated. Three levels of water stress condition were established; well-watered (control), moderately drought-stressed, and severely drought-stressed conditions. Before the treatment, soil in all pots was saturated by watering. To check the loss of water in each pot, the total mass of the container, soil, and tree was measured daily with an electronic balance (FV-150KA1; A & D, Tokyo) having a 50-g sensitivity. The soil mass was calculated by subtracting the total pot mass from the pot mass and plant fresh mass measured at harvesting. The soil moisture deficit was defined as follows: Soil moisture deficit (%) = (measured soil mass – dry soil mass)/(saturated soil mass – dry soil mass) × 100

In addition, the curve was constructed from data for soil moisture deficit versus soil water potential measured by isopiestic psychrometer (Boyer and Knipling, 1965) in the same soil media. Soil water content was determined by weighing soil samples before and after drying at 105 °C in an oven.

During 16 d of treatment, the horizontal diameter was measured daily in 10 fruit in each treatment every day as fruit growth measurement. The relative fruit size was expressed as a percentage, with the diameter at the starting day of treatment considered as 100%. The horizontal diameter of fruit at the starting day of treatment was 4.70 ± 0.65 cm (n = 30). Fruit growth started to decrease in all drought-stressed trees 2 or 3 d after drought treatments were begun. In the well-watered treatment group, 2 L of water was supplied to each pot every day. In the moderately drought-stressed treatment, plants were given 600 to 800 mL of water per day, and fruit growth decreased slightly compared with that of well-watered treatment group. Predawn leaf water potential reached –0.5 to –0.8 MPa and that level was maintained throughout the experiment by applying small amounts as needed based on the amount of water loss. The severely drought-stressed plants were given between 400 and 500 mL of water per day, and fruit growth nearly stopped. Predawn leaf water potential reached about –1.0 MPa at the fourth day after the initiation of water stress and then was maintained at that level throughout the experiment by controlling the water supply in the severely drought-stressed treatment group. Plant tissues of drought-stressed and well-watered trees were collected to measure their water status at each sampling time. At least three trees were used for each treatment.

Water status measurements. The water status of plant tissues was determined using the isopiestic psychrometer (Boyer and Knipling, 1965). Leaves and fruit peels were sampled at predawn (i.e., between 0500 and 0600 HR) to minimize the effect of transpiration on the water status of plants. Three leaf disks were taken from the middle of different individual leaves by using a leaf punch having a 8-mm-diameter hole. About 1.0 cm^2 of peel was excised from each fruit, and the tissue was placed on the bottom of the sampling chamber immediately after excision. Before the sampling, thermocouple chambers were coated with melted and resolidified petrolatum (Boyer, 1967). Immediately after water potential measurements, osmotic potentials were determined on the same tissues by freezing at –70 °C and thawing them at 25 °C, respectively (Ehlig, 1962). Turgor was calculated by subtracting the osmotic potential from the water potential (Nonami et al., 1987). The water status values were the average of at least three replicate leaves and peels.

Measurements of photosynthetic rates and transpiration rates. Net photosynthesis rates and transpiration rates of leaves were measured with a portable leaf chamber unit [Analytical Development Corp. (ADC), Hodesdon, U.K., supplied as model SPBH-3 by Shimadzu Co., Kyoto, Japan] on the 15th day of treatment. The ambient air, which contained 338 ± 2 $mmol \cdot mol^{-1}$ CO_2 and 60% to 70% RH, was dried to <3% RH though the silicagel column and pumped at 400 $mL \cdot min^{-1}$ into the leaf chamber. Measurements were made within a minute after enclosing the lamina into the chamber. PAR was ≈1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and leaf temperatures in all trees were 30 to 33 °C during the measurements. Fifteen leaves of each treatment were selected for measuring photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) calculated by the software supplied by Shimadzu Co., based on von Caemmerer and Farquhar (1981) and Parkinson (1985) as follows:

$$P_N\ (\mu mol\ CO_2/m^2/s) = (C_{ir} - P/(P - e_o) \times C_o) \times W/A$$
$$E\ (mmol\ H_2O/m^2/s) = e_o/(P - e_o) \times W/A$$
$$g_s\ (mol\ H_2O/m^2/s) = w \times e_o/((e_i - e_o) \times A - rb \times W \times e_o)$$
$$C_i\ (\mu mol \cdot mol^{-1}) = ((g_c - E/2) \times C_a - P_N)/(g_c + E/2)$$

where C_{ir}, C_o, and C_a are the CO_2 concentration in air entering the chamber, in air leaving the chamber, and inside the chamber, respectively; e_o and e_i are the vapor pressure of water in air leaving the chamber and saturated vapor pressure at leaf temperature, respectively; P is the atmospheric pressure; W and A are the mass flow and leaf area, respectively; and rb and g_c are the boundary layer resistance to water vapor and leaf conductance.

