ACID CITRUS FRUIT CULTIVAR IMPROVEMENT VIA INTERPLOID HYBRIDIZATION

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Abstract. We have conducted several interploid crosses between fertile somatic hybrids such as ‘Hamlin’ + ‘Femminello’, ‘Key’ lime + ‘Valencia’, ‘Valencia’ + ‘Femminello’, ‘Milam’ + ‘Femminello’ and 4X ‘Femminello’ and various diploid acid fruit cultivars to generate seedless triploids with horticulturally acceptable fruit and improved cold-hardiness (inherited from sweet orange),适合 for production in sub-tropical regions. Embryo rescue was utilized to recover triploid progeny about three months after pollination. Embryos in different developmental stages were grown on Murashige and Tucker medium supplemented with 500 mg/L malt extract. A total of fifty three triploids and twenty two tetraploids were recovered from different cross combinations.

Lemons and limes are not commercially grown in central Florida due to their lack of cold hardiness and their adverse horticultural responses to the semi-tropical condition. Typically, lemon fruits grow too large; and storage quality is negatively affected by peel condition and fungal diseases. ‘Key’ limes are better suited to the warm, humid climate but have no tolerance to frost; also, they are susceptible to lime anthracnose and citrus tristeza virus (CTV). Lemons are not susceptible to CTV, and can tolerate slightly colder winter conditions; however, melanose and scab can complicate cultivation. Citrus canker poses a hazard to both cultivars.

Previously produced somatic hybrids that combine ‘Key’ lime and ‘Femminello’ lemon with ‘Valencia’ or ‘Hamlin’ sweet oranges are flowering and producing pleasant acid-type fruit (Grosser et al., 1989; Tusa et al., 1990 and 1992). Although these somatic hybrid trees are more cold-hardy than cultivated limes and lemons, their fruit is horticulturally inferior to the standard cultivated varieties. Obtaining triploid hybrids will result in seedless fruit, reduced fruit size, and thinner rinds. These improvements should facilitate marketing. Improved cold-hardiness is expected from the use of sweet orange somatic hybrids in the breeding scheme. Using limequat hybrids (which contain Fortunella genes) in the crosses could introduce canker resistance to the new hybrids. Resistance to anthracnose could also be inherited by these new hybrids.

Materials and Methods

‘Key’ lime (Citrus aurantifolia Swing.), five lemon [C. limon (L.) Burm. F.] cultivars, four autotetraploid somatic hybrids (Grosser et al., 1989; Tusa et al., 1990 and 1992) and one autotetraploid grown at The Florida Citrus Arboretum-Division of Plant Industry (Winter Haven, FL) or CREC (University of Florida, Lake Alfred, FL) were selected as breeding parents for interploid crosses. The lemon cultivars, somatic hybrids, and cross combinations are specified in Tables 1 and 2.

Flowers from seed parents were emasculated before anthesis and immediately pollinated with stored pollen collected in the same season. Approximately three months after pollination, the fruits were harvested and stored temporarily at 4°C. Fruits were surface sterilized for at least 30 min. with a solution of 20% bleach (5.25% NaOCl) with three drops of liquid detergent added as a surfactant. Thereafter, the fruits were cut at the equatorial region without damaging the core where the seeds are embedded. After twisting and separating the two halves, seeds were extracted and the embryos carefully excised under a stereo microscope. Embryos in globular, heart and cotyledon stages were cultured on EME medium (Grosser and Gmitter, 1990) under continuous light at 25 to 27°C.

One month later, the germinated embryos were transferred onto RMAN (rooting medium) (Grosser and Gmitter, 1990) for about one month. Embryo germination was induced in Petri dishes (100× 15 mm) and resulting plantlets were grown in Magenta vessels. Seedlings were transplanted into 38-well trays contained a commercial soil mixture (Metro Mix-500®). After acclimatization at 80% RH at 28°C, the plants were moved into a greenhouse under normal conditions.

