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New Tetraploid Breeding Parents for Triploid Seedless Citrus Cultivar Development

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Abstract

Protoplast culture following polyethylene glycol (PEG)-induced fusion resulted in the regeneration of somatic hybrid plants from 'Succari' sweet orange (*C. sinensis* L. Osbeck) with 'Dancy' tangerine (*C. reticulata* Blanco), 'Minneola' tangelo (*C. paradisi* Macf. X *C. reticulata* Blanco), 'Murcott' tangor (purported *C. reticulata* x *C. sinensis*), 'Page' tangelo ['Minneola' tangelo x 'Clementine' mandarin (*C. reticulata*)], and 'Ponkan' mandarin (*C. reticulata*). 'Succari' protoplasts were isolated from ovule-derived embryogenic cell suspension cultures, and protoplasts of the mandarin-type parents were isolated from seedling leaves. Somatic hybrid plants were identified on the basis of leaf morphology, root tip chromosome number, and isozyme and RAPD analyses. These tetraploid plants may have direct cultivar potential; however, their greatest value will be as pollen parents to be crossed with selected monoembryonic diploids to produce seedless triploids.

Introduction

Mandarins and mandarin hybrids, e.g., tangors [sweet orange x mandarin hybrids] and tangelos [grapefruit x mandarin hybrids] are among the most important citrus cultivars for fresh fruit, but many of these cultivars have an undesirable seed content (12). Triploidy may be exploited to produce seedless citrus fruit. Some successful triploid cultivars include 'Tahiti' lime (*C. aurantifolia* [Christ.] Swing.) (10), and 'Oroblanco' and 'Melogold' pummelo x grapefruit hybrids (15, 16).

Somatic hybridization is being utilized in scion improvement plans. Several interspecific somatic hybrids have been produced from cultivars such as 'Washington' navel orange (*C. sinensis*) and 'Hayashi' satsuma mandarin (*C. unshiu* Marc.) (8), to combine cultivar characteristics and overcome barriers to sexual hybridization, such as sterility, polyembryony, and low seed production. Somatic hybridization is important as a means to produce interspecific allotetraploid somatic hybrids (2n = 4x = 36) in which the genomes of complementary parents are combined (3, 4, 6, 7, 9, 11). Such tetraploid hybrids can be crossed with monoembryonic diploid female parents (2n = 2x = 18) to generate triploid zygotic progeny with seedless fruit. Seeds from such crosses generally are not well developed and their germination rate is very low. Zygotic embryo rescue and in vitro culture can overcome this difficulty (17).

Low sugar:acid ratios caused by high acid levels in the fruit of progeny from crosses involving mandarins has been a problem in citrus breeding programs (13). Soost and Cameron (14) indicated that hybrids with high fruit acid levels frequently result from crosses of parents with medium fruit acid levels. 'Succari' sweet orange is a vigorous, early-ripening cultivar with good fruit color. It is virtually acidless at maturity, with a sugar:acid ratio of 90-100:1. According to Barrett (1), 'Succari' transmits the low acidity characteristic to zygotic progeny, more frequently than do other oranges. Therefore, using 'Succari' in interploid crosses may increase the percentage of triploid zygotic progeny that produce fruit with acceptable sugar:acid ratios.

The objective of this research was to produce tetraploid somatic hybrid breeding parents (with 'Succari' sweet

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orange) for mandarin hybrid improvement. Improved seedless mandarin cultivars will allow United States citrus growers to expand their national marketing potential, and to become competitive in the international markets that do not accept seedy fruit.

Materials and Methods

Protoplasts of 'Succari' sweet orange were isolated from an ovule-derived embryogenic cell suspension culture maintained on H+H liquid medium with a 2-week subculture cycle (5). Leaf protoplasts of 'Dancy' tangerine, 'Minneola' tangelo, 'Murcott' tangor, 'Page' tangelo, and 'Ponkan' mandarin were isolated from nucellar seedlings maintained in a growth chamber 5.