Analysis of the distribution of ^{13}C assimilates. To elucidate why sugars were accumulated actively in fruit of the moderately drought-stressed plants, similar-sized trees grown under well-watered, moderately drought-stressed and severely drought-stressed conditions were fed with $^{13}CO_2$. Immediately before the labeling treatment with $^{13}CO_2$, the photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration were measured on the 15th day of drought stress treatment. Citrus trees were transferred from the glasshouse to an controlled-environment greenhouse with 25 ± 2 °C and ≈75% RH under natural sun light to avoid high temperature inside the vinyl bag during feeding ^{13}C treatment. Each tree was enclosed with a vinyl film bag of 60 L with 0.10 mm thickness, and sealed to prevent leaks. A fan located inside the labeling bag ensured good mixing of the air during the labeling with $^{13}CO_2$. The temperature inside the bag was 26 to 28 °C, and RH inside the bag was >90% due to transpiration. Each tree was gradually fed with $^{13}CO_2$, released by adding 50% lactic acid to 10 g $Ba^{13}CO_3$ (98.9 atom %, Isotec. Inc., Ohio),

monitoring the concentration of CO_2 in the atmosphere in the bag with portable photosynthesis/transpiration measurement equipment (SPBH-3; Shimadzu Co.). The CO_2 concentration was between 700 and 800 μmol·mol^{-1} for the first 4 h and then gradually decreased to 300 μmol·mol^{-1} after 5 h once $^{13}CO_2$ feeding started. After this period, the vinyl bags were removed and the atmosphere inside was flushed with fresh air. At 24 h after feeding $^{13}CO_2$, all trees were harvested and separated in 11 components (shown in Table 4). Fruit were divided into three tissues, i.e., peels, locular membranes, and juice sacs. All tissues divided to small pieces were oven-dried with forced air (ND-400, Nito-rika kogyo Co., Japan) at 80 °C, which ensured rapid drying. Furthermore, samples were kept at 80 °C for 3 d, and afterward ground to a fine powder. About 1.0 mg well-mixed powder of each tissues were used for determination of the total carbon and its isotopic ratio between ^{12}C and ^{13}C. A composite juice sample from each tree was filtrated through a 0.45-μm filter (Waters, Millipore Corp., Milford, Mass.), and a 100-μL sample was injected into the HPLC as described below. The collection of sucrose, glucose, and fructose fraction was based on the retention times of a standard sample. After collection, each fraction was added to a small quartz glass cup and dried at 80 C. The total carbon and its isotopic ratio between ^{12}C and ^{13}C in the samples were measured with a combustion method using an infrared $^{13}CO_2$ analyzer (JASCO EX-130S; Japan Spectroscopic Co., Ltd., Tokyo) as described by Hirano et al. (1979) and Okano et al. (1983). The measurement error of this instrument was <1.0% of coefficient in variation, and the samples containing 10 to 40 μg of carbon could be detected (Kouchi et al., 1985). The ^{13}C atom excess % was calculated by the subtraction of ^{13}C atom % between tissues fed with $^{13}CO_2$ and natural tissues (1.1%). Total carbon content was determined by the carbon ratio and the total dry mass in each tissue. The amount of active ^{13}C absorption in each organ was calculated as the following equation: Absorbed ^{13}C content = ^{13}C atom excess % × carbon content. The ^{13}C distribution percentage per each organ was determined by dividing the quantity of ^{13}C absorbed in the organ by the total quantity of excess ^{13}C absorbed in the whole plant. Also, ^{13}C assimilated per unit dry matter for each organ was obtained by dividing the quantity of ^{13}C absorbed in the organ by the dry mass of the organ.

Sugar component and acidity analysis. All fruit were harvested on the day that plants were separated into each organ. SSC were evaluated on ten fruit per treatment using a digital refractometer (PR-100; ATAGO, Tokyo, Japan). Titratable acidity was determined for each sample by titration using 0.1 N NaOH with an endpoint of pH 8.1 and converted to citric acid concentrations in fruit (Chachin, 1986; Sinclair, 1961). To investigate the effect of water stress on sugar accumulation in fruit, the composition of sugars in fruit were analyzed by using ten fruit obtained from each treatment. High-performance liquid chromatography (HPLC) was used to identify and quantify the sugars in the fruit. About 20 g of pulp was homogenized for 5 min with a Polytron homogenizer (Brinkman Instruments, Westburg, N.Y.) in 10 volumes of 80% ethanol (v/v). The extract was filtered through a Whatman glass microfibre filter, and the insolubles were rinsed with the above solution. The filtrate was evaporated on a rotary evaporator (RE-2; Tokyo Rika Kikai Co., Tokyo) at 36 °C under reduced pressure to concentrate the filtrate solution. The concentrated solution was filtered through a 0.45-μm filter (Waters, Millipore Corp.). A 20-μL sample was injected into the HPLC (LC-3A; Shimadzu Co.) equipped with a refractive index detector (RID-2A, Shimadzu Co., Kyoto, Japan). Sugars were separated using a SCR-101N column (Shimadzu Co.) maintained at 40 °C and water as a solvent at a flow rate of 1.0 mL·min^{-1}. Sucrose, glucose, and fructose were identified and quantified by comparison with peaks produced by a known standard sugar solution with a reporting integrator (C-R2X; Shimadzu Co.).

