

Somatic hybridization: a new approach to citrus rootstock improvement

Jude W. Grosser
Frederick G. Gmitter Jr.
William S. Castle
J.L. Chandler

University of Florida, IFAS,
Citrus Research
and Education Center,
700 Experiment Station Road,
Lake Alfred, FL 33850, USA

Somatic hybridization: a new approach to citrus rootstock improvement.

Abstract — Introduction. The two main strategies employing somatic hybridization used at the Citrus Research and Education Center (CREC) in Florida are a combination of existing rootstocks that exhibit complementary traits into allotetraploid somatic hybrids (SH), or wide hybridization of *Citrus* with related genera in efforts to expand the germplasm base for citrus improvement. The SH produced are currently being evaluated for disease, nematode, and pest resistance, and commercial rootstock potential. **Screening for disease and nematode resistance.** The SH produced from [sweet orange + lemon] parentage are consistently showing *Phytophthora* resistance. SH resistant to quick-decline caused by citrus tristeza virus include [sour orange + Rangpur], [sour orange + Volkameriana (zygotic)], and [sour orange + Carrizo citrange]. SH with *Fortunella*, *Citropsis*, *Atalantia*, *Microcitrus*, and *Citrus ichangensis* parentage are significantly more resistant to *Radopholus citrophilus* than the susceptible sour orange control. A SH of [Hamlin sweet orange + Rough lemon] planted in a grove with heavy blight pressure showed no blight symptoms. **Tree size control.** SH containing Flying Dragon parentage are extremely dwarfing and precocious, but [Cleopatra mandarin + Swingle citrumelo] and [sour orange + Rangpur] showed dwarfing potential as well. These improved rootstocks should allow to minimize harvesting costs and facilitate cold-protection via microsprinklers. **Mass propagation.** To date, seeds from SH germinated well and produced vigorous uniform seedlings. **Conclusion.** More than 60 citrus SH have been produced at the CREC; they are being evaluated for direct rootstock potential. Preliminary nursery and field observations of liners and young budded trees are encouraging. (© Elsevier, Paris)

Citrus / plant breeding / plant biotechnology / somatic hybridization / rootstocks / pest resistances

Hybridation somatique : une nouvelle voie pour l'amélioration des porte-greffes d'agrumes.

Résumé — Introduction. Les deux principales stratégies utilisées au CREC (Floride) pour exploiter l'hybridation somatique sont soit la combinaison de porte-greffes existants, portant des caractères complémentaires, dans des hybrides somatiques (HS) allotetraploïdes, soit la large hybridation du genre *Citrus* avec des genres qui lui sont proches, afin d'élargir la base génétique utilisée pour l'amélioration des agrumes. Les HS produits sont en cours d'évaluation quant à leurs résistances aux maladies, nématodes et ravageurs, et à leur potentiel commercial. **Criblage pour la résistance aux maladies et nématodes.** Les HS [oranger + citron] montrent uniformément une résistance au *Phytophthora*. Les HS résistants à la maladie causée par le virus de la tristeza des agrumes sont issus de [oranger + Rangpur], [oranger + Volkameriana (origine zygotique)] et [oranger + citrange Carrizo]. Des HS issus de *Fortunella*, *Citropsis*, *Atalantia*, *Microcitrus* ou *Citrus ichangensis* sont significativement plus résistants à *Radopholus citrophilus* que l'oranger sensible, utilisé en témoin. Un HS [oranger Hamlin + citronnier Rough], planté en zone sous forte pression d'inoculum de Blight, n'en a pas exprimé les symptômes. **Contrôle de la taille des arbres.** Les HS issus de Flying Dragon sont très nanisants et précoces, mais les HS [mandarinier Cléopâtre + citrumelo Swingle] ou [oranger + Rangpur] ont également présenté un potentiel nanisant. L'utilisation de ces porte-greffes améliorés devraient permettre de diminuer les coûts de production et de faciliter la protection des plants contre le froid par utilisation de microasperseurs. **Multiplication.** À ce jour, les graines produites par des HS ont germé normalement et ont donné des plantules vigoureuses et homogènes. **Conclusion.** Plus de 60 HS ont été produits au CREC ; leur potentiel d'utilisation directe en porte-greffes est en cours d'évaluation. Les observations préliminaires de HS clonés et de jeunes arbres greffés sont encourageants. (© Elsevier, Paris)

Citrus / amélioration des plantes / biotechnologie végétale / hybridation somatique / porte-greffe / résistance aux organismes nuisibles

