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Organ-dependent seasonal dynamism of three forms of carbohydrates in Japanese apricot

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ABSTRACT

Changes in concentration of carbohydrates in three forms—soluble sugars, starch, and cell-wall material (CWM) assimilated each month from summer (August; 2 months after harvest) to late fall (November; just before defoliation)—in organs of Japanese apricot (*Prunus mume* Siebold et Zucc.) Nanko" were determined until May of the following season by providing $^{13}CO_2$. ^{13}C concentrations in the nonstructural carbohydrates (soluble sugar and starch) in the foliar and flower buds were higher than those in the current twigs and fine roots, suggesting that these organs are large sinks of reserve carbohydrates. However, they decreased sharply along with growth and were low in all the organs at the beginning of May. In contrast, a large amount of ^{13}C was fixed in the CWM of older twigs. These results indicate that reserve carbohydrates contribute to the growth of young shoots and fruits until the beginning of May, after which their growth depends on current assimilates. Carbohydrates assimilated in August–November were mainly transported to twigs, roots, trunk, and flowers, respectively. These findings show that carbohydrates assimilated in each month contribute to different organs. Thus, leaves should be maintained healthy until defoliation to produce substantial amounts of carbohydrate reserves for stable fruit production from Japanese apricot tree.

1. Introduction

For deciduous trees, the earliest phases of development depend largely on materials supplied by reserve carbohydrates accumulated in the tree during the previous growth season (Hansen and Grauslund, 1973; Tromp, 1983; Worley, 1979). The importance of carbohydrate reserves for initial growth, particularly reproductive growth in the following spring, varies with species or even cultivar (Darnell and Birkhold, 1996). Apple flowers depend on reserves only during their earliest stages of development (Hansen, 1971), whereas stone fruit trees have little leaf area at anthesis; therefore, flowering and initial growth of fruitlets completely depend on reserves (Loescher et al., 1990).

Japanese apricot (*Prunus mume* Siebold et Zucc.) trees are distributed in East Asia, including Japan, Korean peninsula, southeast China, and Taiwan (Horiuchi, 1997). The major cultivar Nanko" in Wakayama Prefecture, Japan bears fruits on leafless 1-year-old twigs, and anthesis occurs well before the development of a competent photosynthetic leaf area on the new twigs. The growth and development of fruits occur from early or mid-February, just after anthesis, through late June, whereas foliation starts in April. The time lag for approximately 2 months between anthesis and foliation results in heavy dependence of initial fruit growth on reserve carbohydrates. Characterization of storage carbohydrate dynamism in Japanese apricot tree, a typical deciduous tree in which fruiting depends heavily on reserves, is important for maintaining abundant yield and sound growth, and it will help elucidate C allocation process in primary growth stages, which is poorly understood in deciduous fruit trees.

Carbohydrate accumulation in the roots of Nanko begins to increase after harvest in July and reaches a peak just before defoliation in November (Okamuro and Tsuchida, 2009). This result appears to indicate that the Japanese apricot tree begins to store carbohydrates after harvest.

Studies of the dynamism of storage carbohydrates using radioisotopes or stable isotopes have been performed in several deciduous trees, such as pear (Teng et al., 1999), fig (Matsuura et al., 2001), grape vine (Okamoto, 1979), apple (Hansen and Grauslund,

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1973; Kandiah, 1979; Quinlan, 1969), and pecan (Lockwood and Sparks, 1978a,b). For Japanese apricot, we previously investigated changes in ^{13}C concentration of total carbohydrates until the following year and reported that the growth of young fruits and new leaves depends on reserve carbohydrates until the beginning of May of the following year (Tsuchida et al., 2012). Although assimilated carbohydrates are present in different forms, such as sugar, starch, and cell-wall material (CWM) (Ho, 1988), it has been unclear whether these three forms of reserve carbohydrate in Japanese apricot are distributed to and metabolized in each organ in the following year. Thus, to further elucidate the use of reserve carbohydrates, we analyzed ^{13}C-labeled carbohydrates in soluble sugar, starch, and CWM until the early growth stage in the following year.

2. Materials and methods

2.1. ^{13}Application and organ sampling

4-year-old Japanese apricot Nanko trees planted in 60-L pots were grown outside at the Japanese Apricot Laboratory, Minabe, Hidaka, Wakayama Prefecture, Japan (latitude 33°82′N, longitude 135°35′E). The mean annual air temperature from 2005 to 2013 was 16.5 °C, and the annual precipitation was 2150 mm. The treatments were designed to feed $^{13}CO_2$ in each month from August to November 2009, respectively, and three trees were used for each treatment. Whole trees were completely enclosed in a clear vinyl bag (0.15-mm thick) to prevent gas leaks. Three Erlenmeyer flasks containing 4.5 g of $Ba^{13}CO_3$ (98%, Kyokuko-Tsusho Co., Ltd., Tokyo, Japan) were placed inside the bag. $^{13}CO_2$ was released by adding 30 mL of 50% lactic acid to $Ba^{13}CO_3$ at 10:00, 11:30, and 13:00. A fan was also placed inside the bag to ensure that the air was mixed well during labeling with $^{13}CO_2$. The vinyl bag was removed at 15:00 to end the $^{13}CO_2$ application. The treatment was performed on 2 consecutive days for each tree (August 18 and 19, September 16 and 17, October 15 and 16, and November 18 and 19). The mean air temperatures during ^{13}C application in August–November were 30.8 °C, 26.8 °C, 22.7 °C, and 12.7 °C, respectively.

