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

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- **Sanitation**
  - Meristem culture
  - *In vitro* thermosterapy

- **Genetic transformation (GT)**
  - *Agrobacterium tumefaciens*
  - *Agrobacterium rhizogenes*
  - Biolistic

- **Alternatives to GT**
  - Somaclonal variation
  - Aneuploids
  - Chimeras

  - 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

- **Competence**
  - Budding
  - S.E. and Protoplasts

Aneuploidy 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, polyploids and cytochimerical genotypes appear.

**Frequencies of ploidy among the genotypes regenerates from the leaves of 3 cytochimeric lines.**

<table>
<thead>
<tr>
<th>Cytokinesis origin</th>
<th>Ploidy level</th>
<th><strong>Fregenates</strong> Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3n</td>
<td>3.5n</td>
</tr>
<tr>
<td>M-1</td>
<td>3n</td>
<td>2.5</td>
</tr>
<tr>
<td>M-1.4</td>
<td>3n</td>
<td>2.5</td>
</tr>
<tr>
<td>Ad21</td>
<td>3n</td>
<td>2.5</td>
</tr>
<tr>
<td>Regenerates Total</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>0.7%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

**Sources of targeted diversification**

- **Aneuploids from targeted apple genotypes**

- **Regenerants bear fruits 4 to 5 years after *ex vitro* grafting.**

**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* increase the frequency and widens the variability occurring 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.

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.

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

Buds from L3 cell layer

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