Protoplasts from suspension cultures and leaves were purified and fused using a modified polyethylene glycol (PEG) method (5). After the fusion, protoplasts of each combination were cultured in BH_3, EME, or a 1:1 (v/v) mixture of the two media. Protoplasts were cultured and plants were regenerated as described by Grosser and Gmitter (5). Chromosome numbers in root-tip meristematic cells from regenerated plants were determined with the modified hematoxylin method (5).

Isozyme banding patterns from crude leaf extracts were determined from the donor parents and putative regenerated somatic hybrid plants. Peroxidase (*Per*), phosphoglucomutase (*Pgm-1*), and phosphoglucose isomerase (*Pgi*) isozymes were separated by electrophoresis on 10% starch gels, using the histidine citrate buffer (pH 5.7) of Cardy et al. (2). Samples were electrophoresed for 3 h at 4° C by constant 60 mA current; gels were stained as described by Vallejos (18).

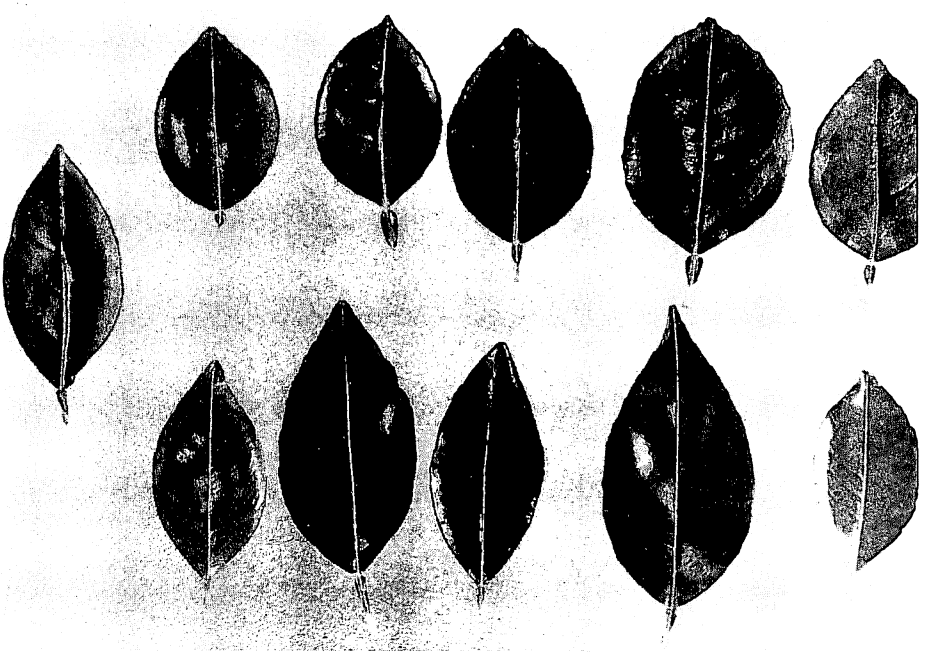

Figure 1. Leaf morphology of the donor *Citrus* parents and the somatic hybrid plants. Left: 'Succari' sweet orange. Lower left to lower right: 'Dancy' mandarin, 'Minneola' tangelo, 'Murcott' tangor, 'Page' tangelo, 'Ponkan' mandarin. Upper left to upper right: Somatic hybrid plants of 'Succari' with 'Dancy,' 'Minneola,' 'Murcott,' 'Page,' and 'Ponkan' respectively.

Additional molecular analysis was performed on the combination 'Succari' + 'Minneola' by random amplified polymorphic DNA (RAPD) analysis using polymerase chain reaction (PCR) on total DNA extracted from somatic hybrids and parental types. DNA extraction procedures and amplification by PCR were carried out using the protocol of Xiao et al. (manuscript in review); random decamers P-10, U-17, W-15, and X-18 (Operon Technologies, Alameda, CA) were used to prime the reactions. DNA amplification products were separated by electrophoresis for 2-3 hours at 25° C using 150 mA constant current, on 1.8% agarose gels with 1x TAE buffer. DNA amplification products were stained with ethidium bromide and photographed under UV light.