Ploidy Analysis

Ploidy level of new seedlings was determined by cytology or flow cytometry analysis. The first technique implies direct microscopic counting of chromosomes from actively growing root tip cells. A modified hematoxylin staining protocol was applied as described by Grosser and Gmitter (1990). For the latter technique, the relative amount of nuclear DNA was determined by flow cytometry. Ploidy determinations by flow cytometry were performed by the ICBR, Flow Cytometry Core, University of Florida, according to the procedure previously described by Arumuganathan and Earle (1991) and using a FacStar PLUS flow cytometer (Becton-Dickinson, Franklin Lakes, NJ).

Results and Discussion

Fruit set and the number of seed per fruit were affected by the pollen parent (Table 1), with the highest number of seeds observed in ‘Lisbon’ and ‘Todo del Año’. The percentage of empty seeds was higher than 25% in most of the diploid-by-tetraploid crosses, except for ‘Todo del año’ by (‘Key’ lime + ‘Valencia’), which showed 6.95% embryoless seeds. Fully developed seeds were collected from the ‘Lisbon’ by (‘Hamlin’+‘Femminello’) crosses; nevertheless, there were no hybrid embryos recovered. This suggests a rapid growth of nucellar embryos and the subsequent death of the zygotic one. Histological studies showed that most of the citrus embryos from diploid (monoembryonic) by tetraploid crosses abort due to the degeneration or abnormal development of the 4X endosperm (Esen and Soost, 1973).

Regardless of the cross combination, the number of recovered plants was low considering the number of extracted seeds and the polyembryonic characteristic of the maternal parents. The number of triploid hybrids was variable. The frequency was determined by the cross combination to a certain extent, and the in vitro germination capacity of the embryos. The number of zygotic embryos was

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very low (1/129) among the total offsprings from crosses that included 4× Femminello as pollen parent (Table 1). The success of embryo rescue was notably affected by the developmental embryo stage. Many embryos in globular and heart stages did not show any further development on EME medium, while embryos in more advanced development germinated successfully. Globular embryos from interspecific citrus crosses also failed to develop on a modified White’s medium supplemented with different addenda (Rangan et al., 1969). Conversely, early embryo stages from *Citrus aurantium* germinated at very high percentage on MS supplemented with malt extract (Carimi et al., 1998). These contrasting results might be related with the triploid characteristic of the new hybrids in addition to sexual incompatibility present between tree parents. Additionally, a few plants died when cultured on RMAN, and a few more during acclimatization. In general, the apical meristems of some seedlings stopped growing before or after the first true leaves, then died.

The occurrence of triploid hybrids was higher from tetraploid-by-diploid crosses than their reciprocals (Tables 1 and 2), although the fruit set and the number of seeds per fruit were lower (Essen and Soost, 1972). Even though the polyembryony present in somatic hybrids can hamper pollination success, this problem can be overcome by increasing the amount of pollinations.

Embryo culture is a useful technique for citrus triploid production. Resulting triploids have been budded to precocious somatic hybrid rootstocks in preparation for field evaluation. Further research is being conducted to improve germination and survival of triploid hybrid embryos, and to identify superior parental combinations. Additional parents were utilized in the spring 2000 crosses, including ‘Lakeland’ limequat to potentially improve disease resistance in resulting triploid progeny.

