In vitro culture technologies to improve fruit trees at the CRA W

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• Sanitation

- Meristem culture
- *In vitro* thermotherapy

If our laboratory deeply invested in virus eradication and fruit growers supply with certified plant material (Druart, 2003), the objectives from the last 15 years are more relevant to set up technologies accelerating the breeding processes.

• Genetic transformation (GT)

- *Agrobacterium tumefaciens*
- *Agrobacterium rhizogenes*
- Biolistic

First approaches were the *Agrobacterium*-mediated transformations of the cultivar 'Jonagold' developed in KULeuven to improve disease resistance (De Bondt et al., 1994) and in CRA-W in order to influence the growth habit.

'*Gus*' gene expression by somatic embryo of 'Inmil' cherry rootstock

(coll. IAM-Vienna)

Alteration of 'Jonagold' growth habit after *A.tumefaciens*-mediated transformation with '*KNAP1*', an apple *kn1*-like homeobox gene (Watillon et al., 2004)

Growth alteration of 'Inmil ' cherry rootstock after *A.rhizogenes*-mediated transformation

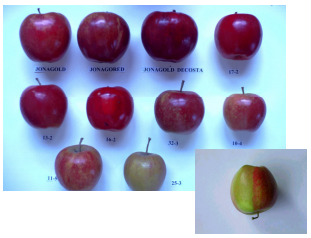
• Alternatives to GT

- Somaclonal variation
- Aneuploids
- Chimeras

In vitro culture techniques revealed very efficient to modify phenotype and genotype at high frequency, in relatively short period of time and without expecting major or irreversible changes in the fruit production process. They also constitute potential alternatives methods to the transformation technologies.

❑ *Anthocyanin in apple by somaclonal variation.*

Adventitious budding induced *in vitro* increases the frequency and widens the varaibilty occuring naturally in the orchard and implies variations of other fruit characteristics such as the shape, the firmness or the maturation that prove to be stable.



cv Jonagold

❑ *Aneuploids from targeted apple genotypes*

Aneuploïdy constitutes a source of genetic diversity for several fruit species. Such genotypes issued from crossing Jonagold (3n) and Mc Intosh ' Wijcik' (2n) (columnar growth) apple varieties declines when growing on their own roots but bear fruits after *in vitro* germination followed by *ex vitro* micrografting (Druart, 2004). The behavior is followed in orchard on the basis of the columnar growth habit.

Genetic instability is detected on adventitious buds after flow cytometry analysis : new aneuploids, polypoids and cytochimerical genotypes appear.

Frequency of the ploidy among the genotypes regenerated from the leaves of 3 cytochimaeric lines.

Cytochimeras origin	Ploidy level								5n				Regenerants Total number
	3n	3.3n	3.4n	3.5n	3.6n	3.7n	4n	5n	+ 3n	+ 3.2n	+ 3.5n	+ 4n	
M9-1			5.7	3.8			1.9	83.0			5.7		53
M13-4		0.8	6.7	3.4	1.7	1.7	1.7	79.8	3.4			0.8	119
Ad-21	1.6		2.3		0.8			94.6		0.8			129
Regenerants Total %	2	1	14	6	3	2	3	261	4	1	3	1	301
	0.7 %	8.6 %					1.0 %	86.7 %	3.0 %				100 %
	Aneuploids								Cytochimeras				

Sources of targeted diversification

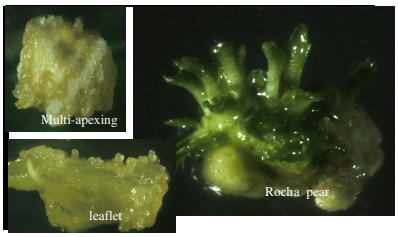
Regenerants bear fruits 4 to 5 years after ex vitro grafting,

• Competence

- Budding
- *S.E. and Protoplasts*

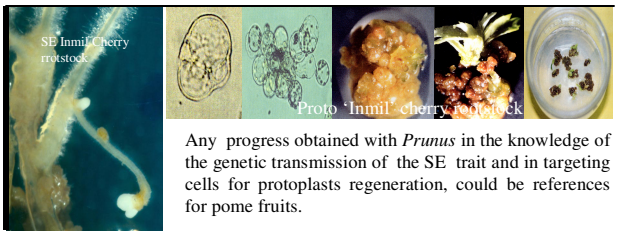
Genetic transformation and alternative technologies as well require efficient and reliable methods of plant regeneration. The regulation of the cell competence is continuously investigated within organs and cell layers of pome (Druart, 2004) and stone fruits.

The unicellular origin of the regenerants constitutes the ultimate objective. Actually, somatic embryogenesis (S.E.) (Druart, 1999) and protoplasts culture (Kondakova and Druart, 2001) are only developed with *Prunus species*



Meristem domes (0.1mm) and leaflets(1-3 mm) implies a minimum of cells in bud neo-formation

Buds from L3 cell layer



Any progress obtained with *Prunus* in the knowledge of the genetic transmission of the SE trait and in targeting cells for protoplasts regeneration, could be references for pome fruits.

Researches on regeneration competence, on induced genetic variation and on the construction of stable chimaeras could improve fruit tree species with trageted traits.

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