Results and Discussion

Somatic hybrid plants were regenerated from each attempted fusion and successfully transferred to soil. No diploid plants were recovered from unfused protoplasts of the embryo-

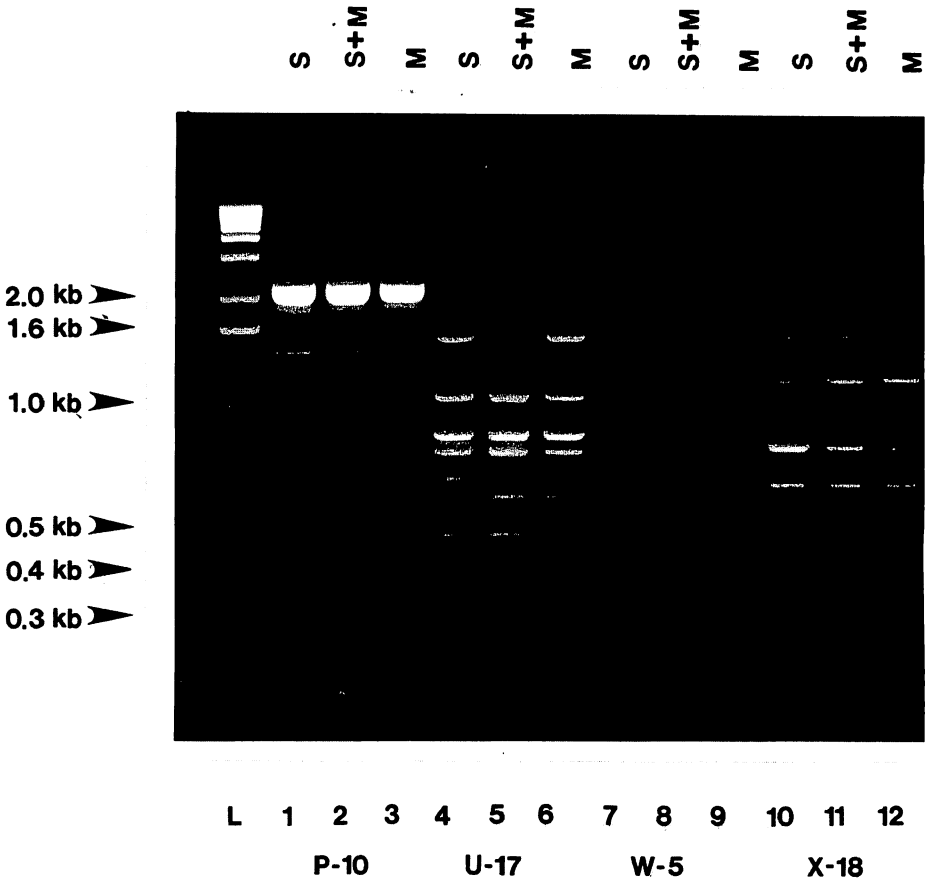

Figure 2. RAPD patterns of interspecific somatic hybrid 'Succari' sweet orange plus 'Minneola' tangelo and its respective parental types. L = DNA Ladder, lanes 1, 4, 7, and 10 = 'Succari,' lanes 2, 5, 8, and 11 = 'Succari' + 'Minneola' somatic hybrid, lanes 3, 6, 9, and 12 = 'Minneola.' Lanes 1 through 3 = P-10 primer, lanes 4 through 6 = U-17 primer, lanes 7 through 9 = W-5 primer, lanes 10 through 12 = X-18 primer.

Table 1. Leaf isozyme genotypes of the donor *Citrus* parents and the somatic hybrid plants for peroxidase *(Per)*, phosphoglucose isomerase *(Pgi)*, and phosphoglucomutase *(Pgm-1)*.

	Per	*Pgi*	*Pgm-1*
Donor citrus parents:			
'Succari' sweet orange	FF	MS	FS
'Dancy' tangerine	FS	MM	FF
'Minneola' tangelo	FF	MS	FS
'Murcott' tangor	FF	MM	FF
'Page' tangelo	FF	MS	IS
'Ponkan' mandarin	SS	MM	FF
Somatic hybrids:			
'Succari' + 'Dancy'	FFFS	MMMS	FFFS
'Succari' + 'Minneola'	FFFF	MMSS	FFSS
'Succari' + 'Murcott'	FFFF	MMMS	FFFS
'Succari' + 'Page'	FFFF	MMSS	FISS
'Succari' + 'Ponkan'	FFSS	MMMS	FFFS

genic 'Succari' suspension, indicating that this suspension line had lost its regeneration capacity. Likewise, no plants were regenerated from any of the leaf parents.

