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Somatic Hybridization in Fruit Crops: Applications and Achievements

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Introduction

Somatic hybridization is the technique of fusing isolated protoplasts from somatic cells and regenerating hybrid plants from the fusion products. It has become an important tool for ploidy manipulation in plant improvement schemes, allowing researchers to combine somatic cells from different cultivars, species, or genera, resulting in novel allotetraploid and autotetraploid genetic combinations. By avoiding some of the challenges associated with traditional sexual hybridization, such as sexual incompatibility, nucellar embryogenesis, and male or female sterility, this strategy can help with conventional breeding, gene transfer, and cultivar development (Grosser and Gmitter, 1990). Furthermore, unlike sexual hybridization or genetic engineering, somatic hybridization offers the unique ability to combine both nuclear and cytoplasmic genes at the same time (Bhojwani and Dantu, 2013).

Types of hybrids produced through somatic hybridization

1. **Symmetrical hybrids:** A "symmetric hybrid" is a combination of two maternal cytoplasmic genomes and two diploid nuclear genomes.
2. **Asymmetrical hybrids:** "Asymmetric hybrids" result from a combination of the parent’s genomes or a combination of one parent's genome and the cytoplasm of another; these are also known as cybrids.

Steps involved in somatic hybridization

1. **Protoplast isolation**: Mechanical or enzymatic methods are utilized to isolate protoplasts from plant tissue. The mechanical process is time-consuming and has several drawbacks such as low protoplast yield and viability. If the cell's protoplasts are to be altered, the cell wall must be degraded. Enzymes such as pectinase,

macerozyme, cellulase, and others are used to hydrolyse the plant cell wall. Protoplasts are primarily obtained from plant leaves.

2. **Fusion of protoplasts obtained from desired genotype:** Various methods can be used to fuse purified protoplasts from two different plants or tissue sources. In general, chemical agents or electrical manipulation are required to cause membrane instability, which leads to protoplast fusion. PEG (polyethylene glycol) is most commonly employed in conjunction with alkaline pH and high calcium concentrations. Another type of cell fusion involves the manipulation of cell membranes by electrical currents. This method includes delivering low-voltage electric pulses into a solution containing protoplasts to be fused in order for them to line up for fusion. The protoplasts are fused by briefly exposing them to a high-voltage electric current, which causes the membrane to change and the surrounding protoplasts to fuse. Electro fusion of plant protoplasts is often preferred over PEG fusion because it does not employ reagents that are toxic to the cells being fused. To obtain optimal effectiveness, electro fusion conditions must be optimized. Electro fusion of protoplasts can produce fusion products with a yield of 20% or higher, compared to less than 1% using PEG.
3. **Culturing hybrid cells:** To culture hybrid cells, several approaches are utilized, which are detailed below:
   i. **Agarose embedded cultures:** This method is similar to the Bergmann's technique of cell plating, which allows following the development of specific individuals. Pure, low gelling temperature agarose, such as SeaPlaque or Sigma type VII and IX are used extensively for protoplast culture. The semisolid media containing embedded protoplasts can be divided into sectors and transferred to bigger plates of liquid culture medium of the same composition.
   ii. **In liquid medium:** The protoplasts are suspended in liquid culture medium at the required plating density and distributed as a thin layer or microdroplets (50-100 ul) into culture dishes (3, 5 or 9 cm diameter). The dishes are sealed with a gas permeable expanding tape, such as Parafilm and Nescofilm. Nutrients can diffuse quickly in a liquid medium.

iii. **Double layer method:** The protoplasts are suspended in a liquid medium and overlaid on a thin layer of the same gelled medium.

iv. **Agarose Droplets or Beads:** In many species, embedding the protoplasts in agarose beads or discs increased plating and regeneration efficiency. When the protoplasts of various resistant species were trapped in agarose droplets in such a way that streaks of locally high cell density were produced, the protoplasts split and regenerated plants. A liquid media is used to cover protoplasts trapped in agarose beads.

4. **Regeneration of hybrid plants:** Plant regeneration occurs via organogenesis or embryogenesis from newly isolated protoplasts or after their fusion.
5. **Selection of somatic hybrids:** To achieve successful somatic hybrid regeneration, the hybrid products must be chosen from among the unfused and homo-fused protoplasts. Several procedures have been used to select or enrich the hybrid cell population. Biochemical mutants, as well as antibiotic and herbicide resistance, are widely employed. In terms of biochemical mutants, chlorophyll or nitrate reductase deficient mutants, as well as albino mutants, have been widely exploited (Bhojwani and Dantu, 2013).

The model of "embryogenic callus protoplast + mesophyll protoplast" was commonly used for citrus somatic hybridization; because mesophyll protoplasts never divide and regenerate into plants, it is actually a half selection system, which is very conducive to identifying mesophyll-parent type cybrids simply by their leaf morphology. Green fluorescence protein (GFP) has recently been used as a marker to select somatic hybrids/cybrids. GFP is form from *Aequorea victora* and can emit stable and distinctive green fluorescence when expressed by living cells, without any cofactors or subtracts but oxygen. For this reason, transgenic plant expressing the GFP gene was used as a parent in somatic hybridization (Guo *et al.,* 2013).