Results

Water status of plants and fruit growth in response to water stress. After 4 d of controlling water supply, the soil moisture deficit of moderately drought-stressed and severely drought-stressed decreased to 68% and 53% in comparison with that at the starting day of treatment, respectively (Fig. 1A). The soil water potential under drought-stressed conditions was between –0.1MPa to –0.2MPa (Fig. 1B). Although the soil water potential of treated soil was much higher than the permanent wilting point, i.e., between –1.2 to –1.5 MPa, Fig. 1 shows that the soil water was progressively less available for treated Satsuma mandarin trees. To check the water status of plants, predawn leaf water potential and daily fruit diameter were monitored throughout the treatments. Water potential of leaves in the well-watered (control) plants ranged from –0.3 to –0.4 MPa during the experiment period. Water potential of leaves in the moderately drought-stressed plants decreased to a range from –0.5 to –0.8 MPa, and that of the severely drought-stressed plants ranged from –0.8 to –1.2 MPa (Fig. 2A). Fruit diameter of the well-watered plants steadily increased (Fig. 2B). Fruit grown in the moderately drought-stressed plants decreased to less than the initial fruit size from the second to the fifth day of treatment. From the sixth day of treatment, fruit growth occurred in the moderately drought-stressed plants (Fig. 2B). Afterwards, the fruit growth of the moderately drought-stressed treatment group was slightly lower than that of the well-watered treatment group (Fig. 2B). In the severely drought-stressed plants, the fruit size often decreased to less than the initial fruit size when

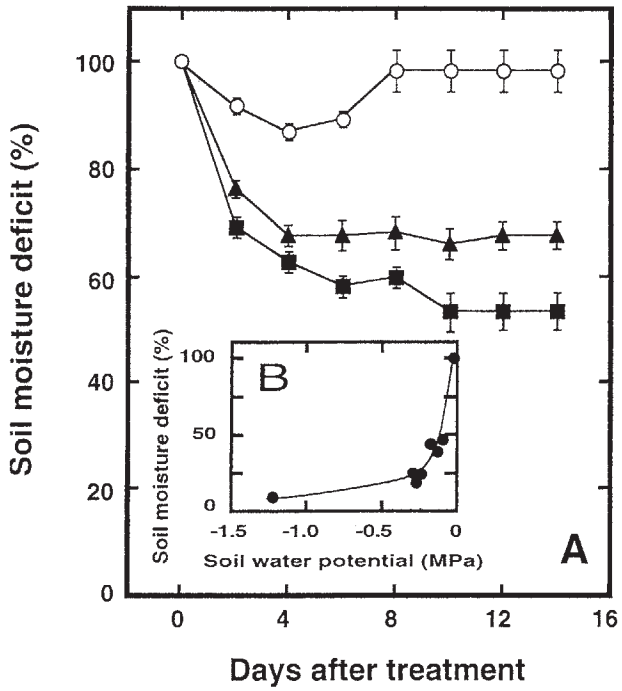

Fig. 1. Changes in soil moisture deficit (A) in pots under well-watered (○), moderately drought-stressed (▲), and severely drought-stressed (■) conditions, and soil water potential at various soil moisture deficit (B, inset) in the same soil media. The soil moisture deficit was defined as follows; Soil moisture deficit (%) = (Measured soil mass – dry soil mass)/(saturated soil mass – dry soil mass) × 100. The saturated soil was the soil condition at 24 h after adequate watering. The maximum water holding capacity of soil media was 53.1% ± 1.7% (w/w) (n = 3). Each point is the mean ± SE of three pots.

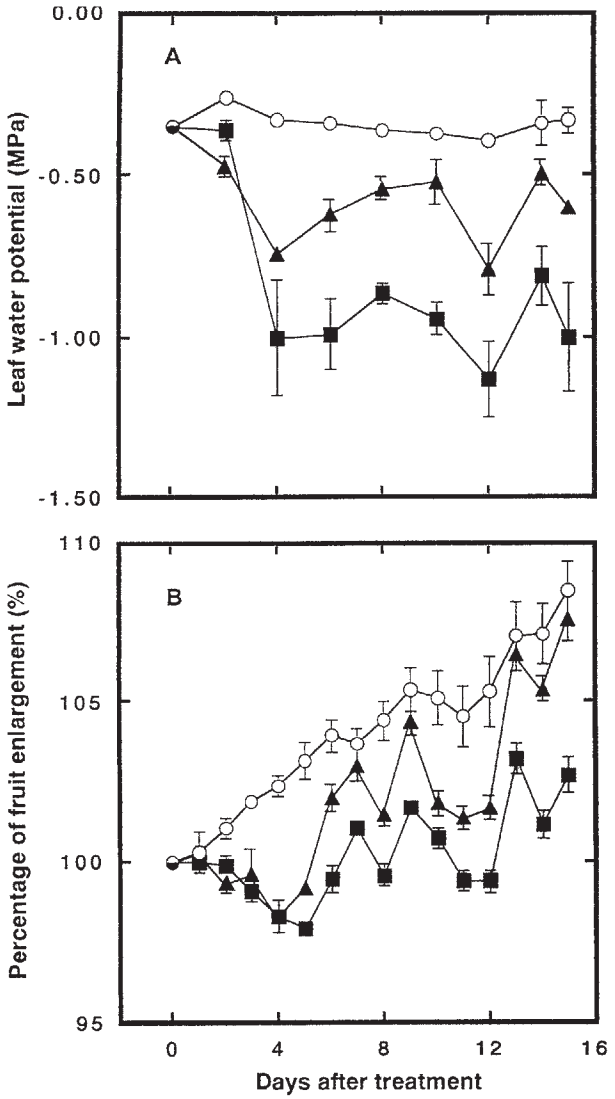

Fig. 2. Changes in water potential of leaves (**A**) and fruit enlargement rate (**B**) of Satsuma mandarin trees which were grown under well-watered (○), moderately drought-stressed (▲), and severely drought-stressed (■) conditions. Fruit enlargement was calculated in percentage, with the horizontal diameter at the starting day of treatment considered as 100%. Each point is the mean ± SE of three leaves (A) and ten fruit (B).

leaf water potential became lower than –1.0 MPa (Fig. 2 A and B).

