Somatic Hybridization, an Integral Component of Citrus Cultivar Improvement: I. Scion Improvement

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Abstract. Seedlessness is an important breeding objective of most citrus scion improvement programs, but production of quality seedless triploid citrus via interploid crosses has historically been limited by the low quality of available tetraploid parents. Production of tetraploid hybrid parents from elite diploid scion cultivars via protoplast fusion is now a practical strategy, and numerous hybrids can be produced on a timely basis from a wide range of parents. Such hybrids can be used as pollen parents in interploid crosses to generate improved seedless triploid fresh fruit cultivars. Herein we report the production of 15 such hybrids from 17 different parents, including sweet orange [*Citrus sinensis* (L.) Osbeck], mandarin/tangerine (*C. reticulata* Blanco), grapefruit (*C. paradisi* Macf.), pummelo [*C. grandis* (L.) Osbeck], tanger (*C. reticulata* × *C. sinensis*), and tangelo (*C. reticulata* × *C. paradisi*) germplasm. All hybrids were confirmed by cytological and RAPD analyses, and have been budded to selected rootstocks to expedite flowering.

Fruit of many commercially important fresh market citrus cultivars in Florida are seedy, especially tangerines, tangors, and tangelos (Saunt, 1990). Seediness limits the domestic marketing potential of Florida citrus for fresh consumption, and excludes most popular Florida cultivars from international markets. One strategy to achieve seedlessness in new cultivars is to generate and select seedless triploids from interploid crosses (Soost and Cameron, 1975; Starrantino and Reforgiato-Recupero, 1981). Two examples of triploid citrus cultivars produced by interploid hybridization are 'Oroblanco' and 'Melogold' pummelo × grapefruit hybrids (Soost and Cameron, 1980, 1985). However, progress using this approach has been limited by a lack of quality tetraploid breeding parents. Unfruitfulness, small fruit size, and high acidity in progeny are also limiting factors, based on hybrids produced by Soost and Cameron (1975).

Recently, autotetraploid breeding parents have been produced by in vitro colchicine induction of chromosome doubling (Gmitter and Ling, 1991; Gmitter et al., 1991) and allotetraploid somatic hybrid breeding parents via somatic hybridization (Grosser and Gmitter, 1990; Grosser et al., 1992a, 1992b). Early reports of interspecific somatic hybrids among

important scion cultivars included only one or a few hybrids, reflecting the difficulty of their production (Grosser et al., 1992a, 1992b; Kobayashi and Ohgawara, 1988; Kobayashi et al., 1988; Ohgawara et al., 1989; Tusa et al., 1990, 1992). Expanded efforts and improvements in techniques (Grosser, 1994) have in-

creased production of elite allotetraploid breeding parents by somatic hybridization (Mourao et al., 1996), offering breeders more appropriate choices of complementary parents in interploid crosses. In our program, two previously reported somatic hybrids, 'Pink Marsh' grapefruit + 'Murcott' tanger (presumed) and 'Nova' tangelo + 'Succari' sweet orange have flowered, and triploid embryos/plantlets have been recovered from interploid crosses using the latter as a pollen parent (F.G. Gmitter, Jr., unpublished data). We report herein the production of 15 new interspecific, tetraploid, somatic hybrid breeding parents (Table 1).

Materials and Methods

Somatic hybrid production. Protoplasts of 'Valencia', 'Rohde Red Valencia', 'Succari', and 'Hamlin' sweet oranges, 'Murcott' tanger, 'Nova' tangelo, and 'Redblush' grapefruit were isolated from either embryogenic callus or suspension cultures maintained on H+H medium according to Grosser and Gmitter (1990). These nucellus-derived lines are part of the Univ. of Florida Citrus Research and Education Center (CREC) citrus embryogenic culture library that maintains >50 genotypes. Leaf protoplasts of 'Minneola' tangelo, 'Page' citrus hybrid, 'Dancy' and 'Ponkan' mandarins, 'Murcott' tanger, three unnamed hybrids (kindly provided by Dr. C.J. Hearn, USDA/ARS, Orlando, Fla.), and 'Hirado Buntan' zygotic pummelo were isolated from seedlings, and leaf protoplasts of hybrids LB8-4 and LB8-9 from newly grafted trees maintained in a growth chamber, according to the methods described by Grosser and Gmitter (1990). Plants providing leaves for protoplast

Table 1. Parental combinations of 15 new somatic hybrid tetraploid citrus breeding parents.

Embryogenic parent	Leaf parent (non-embryogenic)	Number of regenerated somatic hybrid plants
'Valencia' sweet orange	'Minneola' tangelo (('Duncan' grapefruit × 'Dancy' tangerine))	1
'Valencia'	'Murcott' tanger	3
'Valencia'	'Page' citrus hybrid (('Minneola' tangelo × 'Clementine' mandarin))	>10
'Valencia'	USDA-CJH Hybrid 3 ¹ (('Robinson' tangerine × 'Temple' tanger))	2
'Rohde Red Valencia' sweet orange	'Dancy' tangerine	2
'Rohde Red Valencia'	USDA-CJH Hybrid 1 ¹ (('Fortune' mandarin × 'Kinnow' mandarin))	1
'Murcott'	LB8-9 ² (('Clementine' × 'Minneola'))	>10
'Murcott'	USDA-CJH Hybrid 1 ¹	>20
'Murcott'	USDA-CJH Hybrid 2 ¹ (('Wilking' mandarin × 'Valencia' sweet orange))	3
'Redblush' grapefruit	LB8-9 ²	1
'Succari' sweet orange	'Hirado Buntan' zygotic pummelo	23
'Succari'	USDA-CJH Hybrid 1 ¹	6
'Nova' tangelo (('Clementine' × 'Orlando' tangelo))	'Hirado Buntan' zygotic pummelo	>50
'Hamlin' sweet orange	'Ponkan' mandarin	9
'Hamlin'	LB8-4 ² (('Clementine' × 'Minneola'))	>10

¹Nucellar seedling.

