CITRUS CULTIVAR IMPROVEMENT VIA SOMATIC HYBRIDIZATION

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Abstract. The production of intergeneric somatic Citrus hybrids via protoplast fusion has great potential for rootstock and scion improvement by expanding the narrow genetic base of cultivated Citrus. A well-defined protoplast isolation, fusion, and culture system was used to generate an intergeneric somatic hybrid between sexually compatible species Citrus sinensis (L.) Osb. cv. Hamlin and Poncirus trifoliata (L.) Raf. cv. Flying Dragon. This achievement resulted from the fusion of embryogenic suspension culture-derived protoplasts of 'Hamlin' with leaf-derived protoplasts of 'Flying Dragon'; and served as a 'model' system that was slightly modified to produce a second intergeneric somatic hybrid, between the sexually incompatible species C. sinensis cv. Hamlin and Severinia disticha (Blanco) Swing. The latter hybrid was generated by fusing embryogenic suspension culture-derived protoplasts of 'Hamlin' with seedling epicotyl callus-derived protoplasts of S. disticha. The production and importance of these hybrids are discussed.

Intergeneric somatic hybridization of Citrus with sexually incompatible species via protoplast fusion has the potential for expanding the narrow genetic base presently available for citrus cultivar improvement. Somatic hybridization offers a means of by-passing the barriers to sexual hybridization between Citrus and related genera that exhibit such desirable traits as disease and nematode resistance, salt tolerance, and cold hardiness (2). Although many such genera are graft compatible with Citrus, they may not be horticulturally acceptable when used directly as rootstocks. The strong performance of selected sexual intergeneric hybrids between Citrus and Poncirus as rootstocks, suggests potential for intergeneric hybrids between Citrus and other selected genera.

Poncirus trifoliata is a deciduous species that is closely related to Citrus. It is an excellent germplasm source for cold hardiness, polyembryony; resistance to tristeza virus, foot rot (Phytophthora parasitica), and the citrus nematode (Tylenchulus semipenetrans) (3). P. trifoliata is currently being utilized extensively in citrus breeding programs, and several of its hybrids are gaining recognition as valuable rootstocks. Citranges are bigeneric hybrids between P. trifoliata and sweet orange (Citrus sinensis) (4). Citrumelos are bigeneric hybrids between P. trifoliata and grapefruit (C. paradisi). 'Carrizo' citrange and cold hardy 'Swingle' citrumelo are two of the six most important rootstocks used in Florida (6).

My objective was to use a well-defined protoplast isolation, fusion, and culture system to generate an intergeneric somatic hybrid between C. sinensis cv. Hamlin and P. trifoliata cv. Flying Dragon. These two parental species were selected for the development of this 'model' system because they are sexually compatible, thus eliminating the possibility of somatic incompatibility interfering with the development of a successful model. The model system was then modified for the production of an intergeneric hybrid between C. sinensis cv. 'Hamlin' and sexually incompatible Severinia disticha. Repeated efforts to produce sexual hybrids between Citrus and Severinia have been futile both in the United States and in Australia (Don Hutchison, personal communication). The genus Severinia was selected for initial experiments because two available monoembryonic species (S. disticha and S. buxifolia (Poir.) Ten.) exhibit many desirable traits, including cold hardiness, salt and boron tolerance (1), Pytophthora resistance (4), and nematode resistance (5).

Materials and Methods

Embryogenic suspension culture derived protoplasts of C. sinensis cv. Hamlin were fused chemically (using polyethylene glycol) with leaf-derived protoplasts of P. trifoliata cv. Flying Dragon, for the production of the somatic hybrid between 'Hamlin' and 'Flying Dragon'. The protoplast culture medium utilized did not support the mitotic division of unfused 'Flying Dragon' leaf protoplasts. Unfused 'Hamlin' protoplasts, capable of mitosis and limited somatic embryogenesis, but not normal embryo development and germination, acted as nurse culture cells. Somatic hybrid plants regenerated via somatic embryogenesis were identified on the basis of expression of the dominant trifoliate leaf character, donated by 'Flying Dragon'.