Water potentials of fruit peels in the well-watered plants were between –0.4 to –0.5 MPa, those in the moderately drought-stressed plants were between –0.8 to –0.9 MPa, and those in the severely drought-stressed plants were between –1.2 to –1.4 MPa (Fig. 3A). Even though water potential of the fruit peel in the moderately drought-stressed plants was significantly lower than that in the control plants (Fig. 3A), turgor of the fruit peel in the moderately drought-stressed plants gradually increased and recovered to the same level as that of the control plants at the end of treatment (Fig. 3C). The decrease in water potential was compensated for by a decrease in osmotic potential (Fig. 3B). The peel turgor of severely drought-stressed trees, however, remained below that of well-watered trees (Fig. 3C).

Effect of water stress on sugar content of fruit. On the 16th day of treatment, the fruit mass and pulp mass of the moderately drought-stressed trees were similar to those of the well-watered trees, whereas fruit produced in the severely drought-stressed trees were significantly smaller than those of the well-watered trees (Table 1). When the total sugar accumulation in fruit was examined, the sugar content in the pulps of moderately drought-stressed trees was the highest among the treatments (Table 1). However, fruit produced in the severely drought-stressed plants had the highest concentrations of sucrose, glucose and fructose, followed by those of the moderately drought-stressed plants and the well-watered plants. The acidity in fruit produced in both groups of drought-stressed plants was significantly higher than that in fruit produced in the well-watered treatment (Table 1). These results indicate that drought stress increased not only concentrations of sucrose, glucose and fructose but also the total sugar content per fruit.

Active solute accumulation associated with osmotic adjustment. The fruit size of the moderately drought-stressed plant was similar to that of the well-watered plant (Table 1). It is most likely that the number and size of cells in fruit were similar for both moderately drought-stressed and well-watered plants. Cell turgor of fruit of the moderately drought-stressed plant became slightly

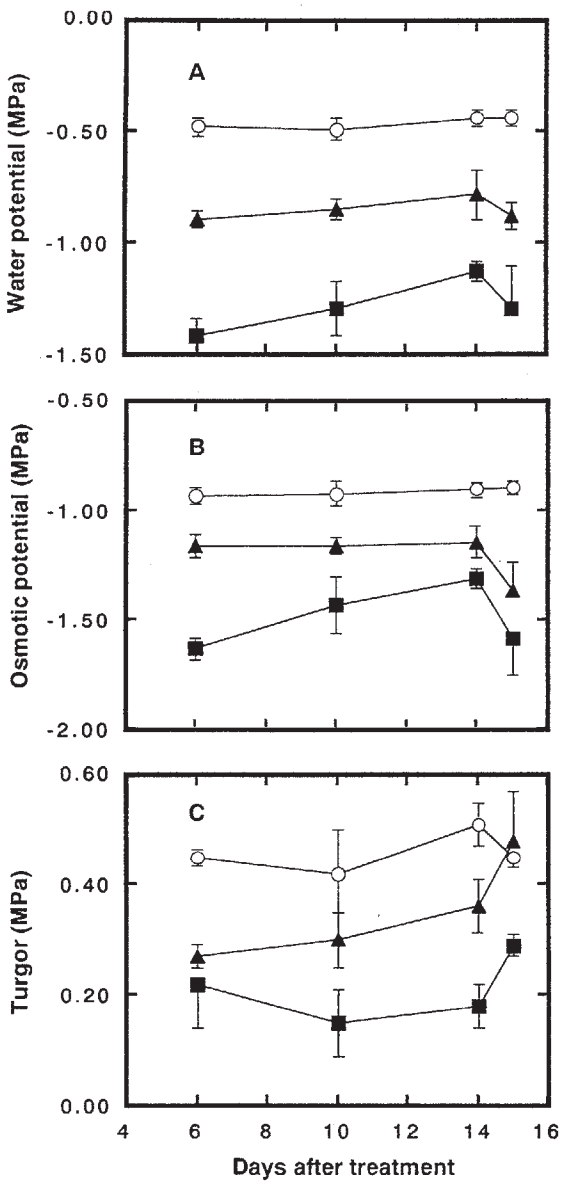

Fig. 3. Changes in water potential (**A**), osmotic potential (**B**), and turgor (**C**) of peel of Satsuma mandarin fruit grown under well-watered (○), moderately drought-stressed (▲), and severely drought-stressed (■) conditions. Each point is the mean ± SE of the three peels, each from a different fruit.

Table 1. Effect of drought stress on fruit mass, pulp mass, soluble solid concentration (SSC), acidity, and sugar content (sucrose, glucose, and fructose) (fresh mass basis) in Satsuma mandarin fruit on the 16th day of watering treatment.