Each regenerated plant was tetraploid (2n = 4x = 36) as determined by root-tip cell chromosome counts. Leaf morphology of regenerants was intermediate to the fusion parents (Fig. 1). Leaf isozyme genotypes for *Per, Pgi,* and *Pgm-1* of the donor *Citrus* parents and the somatic hybrid plants are summarized (Table 1). Somatic hybridity of regenerants was verified by allelic complementation of 'Succari' with 'Dancy' tangerine (*Per, Pgm-1*), with 'Murcott' tangor (*Pgi, Pgm-1*), with 'Page' tangelo (*Pgm-1*), and 'Ponkan' (*Per, Pgi,* and *Pgm-1*).

RAPD analysis revealed complementary banding patterns in the somatic hybrid 'Succari' + 'Minneola,' indicating the presence of DNA from each parent in the corresponding hybrid (Fig. 2). DNA amplified by primers P-10 and W-5 showed the presence of DNA from sweet orange, whereas DNA amplified using X-18 primer confirmed the presence of DNA from 'Minneola.'

Regenerated somatic hybrid plants of each parental combination are currently maintained under greenhouse conditions, and show adequate vigor. These plants may have potential for direct use as mandarin hybrid cultivars, but their greatest value will likely be as tetraploid breeding parents. These hybrids were grafted to citrus rootstocks and planted in the field to hasten flowering. Their pollen will be used subsequently in crosses with selected diploid monoembryonic seed parents.

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Response of Papaya Cultivars to Inoculation with the SMN Papaya Ringspot Viral Strain

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Abstract

Field trials with a wide range of papaya *(Carica papaya)* cultivars inoculated with the severe mottle and necrosis (SMN) papaya ringspot virus (PRV) were evaluated with no control of natural aphid populations. It revealed significant differences in disease severity, ELISA readings, and plant growth rate. Survival rate and symptom development significantly differ among genotypes. 'Sunrise,' 'Mardi' and 'Ostream' were mostly susceptible to SMN strain of PRV. There were no marketable yield in the study. Different sources of 'Cariflora' lines performed similarly. These cultivars tolerated the SMN viral strain, in agreement with reported tolerance in Florida. Monoclone antibodies were applied to distinguish viral strains in healthy looking plants seven months after planting. The extracted sap of healthy-looking plants was inoculated into susceptible hosts to detect plants responding to specific viral infection. This procedure showed that infection of healthy-looking plants was due to severe virulent strains of SMN and severe mottle and deformed (SMD) ringspot virus.

Introduction

Papaya ringspot virus (PRV) is the most serious disease reducing papaya tree life in Taiwan and other papaya production areas (1, 2, 8, 9, 10, 14, 16, 20). The viral pathogen (PRV) enters papaya plants by aphid inoculation (10, 11), and once established in the tissue, the virus symptoms include systemic mottle and necrosis of the leaves, stunt of trees, and eventually death of the tree within one to two years (3, 11, 13). Prior to plant death, leaf losses due to mottle, necrosis, and deformation results in greatly reduced fruit production and fruit quality.

Cook and Zettler (8) inoculated 90 papaya lines and found all to be susceptible after inoculation. Conover (4) reported significant differences in susceptible of 66 papaya cultivars to papaya distortion virus. 'Cariflora,' a cultivar bred in Florida is tolerant to papaya ringspot virus in South Florida and Caribbean (7). Wang (17) evaluated 53 papaya cultivars in the field without artificial inoculation. FL-77-5 and 'Costa Ricoh' lines were selected and used to develop the Tainung No.5 cultivar which is tolerant to papaya ringspot viral disease. Although papaya cultivars differ in their level of tolerance to PRV, no highly tolerant selec-

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