* Correspondence and reprints

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1. introduction

The production of somatic hybrid plants by protoplast fusion requires an efficient system for the isolation, fusion, and culture of citrus protoplasts (wall-less citrus cells). Plant cells are separated and their walls removed by enzyme digestion to liberate large quantities of protoplasts. Embryogenic callus or suspension cultures, initiated from nucellar tissue taken directly from citrus fruits, are used in fusion experiments to provide the capacity for plant regeneration after fusion. The Citrus Research and Education Center (CREC) collection contains cultures from more than 50 citrus varieties. Citrus embryogenic protoplasts are generally fused with leaf-derived protoplasts of the selected complementary parent. Cell fusion is induced by treatment with the chemical polyethylene glycol, and resulting protoplasts are cultured in a complex medium. If successful, somatic hybrid plants can be recovered within 5–8 months [1]. Confirmed somatic hybrid plants are propagated either by tissue culture micropropagation or by a rooted cutting method. Liners are grown out, budded and subjected to appropriate evaluation. Seed trees of each hybrid are also being grown out simultaneously with commercial evaluation. As mentioned, more than 60 somatic hybrid rootstocks have been produced to date and are currently being evaluated [2]. Replicated field trials have been established at three locations, representing the three major citrus growing regions in Florida: the central Florida sandridge; the Indian River district (Atlantic coastal region), and the southwest Florida flatwoods. With few exceptions, trees budded to somatic hybrid rootstocks are growing off quite well, and we expect to begin collecting yield data from the oldest planting next year.

Somatic hybrids differ from conventionally produced sexual hybrids because they theoretically contain all of the nuclear genetic information from both parents. The additive nature of cell-fusion hybridization generally results in genetically uniform allotetraploid hybrid plants,

which greatly simplifies screening. As a result, somatic hybrids have the potential to retain many of the positive attributes of their parents. Two categories of citrus somatic hybrids are being produced at CREC. The first category contains hybrids that combine complementary citrus rootstock germplasm [2], and the second category contains hybrids of *Citrus* with related genera (including sexually incompatible genera) that contain potentially useful traits [3].

2. screening for disease and nematode resistance

Cooperative research to screen selected somatic hybrids for resistance to *Phytophthora*, citrus tristeza virus, citrus blight, and the burrowing nematode has been conducted.

In cooperation with J.H. Graham (CREC), several somatic hybrids have been screened for *Phytophthora* resistance. Most of the hybrids tested are sorting out as expected, showing levels of resistance intermediate to that of their parents. However, somatic hybrids produced from [sweet orange + lemon] parentage are consistently showing resistance. This is surprising because both of these parents are considered to be, and test out, as susceptible.

J.H. Graham is also using this test to identify superior breeding parents. *Poncirus trifoliata* selection 50–7 was identified to have exceptional *Phytophthora* resistance. We have used this selection to make a somatic hybrid with Navel orange, and we are currently evaluating this new somatic hybrid citrange to determine if it has *Phytophthora* resistance.

In cooperation with S.M. Garnsey (USDA-ARS/Orlando, FL), several somatic hybrids containing sour orange parentage have been evaluated for resistance to quick-decline caused by citrus tristeza virus. Somatic hybrids resistant to this disorder include [sour orange + Rangpur], [sour orange + Volkameriana (zygotic)],

and [sour orange + Carrizo citrange]. [Sour orange + Flying Dragon trifoliolate orange] was also resistant to decline, but infected trees were significantly stunted. A somatic hybrid of [sweet orange + sour orange] was extremely susceptible. Additional sour orange hybrids are being assayed as they become available.

Results from a cooperative experiment conducted with L.W. Duncan (CREC) screening wide somatic hybrids for burrowing nematode (*Radopholus citrophilus*) resistance showed that somatic hybrids containing *Fortunella*, *Citropsis*, *Atalantia*, *Microcitrus*, and *Citrus ichangensis* parentage were significantly more resistant than the susceptible sour orange control. A somatic hybrid of [sweet orange + *Severinia*] was not significantly more resistant than sour orange.

Experiments are also underway to determine if field-resistance to citrus blight can be inherited in somatic hybrids containing field resistant sweet orange or sour orange parentage (in cooperation with K.S. Derrick, CREC). Four and a half-year old trees budded to a somatic hybrid of [Hamlin sweet orange + rough lemon], planted as resets in a grove with heavy blight pressure, are showing no blight symptoms, and tested negative for blight specific proteins.

3. tree size control

Preliminary observations of somatic hybrid rootstocks in commercial plantings suggest good potential for tree size control. Somatic hybrids containing Flying Dragon parentage are extremely dwarfing and precocious, even more so than Flying Dragon; yet trees budded to these hybrids generally produce larger fruit than trees on Flying Dragon. Somatic hybrids combining Flying Dragon with sweet orange, grapefruit, Cleopatra mandarin, and sour orange are all much more nursery friendly than Flying Dragon.