Treatment	Fruit mass (g)	Pulp mass (g)	SSC (%)	Acidity (%)	Sugar content				Total pulp sugar content (g)
					Sucrose ($mg \cdot g^{-1}$)	Glucose ($mg \cdot g^{-1}$)	Fructose ($mg \cdot g^{-1}$)	Total ($mg \cdot g^{-1}$)	
Well watered	62.2 ± 2.0 b[z]	48.0 ± 1.6 b	6.5 ± 0.1 a	1.9 ± 0.1 a	19.8 ± 1.0 a	8.5 ± 0.5 a	9.0 ± 0.7 a	37.8 ± 1.9 a	1.94 ± 0.13 a
Moderately drought stressed	58.4 ± 1.8 b	45.6 ± 1.4 b	9.0 ± 0.1 b	2.4 ± 0.1 b	33.8 ± 1.4 b	13.4 ± 0.6 b	13.5 ± 0.8 b	60.7 ± 2.5 b	3.15 ± 0.17 c
Severely drought stressed	48.9 ± 1.8 a	38.2 ± 1.4 a	10.5 ± 0.1 c	3.0 ± 0.1 c	40.1 ± 1.4 c	17.8 ± 0.7 c	17.9 ± 0.8 c	75.8 ± 2.2 c	2.72 ± 0.15 b

[z]Mean separation within columns by Duncan's multiple test, $P \leq 0.05$ (n = 15).

higher than that of the control at the end of the treatment (Fig. 3C), and the osmotic potential became lower as turgor increased (Fig. 3 B and C), indicating that osmotically active solutes accumulated in fruit of the moderately drought-stressed plants. Turgor was high and the size of fruit was similar to fruit of the control plants. It is clear that dehydration was not a cause of solute accumulation in fruit of the moderately drought-stressed plants. Also, the sugar accumulation in fruit of the moderately drought-stressed plants was demonstrated by sugar analysis with HPLC (Table 1). The amounts of sucrose, glucose and fructose extracted from ^{13}C-labeled fruit juice in the moderately drought-stressed plants were significantly larger than those in the well-watered plants. Therefore, it appears that osmotic adjustment occurred in fruit when Satsuma mandarin trees were subjected to moderate drought stress, and that the osmotically active components accumulated during osmotic adjustment were largely sugars.

Photosynthesis and assimilate partitioning of ^{13}C in response to water stress. When net photosynthetic rates and transpiration rates were measured on the 15th day of treatment, the net photosynthetic rate of leaves of the moderately drought-stressed and severely drought-stressed plants was reduced to ≈1/3 and 1/5 of that of the well-watered plants, respectively. The transpiration rates of leaves of the moderately drought-stressed and severely drought-stressed plants were ≈1/2 and 1/3 of that of the well-watered trees, respectively. The stomatal conductance of leaves of the moderately drought-stressed and severely drought-stressed trees were ≈2/5 and 1/4 of that of the well-watered trees, respectively. The intercellular CO_2 concentration of leaves increased in response to drought treatments (Table 2). These data imply that low photosynthetic rates in dehydrated leaves were caused by not only stomatal closure but also the inhibition of photosynthetic metabolism in the mesophyll. However, the total mass of ^{13}C assimilated per tree during the labeling was similar in all treatments (Table 3), indicating that photosynthetic capacity of trees grown under all treatments was similar in a CO_2-enriched atmosphere in the present study.

Because the total mass of ^{13}C per tree was assimilated in about the same quantity in all treatments (Table 3), the difference in ^{13}C partitioning among treatments was related to translocation capability under different water status of plants. In the moderately drought-stressed plant, the ^{13}C distribution percentage of fruit was the highest among the plant organs, followed by new flush leaves, 1-year-old leaves and trunks, whereas in the well-watered and severely drought-stressed plants, the ^{13}C distribution percentage of new flush leaves was the highest among the plant organs, followed by fruit, 1-year-old leaves, and trunks (Table 4). In fruit, the ^{13}C distribution percentage of the moderately drought-stressed plants was much greater than those of well-watered and severely drought-stressed trees (Table 4).

In well-watered plants, the ^{13}C mass per dry mass was highest in new flush leaves, followed by new flush twigs, 1-year-old leaves and fruit (Table 4). In both moderately and severely drought-stressed plants, the ^{13}C mass per dry mass was the highest in new flush leaves, followed by 1-year-old leaves, fruit, and new flush twigs (Table 4). Because $^{13}CO_2$ was absorbed by leaves, it is natural to see the highest absorption of ^{13}C in leaves 24 h after the labeling treatment. Hence, the sink activity in cells tends to be high in newly grown or growing organs such as new flush twigs under the well-watered condition, and when plants were drought-stressed, the sink activity seems to have been shifted to storage organs such as fruit. The sink activity in fruit of the moderately drought-stressed plant was very high and the highest among fruit grown under the treatments (Table 4).

When the location of ^{13}C in fruit tissues was examined in response to drought stress, there was no significant difference in ^{13}C distribution percentage (5.0% to 7.5%) of the peel (Fig. 4). However, ^{13}C distribution accumulated in locular membranes and juice sacs of the moderately drought-stressed plants greater than those of the well-watered or severely drought-stressed plants (Fig. 4). In those tissues, the sink activity in the moderately drought-stressed plant was the highest when the activity was estimated by measurements of ^{13}C absorbed per unit dry mass.

Discussion

In the present study, the water status of soil in pots was carefully

Table 2. Photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate of Satsuma mandarin trees grown under well-watered, moderately drought-stressed, and severely drought-stressed conditions on the 15th day of the watering treatment.

