Somatic Hybridization, an Integral Component of Citrus Cultivar Improvement: II. Rootstock Improvement

J.W. Grosser¹, J. Jiang, E.S. Louzada, J.L. Chandler, and F.G. Gmitter, Jr.

University of Florida, Institute of Food and Agricultural Sciences, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850-2299

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Abstract. Production of tetraploid somatic hybrids that combine complementary diploid rootstock germplasm via protoplast fusion has become a practical strategy for citrus rootstock improvement, with the overall objective of packaging necessary disease and pest resistance into horticulturally desirable, widely adapted rootstocks. Citrus somatic hybridization techniques have been advanced to the point where numerous somatic hybrid rootstocks can now be produced and propagated for evaluation on a timely basis. Herein we report the production of 11 new somatic hybrid rootstock candidates from 12 different parents, including Milam lemon hybrid (*Citrus jambhiri* Lush.), Cleopatra mandarin (*C. reticulata* Blanco), sour orange (*C. aurantium* L.), 'Succari' sweet orange [*C. sinensis* (L.) Osbeck], 'Redblush' grapefruit (*C. paradisi* Macf.), 'Nova' tangelo [*C. reticulata* × (*C. paradisi* × *C. reticulata*)], 'Kinkoji' (*C. obovoidea* Hort. Ex Takahashi), Swingle citrumelo [*C. paradisi* × *Poncirus trifoliata* (L.) Raf.], Carrizo citrange (*C. sinensis* × *P. trifoliata*), rough lemon 8166 (*C. jambhiri*), and Palestine sweet lime (*C. limettoides* Tan.). All hybrids were confirmed by cytological and VNTR-PCR analyses, and have been propagated, budded with a commercial scion, and field-planted for performance evaluation.

Commercial citrus rootstocks usually do not satisfy all selection criteria for a specific location, e.g., disease and nematode resistance, cold hardiness, adaptation to various soils and conditions, appropriate tree size, and high yield of quality fruit (Castle, 1987). One strategy for rootstock improvement is to produce allotetraploid somatic hybrids that combine the intact nuclear genomes of complementary rootstocks (Grosser and Gmitter, 1990). Somatic hybridization techniques (Grosser, 1994) have resulted in the production of >20 citrus rootstocks (Grosser et al., 1992, 1994; Louzada et al., 1992). Preliminary observations of tree survival and early fruit production on scions budded to these hybrids in field trials have been encouraging (Grosser et al., 1995). We report herein the production of 11 additional tetraploid somatic hybrids that combine complementary diploid rootstock germplasm (Table 1).

Materials and Methods

Somatic hybrid production. Protoplasts of Milam lemon hybrid, sour orange, 'Nova' tangelo, Cleopatra mandarin, 'Succari' sweet orange, and 'Redblush' grapefruit were isolated either from 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 'Kinkoji', Swingle citrumelo, Carrizo citrange, Palestine sweet lime, rough lemon 8166, and sour orange were isolated from nucellar seedlings maintained in a growth chamber according to the methods described by Grosser and Gmitter (1990). Plants providing leaves for protoplast isolation were rotated between a greenhouse and a 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 plants were determined by counting chromosome numbers in mitotically active root-tip cells, using the modified hematoxylin staining procedure described by Grosser and Gmitter (1990). Variable number of tandem repeats-polymerase chain reaction (VNTR-PCR) analyses using total DNA extracted from somatic hybrids and parents and amplified with three short repetitive nucleotide sequences, (TCC)5, (GACA)4, and (GTG)5, were performed according to Luro et al. (1995).

Results and Discussion

Regenerated somatic hybrid plants from the various parental combinations attempted are summarized in Table 1. Regenerated somatic hybrid plants were morphologically and genetically uniform within parental combinations, as expected. Cytological analysis revealed that all regenerated somatic hybrid plants were tetraploid ($2n = 4x = 36$). Primer identification and results are provided in Fig. 1. Complementary banding patterns were observed for all hybrids. Somatic hybrids were verified using primer (TCC)5 for all except Milam + Kinkoji and Milam + Carrizo, which were verified using primer (GACA)4. Many of the hybrids were also verified using primer (GTG)5 (data not shown). The number of regenerated somatic hybrid plants per parental combination (Table 1) was indicative of the general level of difficulty in producing each somatic hybrid.

Swingle citrumelo, Carrizo citrange, Cleopatra mandarin, rough lemon, and sour orange are commercially important rootstocks in Florida; Palestine sweet lime, Milam, and sweet orange are used to a minor extent (Castle, 1987). Attributes of these rootstocks have been described by Castle (1987). 'Kinkoji' is similar to sour orange, but unlike sour orange it is resistant to quick decline disease caused by citrus tristeza virus (CTV) in Florida (Castle et al., 1992). Rough lemon 8166 was reported to be a superior clone of rough lemon because of its slight susceptibility to *Phytophthora*-induced disease, high resistance to *Alternaria* leaf spot (*Alternaria citri*) in nurseries, and

Table 1. Parental combinations of 11 new somatic hybrid, tetraploid rootstock candidates.

