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Research article

Influence of crop load on the expression patterns of starch metabolism genes in alternate-bearing citrus trees

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ABSTRACT

The fruit is the main sink organ in *Citrus* and captures almost all available photoassimilates during its development. Consequently, carbohydrate partitioning and starch content depend on the crop load of *Citrus* trees. Nevertheless, little is known about the mechanisms controlling the starch metabolism at the tree level in relation to presence of fruit. The aim of this study was to find the relation between the seasonal variation of expression and activity of the genes involved in carbon metabolism and the partition and allocation of carbohydrates in 'Salustiana' sweet orange trees with different crop loads. Metabolisable carbohydrates, and the expression and activity of the enzymes involved in sucrose and starch metabolism, including sucrose transport, were determined during the year in the roots and leaves of 40-year-old trees bearing heavy crop loads ('on' trees) and trees with almost no fruits ('off' trees).

Fruit altered photoassimilate partitioning in trees. Sucrose content tended to be constant in roots and leaves, and surplus fixed carbon is channeled to starch production. Differences between 'on' and 'off' trees in starch content can be explained by differences in ADP-glucose pyrophosphorylase (AGPP) expression/activity and α-amylase activity which varies depending on crop load. The observed relation of AGPP and UGPP (UDP-glucose pyrophosphorylase) is noteworthy and indicates a direct link between sucrose and starch synthesis. Furthermore, different roles for sucrose transporter SUT1 and SUT2 have been proposed. Variation in soluble sugars content cannot explain the differences in gene expression between the 'on' and 'off' trees. A still unknown signal from fruit should be responsible for this control.
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1. Introduction

The amount of carbon partitioned to different sink organs may be limited by both source and sink ability to provide and utilise assimilates (Wareing and Patrick, 1976). Limitations at the sink level depend on organ genetic features and developmental stage, whereas source limitations may be affected by whole plant status, developmental stage and environmental conditions.

The major component of carbohydrate partitioning is the translocation of sugars from photosynthetic sources to non-photosynthetic sink tissues (Slewinski and Braun, 2010). In *Citrus*, as in most plants, sucrose is the main transported sugar (Zimmermann and Ziegler, 1975). Diverse transport proteins and enzymes are involved in this process. Phloem-localised sucrose transporters are essential for phloem loading, for maintenance of phloem flux, and for sucrose release by apoplastic unloaders (Sauer, 2007). Other enzymes, such as invertases or sucrose-phosphate synthase, allow the fine regulation of sugar accumulation and distribution in the plant (Roitsch, 1999; Li et al., 2012). Another component of carbohydrate partitioning is the mobilisation of carbohydrate reserves. Starch is the main reserve carbohydrate in plants and acts as a major integrator in plant growth regulation. Marked regulatory properties have been found for ADP-glucose pyrophosphorylase, which is involved in starch biosynthesis and is subjected to multilevel regulation (Geigenberger, 2011). Starch degradation occurs via a network of reactions that includes amylases and debranching enzymes (Stitt and Zeeman, 2012). The distribution of carbon units between starch and sucrose biosynthetic pathways is tightly regulated to respond to carbon demands throughout the day and night, and starch synthesis is a key process in the regulation of photoassimilate partitioning and carbon allocation within the plant (Preiss, 1982; Zeeman et al., 2007).

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In deciduous trees, carbohydrate reserves which accumulate in winter are crucial for development as they supply the required energy and carbon skeletons to sustain emergence and growth of new plant organs at the beginning of the growing season (Naschitz et al., 2010). By contrast, in most of the studied evergreens, budbreak and sprouting are exclusively sustained by the recent photosynthates of the existing leaves and reserves are used for root and stem growth (Epron et al., 2012). However, in *Citrus* trees, with periods of intense root growth alternating with flushes of shoot growth (Bevington and Castle, 1985), the behaviour is similar to that of deciduous trees. *Citrus* accumulate reserves in the winter and mobilise them in spring when bud sprouting occurs and vegetative sprouts and flowers are formed (Goldschmidt and Koch, 1996; Monerri et al., 2011). These reserves are stored mainly in roots, although high concentrations can also be found in leaves and bark (Goldschmidt and Golomb, 1982). After fruit set, most fixed carbon accumulates in the fruit. Both the accumulation and mobilisation of reserves have been related to fruit load in *Citrus* (Monerri et al., 2011).

Some citrus cultivars present an intense alternate bearing habit. Trees form a huge number of flowers, resulting in a heavy crop load ('on' year), followed by a year with very few or no flowers ('off' year). Hormonal factors and changes in carbohydrate and mineral status appear to participate in the regulation of these processes (Monselise and Goldschmidt, 1982). In alternate bearing sweet orange 'Salustiana', the accumulation of reserves is inversely related to crop load (Monerri et al., 2011), and changes in carbohydrate reserves during the year reflect variations in supply and demand. Fruiting trees accumulate most fixed carbon in fruits, while no accumulation is observed in roots before harvest. In the non-fruiting trees, however, most fixed carbon is transported to roots and utilised in growth processes, and after December, stored as reserves. Reserve carbohydrate accumulation in leaves starts by early December, and the levels in leaves are, until bud sprouting, the same in both the 'on' and 'off' trees. The heavy flower formation which follows an 'off' year causes the rapid mobilisation of the stored reserves, which are exhausted at full bloom.