Treatment[z]	Photosynthetic rate (CO_2) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Transpiration rate (H_2O) ($mmol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance (H_2O) ($mol \cdot m^{-2} \cdot s^{-1}$)	Intercellular CO_2 concn ($\mu mol \cdot mol^{-1}$)
Well watered	8.3 ± 0.5 b[y]	0.63 ± 0.02 c	0.20 ± 0.02 c	231 ± 3 a
Moderately drought stressed	2.4 ± 0.3 a	0.33 ± 0.03 b	0.08 ± 0.01 b	255 ± 7 b
Severely drought stressed	1.8 ± 0.3 a	0.23 ± 0.03 a	0.05 ± 0.01 a	242 ± 9 a b

[z]Values represent the mean ± the standard error (n = 15).

[y]Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$.

Table 3. The total dry matter mass (DM) of trees labeled with $^{13}CO_2$, the total mass of ^{13}C assimilated per tree, and the concentration of ^{13}C per unit dry matter mass on the 16th day after Satsuma mandarin trees grown under well-watered, moderately drought-stressed, and severely drought-stressed conditions. The labeling of $^{13}CO_2$ was conducted for 5 h on the 15th day of the watering treatment.

Treatment	Total tree dry mass (g)	^{13}C/tree (mg)	^{13}C/unit DM ($mg \cdot g^{-1}$)
Well watered	454.5 ± 18.5 [z]	422.2 ± 13.9	0.93 ± 0.01
Moderately drought stressed	427.5 ± 43.2	468.2 ± 36.1	1.10 ± 0.03
Severely drought stressed	464.6 ± 22.1	471.2 ± 46.9	1.01 ± 0.09

[z]Data are the mean ± SE of three trees.

controlled to impose adequate water deficit on Satsuma mandarin trees. To adjust for changes in the evapotranspiration demand due to changes in weather, the amount of watering was critically regulated by checking the mass of the entire pot, the fruit growth rate and the predawn leaf water potential. Thus, trees grown under the moderately drought-stressed condition experienced neither too much dehydration nor too much hydration, and had acclimated to the growing condition so that fruit growth could be sustained at moderately low water potentials.

In a previous paper (Yakushiji et al., 1996), we reported that when Satsuma mandarin trees were grown under mulch cultivation, osmotic adjustment occurred in fine roots, fruit peels and fruit juice sacs, and the total sugar content of mulch-grown fruit were significantly higher than in well-watered fruit at harvest. On the other hand, when fruit growth nearly stopped for 30 d due to salt stress under hydroponic culture, the total sugars of fruit showed no significant differences between salt-stressed and control groups (Yakushiji et al., 1997). In the present study, we reproduced a management condition similar to mulch cultivation in the moderately drought-stressed plants, and a cultivation condition similar to the salt stress condition in the severely drought-stressed plants in terms of fruit growth. Fruit mass of the moderately drought-stressed plants was similar to that of well-watered plants (Table 1). Fruit of the well-watered (control) plants grew continuously, while fruit stopped growing when leaf water potential at predawn dropped below about –0.8 MPa under drought-stressed conditions. This relationship was consistent with results reported in Satsuma mandarin trees (Maotani et al., 1977). In moderately drought-stressed trees, even though fruit exhibited repeated shrinkage and expansion, fruit gradually grew in size during the treatment. The fruit grown under severely drought-stressed condition sometimes shrank below the initial fruit size.

Anatomical studies of Satsuma mandarin fruit (Kuraoka and Kikuchi, 1961) showed that juice sac primordia appeared in mid-May, and the epidermal cells at the base of juice sacs began to elongate in early June while the epidermal cells near the apex of the sacs continued to divide until much later stages. The cell division for juice sac cells in Satsuma mandarin fruit is normally completed by early June to mid-June (Kikuchi et al., 1964). Because watering was controlled from the 1st of September, when cell division in fruit was presumably completed, it is safe to say that differences in fruit growth among the watering treatments in this study were caused by differences in cell expansion in fruit under different levels of drought stress conditions. When osmotic adjustment occurs under water deficit conditions, cell size and cell turgor should be maintained due to active solute accumulation in cells at low water potentials (Morgan, 1984). Cell division and organ formation in fruit could be considered almost completed prior to water deficit treatment in September because of fruit thinning at the young fruit stage (20 to 30 g) in August. The growth of fruit in early September was mainly the result of cell expansion in both well-watered control and drought- stressed trees.

Cell expansion is very sensitive to water stress, which changes the water potential gradient associated with growth (Nonami and

Table 4. Distribution percentage of ^{13}C and ^{13}C assimilated per unit dry mass in various organs of 3-year-old Satsuma mandarin trees measured on the 16th day after grown under well-watered, moderately drought-stressed, and severely drought-stressed conditions. The labeling of $^{13}CO_2$ was conducted for 5 h on the 15th day of the watering treatment.