Embryogenic parent	Leaf parent (nonembryogenic)	No. of regenerated somatic hybrid plants
Milam lemon hybrid (purported)	'Kinkoji'	1
Milam lemon hybrid	Swingle citrumelo	>100
Milam lemon hybrid	Carrizo citrange	15
Cleopatra mandarin	Carrizo citrange	>100
Sour orange	Carrizo citrange	>100
'Succari' sweet orange	Sour orange	>50
'Succari' sweet orange	'Kinkoji'	6
'Succari' sweet orange	Rough lemon 8166	>50
Cleopatra mandarin	Rough lemon 8166	>20
'Redblush' grapefruit	Rough lemon 8166	>20
'Nova' tangelo	Palestine sweet lime	12

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¹To whom reprint requests should be addressed.

high tolerance to cold (Whiteside and Knorr, 1978). Autotetraploid rootstocks have good potential for tree size control (Lee et al., 1990), and preliminary observations of some allotetraploid somatic hybrid rootstocks also suggest this (J.W. Grosser, unpublished data). Tree size control to reduce harvesting costs and maximize the efficiency of cold protection technology has become an important rootstock breeding objective. All hybrids reported herein have potential to control tree size. The Milam + Swingle and Milam + Carrizo somatic hybrids were produced in an effort to combine the burrowing nematode resistance of Milam with the citrus nematode resistance of Swingle and excellent horticultural performance of Carrizo, and to improve the adaptability of Swingle and Carrizo to high pH and/or calcareous soils. The Cleopatra + Carrizo somatic hybrid may combine the broader adaptability and moderate blight resistance of Cleopatra with the productivity of Carrizo. The sour orange + Carrizo somatic hybrid was made in an effort to combine the wide adaptability and blight resistance of sour orange with the resistance to CTV-induced quick decline of Carrizo. 'Succari' sweet orange was hybridized with sour orange in an attempt to improve the CTV tolerance of sour orange. The three hybrids made with rough lemon 8166, and the Nova + Palestine sweet lime hybrid, are attempts to produce vigorous rootstocks with improved blight resistance and fruit quality. Milam was hybridized with 'Kinkoji' to provide burrowing nematode resistance. 'Kinkoji' has adequate resistance to *Phytophthora*, but its blight resistance is yet to be determined. The 'Succari' sweet orange + 'Kinkoji' somatic hybrid should have excellent blight resistance.

All somatic hybrids reported herein have been propagated, budded with sweet orange, and field-planted in replicated trials to evaluate commercial performance and to identify alternative rootstocks for specific citriculture schemes. General nursery performance and bud take of these new somatic hybrids were comparable to those of standard commercial rootstocks, with the exception of 'Succari' + 'Kinkoji', which showed lower vigor, and Milam lemon hybrid + Swingle citrumelo, which exhibited dieback following budding. Selected somatic hybrids reported herein are also being screened to determine their resistance to CTV, *Phytophthora*, and nematodes. Determination of the genetic control of inheritance of important traits in these new somatic hybrids should prove valuable for selecting parents for future hybridizations. This research demonstrates that somatic hybridization in citrus has advanced beyond the point of being an academic exercise to where it can make a significant and continuous contribution to citrus rootstock improvement.

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Fig. 1. VNTR-PCR analyses of genomic DNA of somatic hybrids of citrus and their parents. Gel A products amplified with short repetitive nucleotide sequence (TCC)5, and Gel B with (GACA)4. Lanes 1 and 30—1Kb ladder; 3—Swingle citrumelo; 4—Milam + Swingle somatic hybrid; 5—Milam; 6—Milam + 'Kinkoji' somatic hybrid; 7—'Kinkoji'; 8—'Succari' sweet orange + 'Kinkoji' somatic hybrid; 9—'Succari' sweet orange; 10—'Succari' + sour orange somatic hybrid; 11—sour orange; 12—sour orange + Carrizo citrange somatic hybrid; 13—Carrizo; 14—Milam + Carrizo somatic hybrid; 15—Milam; 16—Carrizo; 17—Cleopatra mandarin + Carrizo somatic hybrid; 18—Cleopatra mandarin; 19—Cleopatra mandarin + rough lemon 8166 somatic hybrid; 20—rough lemon 8166; 21—'Succari' + rough lemon 8166 somatic hybrid; 22—'Succari'; 23—'Redblush' grapefruit; 24—'Redblush' + rough lemon 8166 somatic hybrid; 25—rough lemon 8166; 26—'Nova' tangelo; 27—Nova + Palestine sweet lime somatic hybrid; 28—Palestine sweet lime.