Protoplast Fusion in *Citrus*

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Fruits, vol. 49, n°5-6
p. 401-403 (English)
p. 481-482 (French)

Protoplast fusion, a prelude for regeneration of interspecific and intergeneric somatic hybrids and cybrids, is a promising area of research for the improvement of Citrus *rootstock and cultivars.*

introduction

Protoplast fusion is an increasingly important component of citrus breeding programmes (Ollitrault and Luro, 1995). It enables breeders to overcome some constraints linked with sexual hybridization and is applied to develop new rootstocks and cultivars for high quality, sustainable and environment-friendly citrus cropping.

Protoplast fusion can be used in rootstock improvement programmes to accumulate genes with resistance or tolerance to biotic and abiotic factors, irrespective of the heterozygosis level of the parents.

In addition, there is a very broad range of genetic resources that can be utilized since genomes from sexually incompatible species and genera can be combined by this fusion technique (Gmitter *et al.*, 1992).

Protoplast fusion also shows promise for diversification programmes through the development of triploid cultivars, especially in mandarin (Ollitrault *et al.*, 1995). The pool of tetraploid progenitors, used in sexual hybridization with diploids to produce triploid cultivars, could be enriched considerably.

Fertility has also been restored in somatic hybrids obtained from sterile elite cultivars (Ohgawara *et al.*, 1991). Results obtained by CIRAD[1] and INRA[4] in this area are presented.

obtaining intergeneric somatic hybrids

Research conducted in France over the past 5 years by CIRAD-FLHOR[2], CIRAD-BIOTROP[3] and INRA[4] (agricultural research station at San Giuliano, Corsica) on embryogenesis in citrus materialized in 1994 when the first *Citrus reticulata* (mandarin) + *Fortunella japonica* (kumquat) intergeneric hybrids were obtained in the CIRAD-BIOTROP laboratories. About a hundred plants were thus regenerated after electrofusion of *C. reticulata* embryogenic callus protoplasts and *F. japonica* leaf protoplasts (Photos 1-3).

Flow cytometry was used to determine ploidy levels (Fig. 1), and isoenzyme analysis confirmed their hybrid status. These tetraploid hybrids coud be sexually hybridized with diploid cultivars to introgress flavours, lateness, cold tolerance and kumquat bacterial canker resistance in mandarin-type triploid cultivars.

cybrid identification

As part of a cooperative project with IFAS[5] (Dr. J. Grosser, Florida, USA), diploid cybrids (nuclear genome from one species combined with cytoplasmic genomes of another species) were identified by two techniques: flow cytometry and RFLP marker analysis of nuclei and cytoplasm (Photos 4 & 5) from plants regenerated following fusions between *C. deliciosa* (mandarin) callus protoplasts

(1) CIRAD: Centre de coopération internationale en recherche agronomique pour le développement.

(2) CIRAD-FLHOR: Département des productions fruitières et horticoles.

(3) CIRAD-BIOTROP: Laboratoire des biotechnologies appliquées àl'amélioration des plantes tropicales.