Organ	Well watered		Moderately drought stressed		Severely drought stressed	
	Distribution percentage (%)	^{13}C mass/ dry mass ($mg \cdot g^{-1}$)	Distribution percentage (%)	^{13}C mass/ dry mass ($mg \cdot g^{-1}$)	Distribution percentage (%)	^{13}C mass/ dry mass ($mg \cdot g^{-1}$)
New flush leaves	32.5 ± 2.7[z] (1)[y]	2.49 ± 0.53 (1)	34.6 ± 4.1 (2)	2.41 ± 0.30 (1)	37.7 ± 6.1 (1)	2.64 ± 0.21 (1)
1-year-old leaves	8.7 ± 0.1 (3)	1.45 ± 0.14 (3)	6.9 ± 1.1 (3)	1.47 ± 0.17 (2)	10.4 ± 1.7 (3)	2.11 ± 0.05 (2)
New flush twigs	6.2 ± 0.9	2.43 ± 0.39 (2)	3.3 ± 0.5	1.25 ± 0.16 (4)	2.6 ± 0.6	0.88 ± 0.26 (4)
1-year-old twigs	4.4 ± 0.5	0.73 ± 0.09	3.0 ± 0.6	0.81 ± 0.14	2.9 ± 0.7	0.73 ± 0.09
2-year-old twigs	5.6 ± 0.4	0.70 ± 0.10	3.1 ± 0.6	0.59 ± 0.04	3.8 ± 0.8	0.55 ± 0.19
Trunk	7.4 ± 0.6 (4)	0.61 ± 0.09	4.0 ± 0.4 (4)	0.43 ± 0.02	5.2 ± 1.5 (4)	0.48 ± 0.17
Main root	5.3 ± 0.9	0.48 ± 0.02	3.1 ± 0.6	0.33 ± 0.03	4.2 ± 1.1	0.47 ± 0.14
Fibrous roots (<2 mm[x])	3.4 ± 1.3	0.39 ± 0.17	2.5 ± 0.7	0.35 ± 0.05	3.4 ± 0.6	0.36 ± 0.09
Middle roots (2–10 mm)	3.4 ± 0.3	0.45 ± 0.06	1.3 ± 0.3	0.24 ± 0.05	2.8 ± 1.2	0.32 ± 0.12
Large roots (>10 mm)	1.5 ± 0.7	0.36 ± 0.09	0.7 ± 0.2	0.25 ± 0.03	0.9 ± 0.1	0.35 ± 0.16
Fruit	21.6 ± 2.5 (2)	0.91 ± 0.03 (4)	37.5 ± 5.7 (1)	1.41 ± 0.13 (3)	26.1 ± 1.7 (2)	1.16 ± 0.14 (3)

[z]Data are the mean ± SE of three organs from different trees.
[y]Top four numbers from the largest in the column.
[x]Root diameter.

Boyer, 1990, 1993; Nonami et al., 1997). Under moderately drought-stressed conditions, osmotic adjustment occurred and growth was recovered in soybean seedlings (Meyer and Boyer, 1981; Nonami and Boyer, 1989, 1990; Nonami et al., 1997). Osmotically active components found in soybean were sugars and amino acids which originated from photosynthates accumulated in cotyledons (Meyer and Boyer, 1981). It is possible to sustain growth at low water potentials if sufficient osmotic adjustment takes place in plants. Therefore, it is possible for Satsuma mandarin fruit to sustain growth by accumulating sugars for osmotic adjustment under the moderately drought-stressed condition.

The rates of photosynthesis and transpiration were reduced by drought stress, and these were shown to have a close relationship with both stomatal conductance and intercellular CO_2 concentration among treatments (Table 2). Increased intercellular CO_2 concentration with decreasing photosynthetic rate generally indicates a nonstomatal limitation to gas exchange (Farquhar and Sharkey, 1982). When the ambient CO_2 concentration was increased by $^{13}CO_2$ enrichment, trees grown under drought-stressed conditions assimilated CO_2 equivalently to the control (Table 3), indicating that the photosynthetic enzymes and apparatus were not severely inhibited under drought stress in the present study. It is known that stomatal closure is more sensitive to drought stress than