Embryogenic suspension culture derived protoplasts of 'Hamlin' were fused chemically with seedling epicotyl cal- lus (capable of organogenesis) derived protoplasts of S. disticha for the production of the somatic hybrid between C. sinensis cv. Hamlin and S. disticha. The protoplast culture medium utilized did not support the mitotic division of unfused S. disticha protoplasts. Unfused 'Hamlin' protoplasts again acted as nurse culture cells. Recovered somatic hybrid embryos would not germinate but underwent shoot-bud induction when placed on a medium designed to induce organogenesis. Regenerated somatic hybrid shoots were identified by pubescence and red pigment in newly forming leaves (donated by S. disticha), and by winged petioles, donated by 'Hamlin'.

In both cases, verification of somatic hybrid identities was also provided by preliminary examination of HPLC (high performance liquid chromatography) ultraviolet and fluorescence profiles. This work is being performed by R. L. Rouseff (DOC Research Scientist, CREC, Lake Alfred, FL) and will be published upon the completion of pattern recognition analyses.

Characterization of both of these intergeneric somatic hybrids on the basis of electrophoretic analyses of isozyme banding patterns (in conjunction with F. G. Gmitter, IFAS Citrus Breeder at CREC, Lake Alfred, FL), morphology, and cytology is in progress. Detailed information regarding the techniques used for the isolation, fusion and culture of protoplasts, media formulations, and characterization of the somatic hybrid plants will be published in the near future.

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Results and Discussion

A model system for the production of intergeneric somatic citrus hybrids was developed by the successful somatic hybridization of *C. sinensis* cv. Hamlin and the sexually compatible species, *P. trifoliata* cv. Flying Dragon. Over 125 plants of this somatic hybrid, all exhibiting a typical 'citrange' appearance, have been regenerated, rooted, and transferred to pots containing a commercial potting mixture. Application of the model system resulted in the production of another somatic hybrid, between *C. sinensis* cv. Hamlin and the sexually incompatible citrus relative *S. disticha*. Over 35 plants of this somatic hybrid, intermediate between the parents in appearance, have been regenerated, rooted, and potted. Both of these intergeneric somatic hybrids are vigorous and can be propagated *in vitro* via shoot multiplication. A profile of leaf morphology of each hybrid and the parents is exhibited in Fig. 1. Evaluations of disease and nematode resistances for the hybrids are in progress, and both hybrids will be entered into extensive rootstock testing programs.

The *C. sinensis* cv. Hamlin x *S. disticha* hybrid is the first example of a somatic hybrid produced between sexually incompatible woody species. This research demonstrates that it is possible to by-pass barriers to sexual hybridization between *Citrus* and related genera in order to access previously unavailable germplasm. These results suggest potential for the generation of many other interesting intergeneric somatic citrus hybrids. Moreover, such hybrids may be useful directly as rootstocks. If fertile, such hybrids would also provide a unique source of breeding material with potential in scion and rootstock improvement.

Literature Cited


**GROWTH OF CO₂-ENRICHED SOUR ORANGE SEEDLINGS TREATED WITH GIBBERELLINS/CYTOKININS**

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Abstract. Enriched CO₂ atmospheres and specific plant growth regulators are known to stimulate plant growth, but their combined effects on citrus seedlings have not been studied. Sour orange (*Citrus aurantium* L.) seedlings were treated with plant growth regulators (6-benzyladenine <BA> [250 μl/l]; 6 benzyladenine and gibberellic acid <BA + GA> [250 μl/l]; gibberellin 3 <GA₃> [450 μl/l] and gibberellin 4+7 <GA₄+₇> [250 μl/l]) and grown at either ambient or elevated CO₂ levels (330 or 660 μl/l).

Seedlings treated with GA₄+₇ and grown at elevated CO₂ levels were taller and had greater leaf weight than plants given all other treatments. Leaf number increased under elevated CO₂ levels when BA or GA₄+₇ were applied. Stem weight was unaffected by growth regulators except when GA₄+₇ was applied to plants grown under high CO₂ levels. Stem caliper increased slightly under high CO₂ levels, especially when GA₄+₇ was applied.

The response of food, fiber, forestry and ornamental crop plants to elevated CO₂ levels has been well documented (4, 9) and CO₂ enrichment has become an established practice, especially in greenhouse vegetable production (3, 9). Growth increases have also been re-