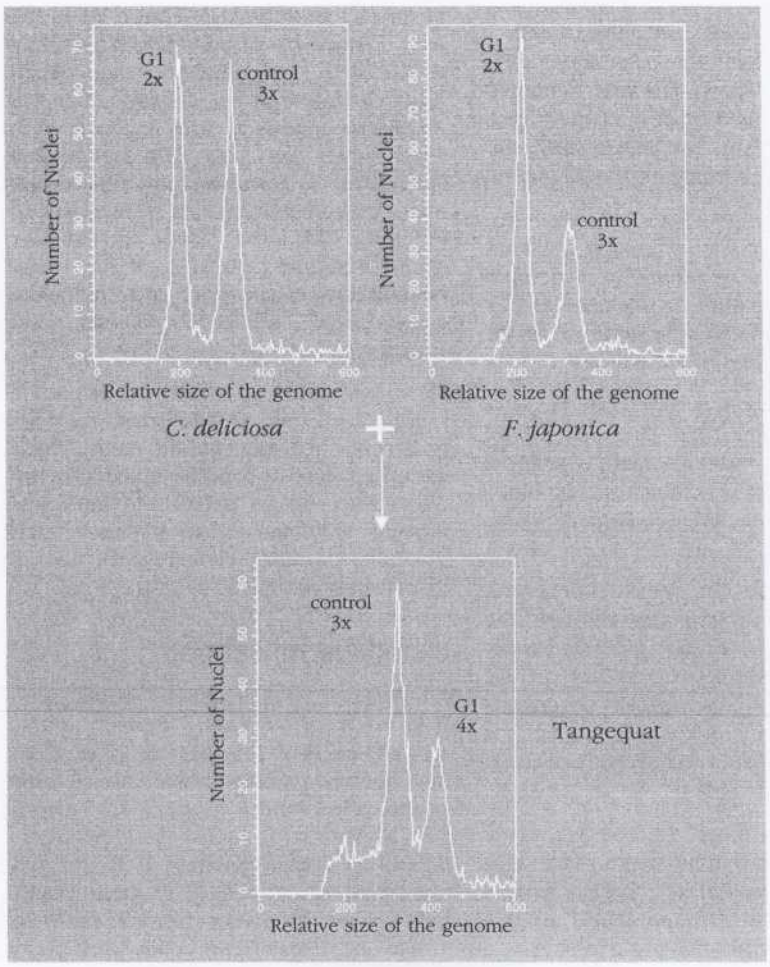
(4) INRA: Institut national de la recherche agronomique.

(5) IFAS: Institute of Food and Agricultural Sciences (University of Florida).

and *C. sinensis* (sweet orange) leaf protoplasts, as well as between *C. deliciosa* callus protoplasts and *C. paradisi* (grapefruit) leaf protoplasts (LURO *et al.*, 1995).

The studies highlighted the systematic presence of nonrecombined mitochondrial and chloroplast genomes from the *C. deliciosa* parent (embryogenic calli) associated with nuclear genomes from *C. deliciosa, C. sinensis* or *C. paradisi.* The results were similar to those obtained by TUSA *et al.* (1990) and SAITO *et al.* (1993). They suggest that *Citrus* somatic embryogenesis regeneration capacity is under cytoplasmic control. Cybrids obtained in this way are ideal for assessing the involvement of cytoplasmic genomes and nucleus-cytoplasm interactions in determining phenotypic characters.

Figure 1
Analysis of ploidy levels of plants regenerated after somatic fusion between C. deliciosa *and* F. japonica.



prospects

Somatic hybridizations of various interspecific [*C. reticulata* + *C. sinensis*; *C. reticulata* + *C. paradisi*; *C. reticulata* + *C. lemon* (lemon); *C. reticulata* + *C. aurantifolia* (lime)] and intergeneric (*C. reticulata* + *Poncirus trifoliata* and *C. aurantium* + *Eremocitrus glauca*) combinations have been carried out and embryos are currently being propagated. These hybridizations are to be used in rootstock and cultivar improvement programmes and should also provide valuable information on polyploid genetics .

Fusions between diploid protoplasts and haploid protoplasts (derived from haploid plantlets obtained by induced gynogenesis) will soon be performed in order to synthesize triploids directly. The origins of plants regenerated in these different programmes will be checked with molecular markers and by flow cytometry. All plant material obtained by protoplast fusion will then be introduced in the CIRAD-FLHOR/INRA multilocation experimental network to assess their potential under Mediterranean and tropical conditions and their tolerance to the main diseases affecting such crops. ●

acknowledgements

We thank Mr. R. Haicourt and Mr. D. Sibacharkr for their valuable advice concerning the protoplast electrofusion technique.