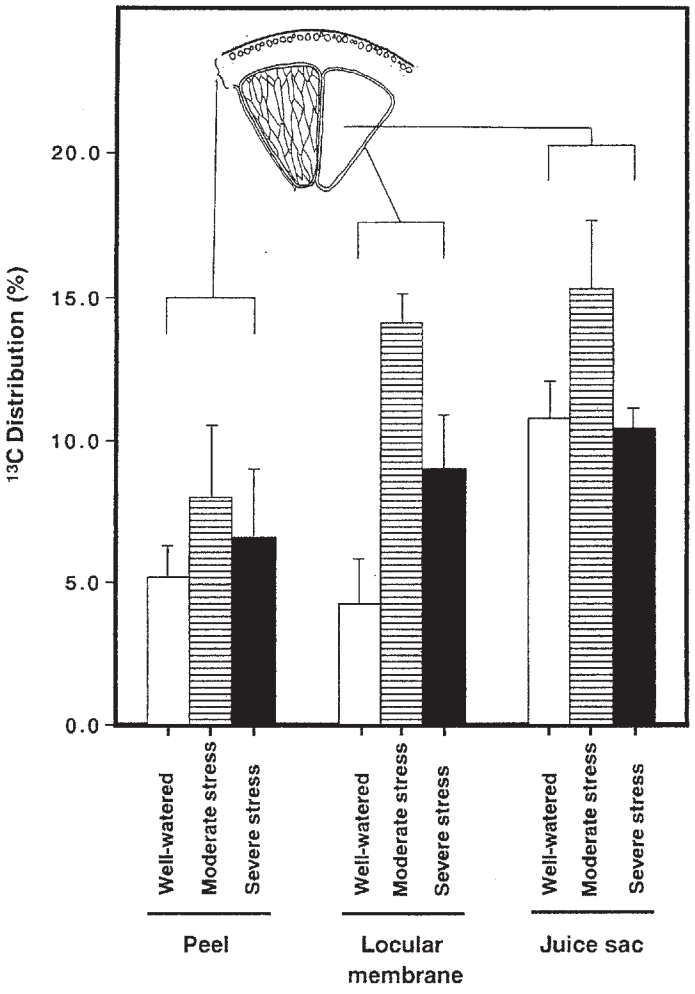

Fig. 4. The distribution percentage of the assimilated ^{13}C in peels, locular membranes and juice sacs of Satsuma mandarin fruit grown under well-watered (open column), moderately drought-stressed (laterally striped column), and severely drought-stressed (shaded column) conditions. Each error bar indicates the mean ± SE of three tissues from a different tree. Peel, locular membrane, and juice sacs of Satsuma mandarin fruit are shown in a schematic diagram.

CO_2 assimilation (Hsiao, 1973). The CO_2 enrichment could compensate for photosynthesis by nullifying the effect of both stomatal closure and mesophyll response under drought stress in sunflower plants (Graan and Boyer, 1990), and we observed a similar effect of CO_2 enrichment on CO_2 assimilation in Satsuma mandarin trees during the labeling with $^{13}CO_2$. Although the total amount of CO_2 assimilated during the labeling was not significantly different among the treatments, the assimilation measured by ^{13}C fixed per unit dry matter mass was the highest in the moderately drought-stressed tree (Table 3). This CO_2 enrichment treatment with ^{13}C gave us an opportunity to analyze the translocation and partitioning of CO_2 assimilates under different water status conditions in Satsuma mandarin trees, owing to nullification of photosynthesis inhibition caused by decreases in mesophyll capacity and/or by the stomatal closure. Thus, the distribution percentage is related to the sink capacity as a whole organ, and the quantity of ^{13}C assimilated per unit dry mass is related to the sink activity in organ cells.

Fruit grown under the moderately drought-stressed condition had the highest absorption of ^{13}C-labeled assimilates in the fruit among the treatments (Table 4). Similar observations have been reported for Satsuma mandarin fruit by Asakura et al. (1991) using steady state ^{13}C feeding technique. These findings indicate that the sugar accumulation in the fruit is caused by an increase in the partitioning rate in translocation of photosynthates into fruit under drought stress in spite of a presumed decrease in biochemical photosynthesis. Thus, fruit was considered to become a large sink for photosynthate translocation when Satsuma mandarin plants were drought stressed moderately at low water potentials.

Kadoya (1973) reported that the ^{14}C activity of the ethanol soluble fraction in the fruit increased in comparison with that of the ethanol-insoluble fraction with decreasing moisture supply. The increase of the ^{14}C activity in the ethanol soluble fraction is thought to be an accumulation of solutes which mostly consist of sugars, amino acids and organic acids during osmotic adjustment in the juice sacs. Yen and Koch (1990) reported that exported products of $^{14}CO_2$ fixation under light condition in leaves mostly consisted of the ethanol-soluble fraction in juice tissues of grapefruit. In the present study, the distribution and absorption of photoassimilates in juice tissues (locular membranes and juice sacs) of the moderately drought-stressed plants proved to be the highest among the fruit organs under all treatments (Fig. 3). Thus, the sugar accumulation in the fruit produced under moderately drought-stressed condition could be accounted for by an increase in the photosynthate transport rate from the source to the juice sac cells, which had a high sink activity.

The sugar analysis and water status measurements in the present study demonstrate the active accumulation of sugars in fruit and hexoses to be active components of fruit for osmotic adjustment (Table 1). In grapefruit, it is apparent that unloading occurs from the vascular tissues within the albedo and is followed by transport of the assimilates through the threadlike stalks into the juice sacs (Koch, 1984). Garcia-Luis et al. (1991) pointed out that a symplastic pathway for solute entry into the juice sacs is possible due to the presence of numerous plasmodesmata in both the parenchyma of the albedo and juice stalk of Satsuma mandarin fruit. Since sucrose is the primary substance for translocation and phloem unloading in citrus fruit (Garcia-Luis et al., 1991; Kriedemann, 1969; Sawamura et al., 1975), degradation of sucrose to fructose and glucose must be promoted in juice sacs during the process of active osmotic adjustment in Satsuma mandarin fruit as implicated by Yakushiji et al. (1996). It is evident that metabolism related to translocation was modified under drought stress condition, and translocation of photosynthates into fruit was promoted

under the moderately drought-stressed condition. These results suggest that the activities of sucrose-metabolizing enzymes might be increased significantly in fruit and that such enzymatic activities may be linked to osmotic adjustment in fruit under drought stress conditions and result in active sugar accumulation in Satsuma mandarin fruit

In our study, the labeling experiment in fruit confirmed that photosynthates translocated from leaves were stored as sugars in fruit as sucrose, glucose and fructose within a relatively short period under moderately drought-stressed condition, and it is most likely that those sugars were used as osmotically active solutes for osmotic adjustment in fruit at low water potentials. Thus, photosynthates were most actively translocated in fruit of the moderately drought-stressed plants in relation to osmotic adjustment because the sink activity was promoted in fruit at moderately low water potentials. These findings indicate that sugar accumulation in fruit grown under moderately drought-stressed conditions was caused by an increase in translocation of photosynthates into fruit, especially into the juice sacs.

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