Other somatic hybrids showing dwarfing potential include [Cleopatra mandarin

+ Swingle citrumelo] and [sour orange + Rangpur]. Somatic hybrid rootstocks producing small to medium size trees include [sour orange + Volkameriana (zygotic)] and [Cleopatra mandarin + Argentine trifoliolate orange].

Selected somatic hybrids should therefore fit well into high density planting schemes designed to reduce harvesting costs, facilitate mechanical harvesting, and/or to maximize the efficiency of cold protection systems that use elevated microsprinklers.

4. mass propagation

Due to the dominant nature of polyembryony in citrus, somatic hybrids produced from two polyembryonic parents are expected to be polyembryonic. To date, a few seed trees of somatic hybrid rootstocks have flowered, and two hybrids have produced a significant amount of seedy fruits. Seed from the [Hamlin sweet orange + Rough lemon] and [sour orange + Rangpur] somatic hybrids was commercially extracted by Reed Brothers Nursery (Dundee, FL, USA). In both cases, seed germinated well and produced vigorous uniform seedlings. We expect that many of the somatic hybrids will be amenable to propagation by standard methods. However, in the event that promising hybrids do not produce adequate polyembryonic seed, alternative methods of propagation are available. Some selections are amenable to in vitro micropropagation, and all can be propagated from cuttings with good efficiency.

5. conclusion

More than 60 citrus somatic hybrids have been produced at the CREC that are being evaluated for direct rootstock potential. Preliminary nursery and field observations of liners and young budded trees are encouraging. Determination of

their survivability, adaptation to various soil types, and horticultural performance regarding yield and fruit quality with various scions over time should result in providing growers with alternative choices for specific citriculture schemes. Along with this, determination of the genetic control of important traits will provide valuable feedback that will be used in the selection of parental combinations for future somatic hybridization experiments.

references

- [1] Grosser J.W., Gmitter F.G. Jr., Protoplast fusion and citrus improvement, *Plant Breed. Rev.* 8 (1990) 339–374.
- [2] Grosser J.W., Gmitter F.G. Jr., Castle W.S., Chandler J.L., Production and evaluation of citrus somatic hybrid rootstocks: progress report, *Proc. Fla. State. Hort. Soc.* 108 (1995) 140–143.
- [3] Grosser J.W., Mourao-Fo F.A.A., Gmitter F.G. Jr., Louzada E.S., Jiang J., Baergen K., Quiros A., Cabasson C., Schell J.L., Chandler J.L., Allotetraploid hybrids between citrus and seven related genera produced by somatic hybridization, *Theor. Appl. Genet.* 92 (1996) 577–582.

Hibridación somática: una nueva vía para el mejoramiento de los porta injertos de agrios.

Resumen — Introducción. Las dos principales estrategias utilizadas en el CREC (Florida) para explotar la hibridación somática son ya sea la combinación de porta injertos existentes, llevando caracteres complementares, en híbridos somáticos (HS) alotetraploides, ya sea la amplia hibridación del género *Citrus* con géneros que se asemejan, a fin de ensanchar la base genética utilizada para mejorar los agrios. Se está evaluando los HS producidos para sus resistencias a las enfermedades, nematodos y plagas, y a su potencial comercial. **Cribado para la resistencia a las enfermedades y nematodos.** Los HS [naranja + limonero] muestran uniformemente una resistencia al *Phytophthora*. Los HS resistentes a la enfermedad provocada por el virus de la tristeza de los agrios procedentes de [naranja + Rangpur], [naranja + Volkameriana (origen cigótico)] y [naranja + citrange Carrizo]. HS oriundos de *Fortunella*, *Citropsis*, *Atalantia*, *Microcitrus* o *Citrus ichangensis* son significativamente más resistentes a *Radopholus citrophilus* que el naranja sensible, utilizado de testigo. Un HS [naranja Hamlin + limonero Rough], sembrado en zona bajo fuerte presión de inoculo de Blight, no ha manifestado sus síntomas. **Control del tamaño de los árboles.** Los HS procedentes de Flying Dragon son muy reductores y precoces, pero los HS [mandarinero Cléopâtre + citrumelo Swingle], o [naranja + Rangpur] también presentaron un potencial reductor. La utilización de estos porta injertos mejorados debería permitir disminuir los costos de producción y facilitar la protección de las plantas contra el frío al utilizar micro aspersores. **Multiplificación.** A la fecha, las semillas producidas por HS germinaron normalmente y dieron plántulas vigorosas y homogéneas. **Conclusión.** Más de 60 HS fueron producidos en el CREC; se está evaluando su potencial de utilización directa en porta injertos. Las observaciones preliminares de HS clonados y de jóvenes árboles injertados son esperanzadores. (© Elsevier, Paris)

Citrus / fitomejoramiento / biotecnología vegetal / hibridación somática / portainjertos / resistencia a las plagas