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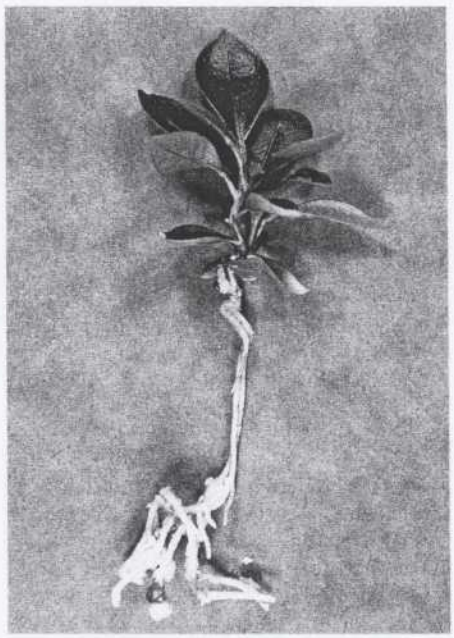

Photo 3
Somatic hybrids between C. deliciosa *and* F. japonica.

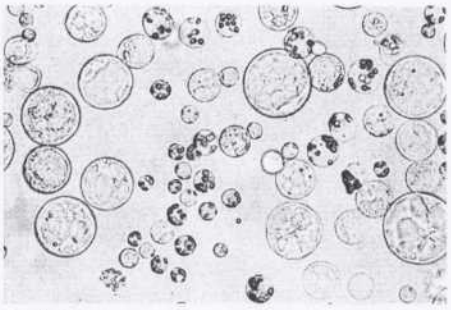

Photo 1
Mixture of Citrus deliciosa *embryogenic callus protoplasts and* Fortunella japonica *leaf protoplasts.*

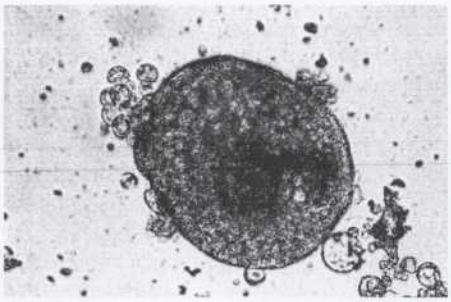

Photo 2
Proembryos 28 days after electrofusion of C. deliciosa *and* F. japonica *protoplasts.*

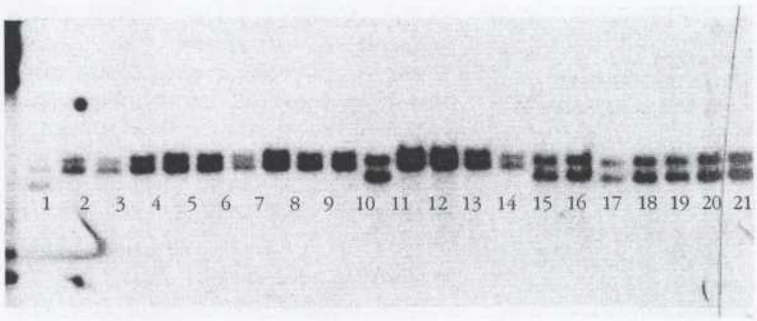

Photo 4
Checking the origins of nuclei from plants regenerated after protoplast fusion between C. deliciosa *and* C. sinensis.
1: C. deliciosa
2: C. sinensis.
Plants regenerated after fusion:
3-10 and 12-15: cybrids
11 and 16 to the end: plants regenerated from unfused C. deliciosa *protoplasts.*

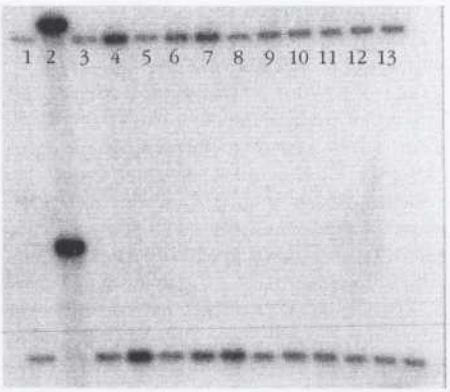

Photo 5
Checking the origins of cytoplasm (mitochondrial probe) from plants regenerated after protoplast fusion between C. deliciosa *and* C. sinensis.
1: C. deliciosa
2: C. sinensis
3-13: plants regenerated after fusion.