²Zygotic seedling, open pollination.

³Seedling type undetermined.

⁴Newly grafted tree.

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isolation were rotated between a greenhouse and growth chamber to promote continuous healthy growth flushes. Protoplast purification, fusion, culture, and subsequent plant regeneration were conducted as described previously (Deng et al., 1992; Grosser and Gmitter, 1990).

Somatic hybrid verification. Ploidy levels of regenerated fusion products were determined by counting chromosome numbers in mitotically active root-tip cells, using the modified hematoxylin staining procedure described by Grosser and Gmitter (1990). Analyses by RAPD using total DNA extracted from somatic hybrids and parents were according to Gmitter et al. (1996), using random decamer primers from Operon Technologies, Alameda, Calif. (Figs. 1, 2).

Results and Discussion

Regenerated somatic hybrid plants from the various parental combinations attempted are listed in Table 1. Cytological analysis revealed that all were tetraploid ($2n=4x=36$). All somatic hybrid plants were verified by RAPD analyses (Figs. 1, 2). Complementary banding patterns were observed for all hybrids. Because the PCR primers used in this study cannot distinguish among various sweet oranges, DNA from 'Valencia' was used for all sweet orange parents in the RAPD analyses. The number of regenerated plants per parental combination (Table 1) was indicative of the general level of difficulty in producing each somatic hybrid. Producing such hybrids for scion improvement is generally more difficult than for rootstock improvement, because parents selected for the former are generally more closely related, which may reduce somatic hybrid vigor.

These new somatic hybrids may have potential for direct use as improved cultivars, but their greatest value probably will be as tetraploid pollen parents in interploid crosses to generate seedless triploids. These hybrids have been budded to citrus rootstocks and field-planted to hasten flowering and subsequent crosses with selected diploid monoembryonic seed parents. Expanded quality tetraploid germplasm will provide breeders with opportunities to manipulate important traits such as fruit color, size, flavor, peelability, and maturity date, and should result in the development of improved seedless cultivars.

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Fig. 1. RAPD patterns of interspecific somatic hybrids and their parents. First and last lanes = DNA ladder. Fragments in lanes 1-18 were amplified by Operon primer A-07, and 19-21 by X-18. Lane 1—'Murcott' tangelo; 2—'Murcott' + LB8-9 somatic hybrid; 3—LB8-9; 4—'Red Marsh' grapefruit + LB8-9 somatic hybrid; 5—'Red Marsh' grapefruit; 6—USDA-CJH Hybrid 2; 7—'Murcott' + USDA-CJH Hybrid 2 somatic hybrid; 8—'Murcott'; 9—'Murcott' + USDA-CJH Hybrid 1 somatic hybrid; 10—USDA-CJH Hybrid 1; 11—'Rohde Red Valencia' + USDA-CJH Hybrid 1 somatic hybrid; 12—'Succari' sweet orange + USDA-CJH Hybrid 1 somatic hybrid; 13 and 16—sweet orange; 14—'Dancy' tangerine; 15—'Rohde Red Valencia'; 17—'Dancy' somatic hybrid; 18—'Hamlin' sweet orange + 'Ponkan' mandarin somatic hybrid; 19—'Nova' tangelo; 20—'Nova' tangelo + 'Hirado Buntan' zygotic pummelo somatic hybrid; 21—'Hirado Buntan' zygotic pummelo.

Fig. 2. RAPD patterns of interspecific somatic hybrids and their parents. First and last lanes = DNA ladder. Fragments in lanes 1-10 were amplified by Operon primer AA-07, 11-13 by primer X-18, 14-18 by primer P-10, and 19-21 by primer AK-15. Lane 1—'Murcott' tangelo; 2—'Valencia' + 'Murcott' somatic hybrid; 3, 8, 13, 16, and 19—sweet orange; 4—'Valencia' sweet orange + 'Page' somatic hybrid; 5—'Page' citrus hybrid; 6—'Hirado Buntan' zygotic pummelo; 7—'Succari' sweet orange + 'Hirado Buntan' zygotic pummelo somatic hybrid; 9—'Hamlin' sweet orange + LB8-4 somatic hybrid; 10—LB8-4; 11—'Minneola' tangelo; 12—'Valencia' + 'Minneola' somatic hybrid; 14—'Minneola' tangelo; 15—'Valencia' + 'Minneola' somatic hybrid; 17—'Valencia' + USDA-CJH Hybrid 3 somatic hybrid; 18—USDA-CJH Hybrid 3; 20—'Valencia' + USDA-CJH Hybrid 3 somatic hybrid; 21—USDA-CJH Hybrid 3.

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