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Increase in final fruit size of tangor (*Citrus reticulata* × *C. sinensis*) cv W. Murcott by application of benzyladenine to flowers

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ABSTRACT

Fruit development involves the phenomena of cell division and cell elongation. The process is signalled and regulated by the combined action of hormonal substances such as gibberellins, cytokinins and auxins. Benzyladenine (BA) in the presence of adequate internal auxin concentrations is able to stimulate cell division. When BA is applied to mandarins immediately after flowering, initial fruit development can increase, coinciding with endogenous cytokinin content behaviours. Different BA concentrations were therefore applied through immersion of flowers of tangor cv. W. Murcott during anthesis: 30 ppm (BA30); 60 ppm (BA60), 90 ppm (BA90) and an untreated control. BA effects were first determined on the development of different tissues during early fruit growth stages and final fruit size. In the first growth stage, BA90 led to an increase in the number and size of pericarp cells, with size increases also seen in locules and the central axis. This effect led to larger fruit size at the end of development. There was also an increase in the fruit number retained when treated with BA. No effect was seen on internal quality parameters. The effect of application of different BA concentrations on W. Murcott tangor trees is yet to be determined.

1. Introduction

Fruit size is a decisive external quality parameter in mandarins. There is a constant search for new farming techniques to obtain larger fruit. Fruit development involves the phenomena of cell division and cell elongation. Differences or imbalances in some of these events or their combination result in fruit size variation (Bons et al., 2015; El-Otmani et al., 2000; Ozga and Reinecke, 2003). Three growth stages have been identified during fruit development and are seen to overlap. In the first stage, the number of cells in ovary tissue increases as a result of cell division, especially in the pericarp. This stage also sees the beginning of cell differentiation in all fruit tissues. In the second stage, cell elongation begins; it is important for increasing the volume and weight of the fruit resulting from accumulation of dry matter and water. The third stage begins with the development of phenomena associated with fruit maturity, while the growth rate decreases drastically (Bain, 1958; Farooq et al., 2011; Tadeo et al., 2003). The pericarp reaches approximately three-quarters of the fruit radius by the end of the first stage. In the second stage, the pericarp becomes longer and thinner, taking up 20% of the total radius (Dalal et al., 2012; Guardiola and Lázaro, 1987). Locules are structures that determine the final fruit size. They originate from endocarp walls and contain juice vesicles. The growth and development of juice vesicles can be further divided into three stages: cell division, elongation and maturation. These coincide with fruit growth stages (Burns et al., 1992).

During development, the fruit acts as a significant sink of photosynthates and water. The transport of photoassimilates to fruit is determined by availability in nearby sources and sink strength used to attract them (Guardiola and García-Luis, 2000, 1998), not by their ability to be transported through the phloem (García-Luis et al., 2002). In some cultivars, the restriction of carbohydrate availability at the end of the first stage or during the period of highest demand for photosynthates limits fruit development (Goldschmidt, 1999). However, Bustan et al. (1996) shows that in Murcott, an increase in photosynthate availability during the initial part of the first growth stage does not affect fruit development, as this is more significantly affected by the initial floral ovary size.

Fruit development is a process that is signalled and regulated by the combined action of hormonal substances, such as gibberelins, cytokinins and auxins. The response is modulated by different levels of these substances (Kumar et al., 2014). These hormones, considered promoters of fruit growth, differ in their effects and their action (Talon et al., 1998). In citrus species, several cytokinins present in the ovary have been identified: ribosyl zeatin, zeatin, isopentenyl adenosine and

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isopentenyl adenine (Hernández Miñana et al., 1989). In pirenic mandarins such as Cleopatra, cytokinin content measured in ovary tissue was found to depend on ovule fertilisation (Hernández Miñana and Primo-Millo, 1990). In others such as Murcott, cytokinin content in ovary tissue is independent of this phenomenon (Bermejo et al., 2015). In apirenic mandarins, the highest level of cytokinin activity was observed at anthesis, BBCH stage 60 (Agustí et al., 1995), and fell rapidly over a few days, suggesting a direct connection between this growth regulator and the initial fruit development stage.

Exogenous application of growth regulators has been described as an important tool for manipulating fruit development (Monselise, 1979). Auxins applied at the end of the natural drop lead to increased final fruit size, due to an increase in sink capacity, increased sugar accumulation (sucrose and hexose) with subsequent cell growth in all tissues, and an increase in dry matter content of retained fruit (Agustí et al., 2002, 1996; Yildirim et al., 2011). However, there is still very little information regarding exogenous cytokinin application to stimulate fruit development (Mariotti et al., 2011). The results of applications in previous studies vary with species, variety, type of cytokinin, concentration, timing and application methods (Bound et al., 1991). The most common cytokinin substances belong to the adenine group, such as kinetins and benzyladenines (BA), and the phenylurea group, such as forchlorfenuron (CPPU) and thidiazuron (Bubán, 2000). Tests with CPPU in oranges have shown a low effect on final fruit size, a significant effect on colour and internal maturity delay, and chlorophyll destruction (Abd El Raheem et al., 2013).