**Literature Cited**


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**FACTORS INVOLVED IN SOLUBLE SOLIDS ACCUMULATION IN CITRUS FRUITS**

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Abstract. The direction of photosynthate movement among plant organs is determined by “sink strength”, a model involving the importation of sucrose (or other sugars) by hydrolysis or sequestration. In most plants, sink strength is determined by the activities of two sucrose-cleaving enzymes: sucrose synthase (SS) and invertase (INV). In addition, activities of sucrose phosphate synthase (SPS), sucrose phosphate phosphatase (SPP), and tonoplast-bound ATPase could affect sink strength. Increased activities of membrane-bound sucrose transporters or decreased vacuolar pH could also enhance accumulation of soluble solids. In fruits of many commercial crops, increases in soluble solids have been recorded during drought conditions. This research investigates the determinants of citrus fruit sink strength through drought stress. Potted Hamlin orange trees were grown under watered or drought-stressed conditions and fruit harvested and analyzed for Brix and acids. Stressed fruits had higher acid content and soluble solids, and lower pH than controls. The following components of fruit sink strength were measured: SS, INV, SPS, SPP, ATPase, PPase. In addition, isolated and purified membranes from fruit were tested for the presence of a sucrose symporter at the plasmalemma and an antiporter at the tonoplast. Increased sink strength appeared to be the result of SS, since SS activity was higher in drought-stressed versus well-watered fruit. Activities of other enzymes and transporters were not significantly different between control and treated fruit. We concluded that SS is the predominant factor controlling Brix levels in citrus fruit, although the altered pH could have contributed to sink strength by enhancing acid hydrolysis.

It is well known that some temperate fruits accumulate higher levels of soluble solids during mild drought stress (Behboudian and Mills, 1997). Since fruit quality and production is not compromised by mild late-season drought but rather enhanced, this issue has attracted the interest of many researchers and fruit producers.

The mechanism involved is more than concentration by dehydration as there is active accumulation of solids in fruit (Mills et al., 1996; Yakushiji et al., 1996). Increasing fruit soluble solids during drought involves one or more of the following processes, such as sugar movement, accumulation, and/or storage into fruit. However, the precise mechanisms are still unclear.

The direct movement of assimilated carbon into a particular plant organ is determined by its ‘sink strength’ and by photosynthesis in source tissue. ‘Sink strength’ is the ability of a particular organ to attract photoassimilates (Ho, 1988). During fruit elongation and expansion, fixed carbon is required to provide growing tissues with energy for metabolism and to provide osmotic solutes to maintain turgor pressure. Fixed carbon is transported through the phloem in the form of sucrose, a disaccharide composed of joined molecules of fructose and glucose. To develop a concentration gradient for adequate sink strength, cells must cleave sucrose, or effectively sequester it into the vacuole as in sugar beets (Getz et al., 1991). Therefore, sink strength is determined by the ability of the sink to metabolize sucrose and/or by its capacity for compartmentation and storage. In plants, there are two specific enzymes capable of cleaving sucrose. The first is invertase (INV), whose unidirectional catalytic action yields fructose and glucose. The second enzyme is sucrose synthase (SS) with a reversible reaction using sucrose and UDP to yield UDP-glucose and fructose. Sequestering sucrose in vacuoles permits the sink cell to maintain a sucrose gradient between itself and the phloem, allowing the continuous movement of sucrose toward the sink.

Many storage organs appear to require the resynthesis and storage of sucrose into the vacuole in a seemingly ‘futile’ cycle (Ho, 1988). Related sucrose-metabolizing or synthesizing enzymes that may play a role in sink strength are sucrose phosphate synthase (SPS, UDPG + F-6-P→S-6-P + UDP) and sucrose phosphate phosphatase (SPP, S-6-P→Sucrose + B), although their exact functions in a sink organ are uncertain. Other factors such as low vacuolar pH may be involved in sink strength, since low pH can cleave sucrose (Wienen and Shallenberger, 1988). In citrus, the vacuoles of fruit juice cells can be extremely acidic with a pH of 3 or lower (Echeverria and Burns, 1989). Such low pH is capable of hydrolyzing sucrose into glucose and fructose in vitro (Wienen and Shallenberger, 1988). That same low pH could automatically cleave sucrose entering the vacuole at a rate dependent on the hydronium ion concentration and temperature. A fruit cell could use a sucrose antiport at the tonoplast to sequester sucrose into the vacuole, utilizing the existing pH (Getz et al., 1991).