Organs were sampled from the trees for the determination of ^{13}C concentration. Leaves were taken on the first day after each $^{13}CO_2$ application [i.e., one day after application (1DAA)] and on November 24 (defoliation begins at the beginning of December). Samples of current twigs and fine roots were taken on 1DAA, December 21, February 16, and May 7. Five 10–20-cm long current twigs were taken from each tree. Leaves were obtained from these twigs until defoliation. Two or three middle leaves were taken from one shoot, so that a total of 5 current twigs and 12–15 leaves were obtained at each sampling date.

Approximately 10 g of fresh weight of fine roots was taken clockwise from around the trunk on each sampling date.

The sampled organs were cut into small pieces, oven dried using forced air at 80 °C, and ground into fine powder.

Flower buds (December 21 and February 16 for flowers and May 7 for young fruits) and foliar buds (February 16 and May 7 for new leaves) were obtained evenly from five current twigs. In total, 20 flower buds or flowers or 10 young fruits and 20 foliar buds or new leaves were taken at each sampling date.

The organs were cut into small pieces, oven dried using forced air at 80 °C, and ground into fine powder.

Finally, On May 7 in the following year (2010), the trees were divided into according to the fruits including stone, new leaves, new twigs, 2-year-old twigs (current twigs in 2009), over 3-year-old twigs, trunk (under grafted site), new fine roots (<2 mm in diameter), and large roots (≥2 mm in diameter). All components were dried with forced air at 80 °C and dry weight was then estimated. Dried matter was ground to fine powder.

2.2. Extraction of soluble sugar, starch, and CWM

Approximately 0.1 g of powder of each organ was heated in 80% ethanol (EtOH) for 15 min at 80 °C and centrifuged at 1000 × g for 10 min. The supernatant was designated as the soluble sugar fraction. This treatment was repeated three times. The residue was suspended in 80% EtOH at room temperature and centrifuged, and the supernatant was added to the soluble sugar fraction. This treatment was repeated twice, after which EtOH in the soluble sugar fraction was evaporated in a hot bath at 80 °C. The precipitate was washed with distilled water, dispersed in Na-acetate buffer (pH 6.84), and incubated for 2 h at 80 °C with 500 units of α-amylase (*Bacillus amyloliquefaciens* α-amylase, Sigma Aldrich, USA). The mixture was centrifuged (10 min at 1000 × g), and the supernatant was designated as the starch fraction. The precipitate was centrifuged after the addition of distilled water. The supernatant was added to the starch fraction. The residue was designated as CWM. Both the soluble sugar and starch fractions were lyophilized, and the CWM was dried with forced air at 80 °C.

2.3. Measurement of ^{13}C

Approximately 1 mg of the lyophilized samples of soluble sugar and starch fractions and powder of CWM of each organ was used for determining the total carbon and the isotopic ratio between carbon-12 (^{12}C) and ^{13}C. The ^{13}C atom% in each organ was analyzed using a mass-spectral stable isotope analyzer (model EX-130S, Nihon Bunko Co., Ltd., Tokyo, Japan). The excess ^{13}C atom% was calculated by the subtraction of the ^{13}C atom% in the organs from an untreated tree (1.1 atom%). The total carbon content was determined from the carbon ratio and the total dry weight of each sample. The amount of active ^{13}C absorption by each organ, the ^{13}C content in the sample, was calculated as ^{13}C atom excess% × total carbon content in the sample. The ^{13}C concentration was obtained by dividing the quantity of ^{13}C absorbed in the sample by the dry weight of the sample.

A significance test of total ^{13}C concentration (total sum of soluble sugar, starch, and CWM) among sampling days and applications for each organ was performed using Tukey's multiple range test.

The distribution ratio of the ^{13}C in soluble sugar, starch, and CWM per organ was determined by dividing the amount of ^{13}C absorbed by the organ by the total amount of excess ^{13}C absorbed in the entire plant.

3. Results

3.1. Changes in ^{13}concentration of carbohydrates in leaves, current twigs, and fine roots

On 1DAA, ^{13}C concentration in soluble sugars of the leaves accounted for by far the highest share of the three carbohydrate components; in contrast, ^{13}C was barely absorbed into starch (Fig. 1). Thereafter, ^{13}C concentration in soluble sugars sharply decreased by November 20. ^{13}C concentration in the CWM was constant at a low level.

In current twigs, ^{13}C concentration in the soluble sugar was highest among the three carbohydrate components on the first day after ^{13}C application but decreased greatly by December 21 and continued to decrease until May 7, resulting in the lowest level in total ^{13}C concentration (Fig. 2). In contrast, ^{13}C concentration in the CWM was maintained until the end of the investigation and was highest among the three carbohydrate components after December. Total ^{13}C concentration in the November application was higher than that in the September and October applications on