Applications of SH in crop improvement

1. **Overcoming sexual incompatibility:** In plant breeding programmes, interspecific and intergeneric sexual crossing frequently fails to yield hybrids due to incompatibility barriers that can be addressed via somatic cell fusion.
2. Ploidy modification in scion improvement, *i.e.*, the creation of seedless triploids.

3. Development of Cybrids/Asymmetric hybrids for commercial F_1 hybrid production, reducing the requirement for time-consuming and labour-intensive physical emasculation.
4. **Germplasm Diversification:** Somatic hybridization via protoplast fusion puts two species' genomes together and can be used to convey monogenic or polygenic features. It also generates unique genotypes by merging the cytoplasmic genomes of other cultivars.
5. Used as a bridging material
6. Resistance to diseases/pests/herbicides, *etc*.
7. Increase in alkaloids/Secondary metabolites
8. **Somatic Hybridization Between Haploid and Diploid Protoplasts:** Fusion between somatic (diploid) and pollen (haploid) protoplasts is likely to result in valuable triploid fruit trees. Such triploids would bypass the usual reduction in self-fertility as found in triploid fruit trees obtained by conventional means.
9. **Creation of novel and desirable rootstocks:** Several tetraploid rootstocks have been produced with dwarf growth habits and tolerance to various abiotic and biotic stressors, as well as increased yield and fruit quality.

Achievements

Somatic hybridization achievements in various significant fruit crops are highlighted below:

S. No.	Fruit crops	Somatic hybrids	Modified traits	References
1.	**Banana**	Musa Silk cv. Guoshanxiang (AAB) + *Musa acuminata* cv. Mas (AA)	Disease resistance	Xiao *et al.*, 2009
2.	**Citrus**	"Bonanza" navel orange (*C. sinensis*) + "Red Blush" grapefruit (*C. paradisi*)	Precocious flowering	Guo *et al.*, 2000
		"Rangpur" lime (*Citrus limonia*) + "Caipira" sweet orange (*C. sinensis*),	Drought and blight tolerance	Mendes *et al.*, 2000
		"Hamlin" sweet orange +	Blight, Citrus tristeza	Calixto *et*

		"Singapura" pummelo (*Citrus grandis*)	virus and Phytophthora-induced disease tolerance	*al.,* 2004
		Mandarin (*Citrus reticulata*) + pummelo (*C. grandis*).	Sting nematode (*Belonolaimus longicaudatus* Rau) tolerant	Grosser *et al.,* 2007
		Calamondin (*C. madurensis*) + 'Keen' sour orange (*C. aurantium*)	Tolerance against Citrus tristeza virus (CTV)	Abbate *et al.,* 2019
3.	**Kiwifruit**	*Actinidia chinensis* (2x) + *Actinidia kolomikta* (2x)	Chilling tolerance.	Xiao *et al.,* 2004

References

Abbate, L., Panno, S., Mercati, F., Davino, S. and Fatta Del Bosco, S. (2019). Citrus rootstock breeding: response of four allotetraploid somatic hybrids to Citrus tristeza virus induced infections. *European Journal of Plant Pathology,* **153**(3): 837-847

Bhojwani, S. S., Dantu, P. K., Bhojwani, S. S. and Dantu, P. K. (2013). Parasexual Hybridization. *Plant Tissue Culture: An Introductory Text*, 173-198.

Calixto, M.C., Mourão Filho, F.A., Mendes, B.M. and Vieira, M.L. (2004). Somatic hybridization between *Citrus sinensis* (L.) Osbeck and *C. grandis* (L.) Osbeck. *Pesquisa Agropecuaria Brasileira,* **39**: 721-4.

Grosser, J.W. and Gmitter, F.G. (1990). Protoplast fusion and citrus improvement, Chapter 10. In Plant Breeding Reviews 8. Timber Press Inc, Portland, pp 339–374.

Grosser, J.W., Chandler, J. L. and Duncan, L.W. (2007). Production of mandarin + pummelo somatic hybrid citrus rootstocks with potential for improved tolerance/resistance to sting nematode. *Scientia Horticulture*, **113**: 33-6.

Guo, W. W., Deng X. X. and Yi, H. L. (2000). Somatic hybrids between navel orange (*Citrus sinensis*) and grapefruit (*C. paradisi*) for seedless triploid breeding. *Euphytica*, **116**: 281-5.

Guo, W. W., Xiao, S.X. and Deng, X. X. (2013). Somatic cybrid production via protoplast fusion for citrus improvement. *Scientia Horticulturae.* 1-7.

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Mendes, F. J., Mourao, F. D., Camargo, L.E. and Mendes, B.M. (2000). Caipira sweet orange plus Rangpur lime: A somatic hybrid with potential for use as rootstock in the Brazilian citrus industry. *Genetics and Molecular Biology*, **23:** 661-5.

Xiao, Z., Wan, L. and Han, B. (2004). An interspecific somatic hybrid between *Actinidia chinensis* and *Actinidia kolomikta* and its chilling tolerance. *Plant Cell, Tissue and Organ Culture,* **79**: 299-306.