BA has been described as a natural cytokinin that has the ability to stimulate cell division in the presence of adequate auxin concentrations (Van Staden and Cook, 1986). In apple and pear trees, it has been found that BA acts directly to increase the cell division rate in the fruit (Bubán, 2000; Greene et al., 1992; Ouma, 2012; Wismer et al., 1995). BA application on satsumas immediately after flower opening stimulated initial fruit development, coinciding with endogenous hormone content behaviour (Guardiola et al., 1993). Thus, it is expected that BA concentrations applied to W. Murcott flowers stimulate cell division and final fruit size. Therefore, the objective of this study was to evaluate different BA concentrations applied to the flowers of W. Murcott tangor during BBCH stage 60, corresponding to the first open flowers (Agustí et al., 1995). The BA effect on the development of different tissues during the first fruit growth stage and on the final size was determined.

2. Materials and method

2.1. Plant material and growth regulator

The study was conducted in an orchard in central Chile (latitude 32°56′34.19″S; longitude 71°16′9.62″O). Six-year-old W. Murcott tangor trees (*C. reticulata* × *C. sinensis* (L.) Osbeck) were selected, grafted onto citrange C-35 rootstock (*C. sinensis* × *P. trifoliata*) and planted in a 4 × 2 m framework. The trees were irrigated using drip irrigation, with evaporation from a class A evaporimeter tank as a reference. The nutrition program involved fertigation with nitrogen, phosphorus, potassium and zinc, with additional leaf application of other micronutrients.

At the beginning of anthesis, 40 trees with good, similar vigour, good sanitary condition, and similar flowering intensity (in an "on" year) were selected, discarding trees that were in an "off" year. On each tree, four flowers opening the same day located on single flowered leafy inflorescences (LOF) and eight top flowers opening the same day located on several flowered leafy inflorescences (LSF) were chosen, and the flowers were marked. The treatments consisted of the flower being submerged for 15 s in one of the BA concentrations made with distilled water: 30 ppm (BA30); 60 ppm (BA60), 90 ppm (BA90) and an untreated control. A non-ionic wetting agent, at 0.02% (v/v) concentration, was added to all solutions (Curetti et al., 2011). The treatments were randomly distributed on each tree between flowers of the same type of inflorescence.

2.2. Anatomic study

Sixty days after application (DAA), a sample of 10 fruits from BA90 treatment and 10 control fruits were taken from LOF inflorescences. The fruits were cut along the middle to measure diameter, pericarp thickness, dorsal and radial lengths of locules, and central axis diameter, using the program ImageJ 1.50i (USA). The samples were fixed in FAA solution (95% ethyl alcohol: glacial acetic acid: formaldehyde: water; 10:1:2:7, v/v/v/v). Later, at the Histology Laboratory of Pontificia Universidad Católica de Valparaíso, the samples were prepared for histological sectioning. They were initially subjected to increasing dehydration treatment with butyl alcohol at 50, 70, 80, 96 and 100%. Inclusion was performed with Xylol and Histosec (Merck), and blocks were prepared with paraffin. Sections were cut with a width of 10–14 microns in a manual rotating microtome (LEICA RM 2235, Germany). Hydration was performed with decreasing amounts of alcohol and water. Staining was applied with 1% Safranin, followed by washing and further dehydration with ethanol at 70 and 96%. A second stain, Fast green, was applied, and the samples were dehydrated with 100% ethanol and Xylol, and sealed with Entellan (Merck).

The samples were analysed under a microscope (Olympus CX31, Japan) with an objective Plan CN 10x/0.25 lens. A pericarp area was selected for the study comprised of a strip 140 micros in width from the external epicarp layer to the external locule wall, avoiding passing over oil glands. The number and size of cells on the strip was determined using ImageJ software (v. 1.50i, US National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/) (Fig. 1).

2.3. Monitoring development and fruit drop

The treated fruit diameters were measured periodically with a digital calliper (CALDI- 6MP, Truper, Mexico) from anthesis until harvesting. Fruit drop was also recorded during this period to identify the percentage of retained fruit.

2.4. Acidity and soluble solids

Two hundred and eighty-three days after the treatment application, all fruits were harvested. Five fruits were randomly selected for each type of inflorescence and treatment, and the soluble solids (SS) content was measured using a digital refractometer (HI 96801, Hanna Instrument, USA). Titratable acidity (TA) was measured using a digital meter (GMK-835F, G-Won, Korea), and the correlation between SS and TA was identified.

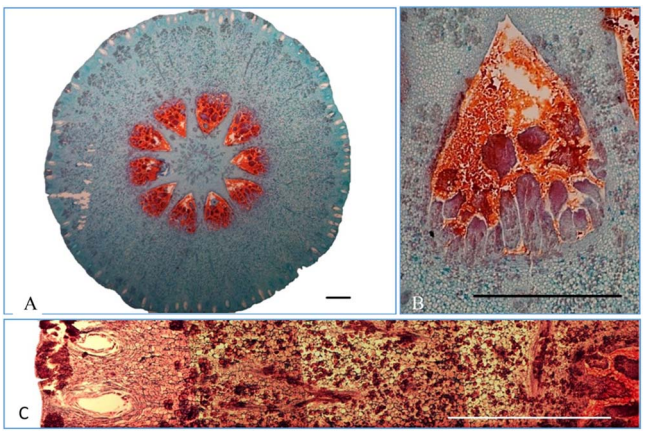

Fig. 1. A. Cross-section of W. Murcott tangor fruit 60 days after anthesis. B. Locule showing the development of juice vesicles; C. Section of pericarp on which a 140-μm strip was selected to measure the number and size of cells. Scale bar = 1 mm.