Regulation of photosynthesis by fruit has been studied in *Citrus* (Iglesias et al., 2002; Syvertsen et al., 2003; Nebauer et al., 2011). It is assumed that photoassimilate production in leaves is modulated by the demand of sinks (Goldschmidt and Koch, 1996), but this effect is not always observable (Nebauer et al., 2011). It has been described that the root system is a strong and unsaturable sink under cropping conditions, and no enhanced photosynthetic rate by high sink strength related to fruiting was found by Nebauer et al. (2013). The photosynthetic rate was similar in trees with high and low crop loads in 'Salustiana' sweet orange (Monerri et al., 2011; Nebauer et al., 2013) when differences in carbohydrate content were highest.

As foregoing information clearly reveals, photoassimilate production and partitioning are highly integrated processes, and understanding how they are controlled will underpin many targets for plant biotechnologists (Halford, 2010).

There are no studies that analyse the effect of fruit on the seasonal expression of carbohydrate metabolism-related genes. It has been shown that the seasonal expression of flowering genes is affected by fruit load (Muñoz-Fambuena et al., 2011; Shalom et al., 2012), although they do not provide enough information to understand the mechanism by which fruit controls the flowering process.

Soluble sugars, like hormones, can act as primary messengers and regulate signals that control the expression of different genes involved in plant growth and metabolism (Rolland et al., 2006; Rosa et al., 2009). The aim of this study was to analyse the influence of fruit load on the seasonal expression and activity of the genes involved in carbon metabolism, and the possible role of soluble sugars as signals controlling the starch metabolism gene expression in citrus trees. The studied genes were selected from previous works which reported on the relation between its expression and changes in carbohydrate levels provoked by girdling (Li et al., 2003a,b,c; Nebauer et al., 2011). After taking into account that field studies may reveal essential roles of genes which cannot otherwise be observed, this work has been carried out in non-manipulated mature trees under cropping conditions during periods when the tree physiology showed distinctive characteristics. Furthermore, in order to assess the effect of fruit on the regulation of the activity of the studied genes, this work was performed in a citrus cultivar that presents an intense alternate bearing habit.

2. Materials and methods

2.1. Plant material

Experiments were performed on 40-year-old trees of the 'Salustiana' cultivar of sweet orange (*Citrus sinensis* [L.] Osbeck) grafted onto a Troyer citrange (*C. sinensis* [L.] Osb. × *Poncirus trifoliata* Raf.) rootstock. Trees were drip-irrigated, and mineral elements were supplied in the irrigation water from February to September.

Trees present an alternate-year bearing habit, and flowering intensity depends on the fruit load of the previous year. Trees alternated between years of abundant flowering and fruit set ('on' year) and years of almost no flowering ('off' year). During each year, 'on' and 'off' trees were found in the same orchard. Mature fruits were harvested by early February. The 'on' trees averaged 3119 fruits per tree in the study orchard during the previous season, whereas only 43 fruits per tree formed in the 'off' trees (Y. Bordón, personal communication). At the beginning of the study (March), the 'on' trees, which entered an 'off' year, formed only 1.6 flowers per 100 nodes, unlike the 54.1 flowers formed in the 'off' trees that entered an 'on' year.

Sampling dates for determinations of carbohydrates, enzymatic activity and gene expression were performed based on previous studies (Monerri et al., 2011): 1) June, after fruit abscission, when the maximum rate of accumulation by the fruit occurred; 2) September and 3) December, in the middle and final period of fruit development, respectively; 4) January and 5) February, just before and after fruit harvest, respectively; and 6) March, after the beginning of Spring bud sprouting. Plant material was sampled between 10:00 h and 11:00 h on all six dates. The mature leaves (4th leaf from the apex) from vegetative shoots formed last Spring and the fibrous roots (1.5–2.5 mm in diameter) bearing new formed feeder roots were used in the study.

2.2. Carbohydrate analysis

The determination of total soluble sugars and starch (as mg per g of dry weight) was performed as described by García-Luis et al. (2002). Three independent extracts, each obtained from nine different trees (five leaves per tree and three trees per extract), were assayed for each treatment in all the determinations. Sucrose was determined by HPLC, as described by Iglesias et al. (2002).

2.3. Gene expression analysis

The expression of sucrose transporters SUT1 and SUT2 (Li et al., 2003c), sucrose synthases SUS1 and SUSA, sucrose-phosphate synthase (SPS, EC 2.4.1.14), α-amylase (AMY, EC 3.2.1.1) and ADP-glucose pyrophosphorylase (AGPP) genes (Li et al., 2003a), involved in carbohydrate metabolism, were studied (Table 1). Leaf tissue was finely ground in liquid nitrogen and total RNA was

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