Plant Genetic variability

- Somatic hybridization
- Mutagenesis
- Somaclonal variability
- Haploids
- Genetic modified plants
Plant Genetic variability

Somatic hybridization
Sexual Crossing

- Microspore mother cell (2n) -> MEIOSIS
- Pollen sac
- Pollen grain
- Stigma
- Anther
- Ovary
- Ovule
- Young embryo (2n)
- Seed coat
- Embryo
- Endosperm (3a)
- Seed (2n)
- Pollen tube
- Formation of pollen tube (n)
- Eight-nucleate embryo sac (megaspore-megaphyte) (n)
- MEIOSIS
- Micropore mother cell (2n)
- Double Fertilization
- Egg
- Adult spermatophyte (2n) with flowers
Somatic Hybridization

Il trasferimento genetico interspecifico tramite ibridazione sessuale ha giocato un ruolo importante nel miglioramento genetico vegetale.

In alcuni casi la fecondazione è impedita:

a) Incompatibilità pre-zigotica

b) Incompatibilità post-zigotica
Somatic Hybridization

Is a technique that combines somatic cells from two different cultivars, species or genera in an effort to regenerate novel germplasm. This technique can circumvent such problems as sexual incompatibility, polyembryony and male or female sterility encountered in sexual crossing.
Somatic hybridization steps

1. Isolation of protoplasts
2. Fusion of protoplasts
3. Wall regeneration
4. Identification and selection of hybrid cells
5. Clump of cells
6. Colony formation
7. Callus tissue
8. Callus differentiation
9. Regenerated plantlet
Protoplast fusion

It involves mixing of protoplasts of two different genomes and can be achieved by either spontaneous or induced fusion.

Induced fusion method are:

- treatment with sodium nitrate
- Calcium ions at high pH
- Polyethylene glycol method
- Electrofusion
This is one of the most successful techniques for fusing protoplast.

The protoplast are suspended in a solution containing high molecular weight PEG which enhance agglutinations and fusion of protoplast in several species.

PEG-induced fusion has advantages of not requiring special equipment, low cost and high frequency of heterokaryon formation.
Electrofusion relies on two different electrical pulses. Protoplast are brought into intimate contact during the first pulse called dielectrophoresis; and the second pulse is a very short burst of intense direct current, which results in membrane fusion. Electrofusion has the advantages of convenience, no cell toxicity, and high frequency heterokaryon formation.
Fig. 6. Electron micrograph showing fusion product of *Vicia hajastana* cell culture protoplast and *Vicia narbonensis* leaf protoplast. The two nuclei (N) have not fused. Both leucoplasts (L) and chloroplasts (Ch) are present. Bar = 10 μm. (Rennie et al. 1980)
Fig. 7. Electron micrograph showing nuclear bridge (arrow) between interphase nuclei from pea (P) leaf and soybean (S) cell culture in a fusion product. Ch chloroplast. Bar = 5 μm. (Fowke et al. 1977)

Fig. 8. Electron micrograph showing nucleus containing both pea (P) leaf chromatin and soybean (S) cell culture chromatin in a fusion product. Ch chloroplasts; L leucoplasts. Bar = 5 μm. (Fowke et al. 1977)
Types of somatic hybrids

Symmetric somatic hybrids: is defined as the combination of nuclear and cytoplasmic genetic information of both parents.

Asymmetric somatic hybrids: is incomplete with the loss of some cytoplasmic or nuclear DNA, and this type of hybridization has been used to introduce fragments of the nuclear genome from one donor into the intact genome of recipient.

Cytoplasm hybrids (cybrids): harbor only one parental nuclear genome and either the cytoplasm genome of the other (non-nuclear) parent or a combination of both parents.
Protoplasti in divisione
Divisione
Selection scheme For successful somatic hybrid regeneration, it is necessary to select the hybrid products from among the unfused and homo-fused protoplasts.

selective media
metabolic inhibitors
complementation systems such as chlorophyll deficiency complementation
auxotroph complementation
resistance markers
double mutants
and application of the green fluorescent protein (GFP) marker gene
Selezione di ibridi

Daucus carota (2AECs)

Fusione in substrato contenente 2AECs e 5MTs

Nessuna colonia

Daucus capillifolius (5MTs)

Formazione di colonie (gli ibridi resistono a 2AEC, 5MT)

Nessuna colonia

2AEC = S 2-aminoetil-cisteina
5MT = 5-metil-triptofano
Selezione di ibridi

Datura innoxia (piante albine)

Atropa belladonna (Piante normali)

Callo verde pubescente

Callo bianco e pubescente

Callo verde e non pubescente

Callo verde e non pubescente
Methods to characterize somatic hybrids and their organelles genome

Methods include:

- morphological evaluation
- cytological evaluation by chromosome counting and flow cytometry analysis
- isozyme analysis
- DNA molecular markers

**Techniques**:

- RAPD: random amplified polymorphic DNA
- RFLP: restriction fragment length polymorphism
- AFLP: amplified fragment length polymorphism
- SSR: simple sequence repeat
- GISH: genomic *in situ* hybridization
- CAPS: cleaved amplified polymorphic sequence
Esempi di fusione somatica

Nel 1978 G. Melchers e collaboratori riuscirono a fondere e rigenerare ibridi somatici tra pomodoro (2n=24) e patata (2n=48), specie sessualmente incompatibili, che però non hanno trovato nessuna applicazione pratica in quanto presentavano l’apparato ipogeo della prima specie ed quello epigeo della seconda.
Table 1
Recent examples of the transfer of useful agronomic traits by protoplast fusion

<table>
<thead>
<tr>
<th>Species</th>
<th>Useful traits transferred</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brassica</em></td>
<td></td>
<td></td>
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<tr>
<td>B. napus (+) B. rapa</td>
<td>Increased biomass and yield*</td>
<td>Qian et al. (2003)</td>
</tr>
<tr>
<td>B. napus (+) <em>Crambe abyssinica</em></td>
<td>Increased erucic acid content in seeds*</td>
<td>Wang et al. (2003)</td>
</tr>
<tr>
<td>B. napus (+) <em>Orychoparagous violacea</em></td>
<td>Improved fatty acid composition in seeds*</td>
<td>Hu et al. (2002b)</td>
</tr>
<tr>
<td>B. napus (+) <em>Sinapis arvensis</em></td>
<td>Enhanced resistance to Blackleg</td>
<td>Hu et al. (2002a)</td>
</tr>
<tr>
<td>B. oleracea (+) <em>Moricandis arvensis</em></td>
<td>Introduction of the C3–C4 intermediate trait†</td>
<td>Ishikawa et al. (2003)</td>
</tr>
<tr>
<td><em>Raphanus sativus</em> (+) <em>Diploptera tensifolia</em></td>
<td>Introduction of the C3–C4 intermediate trait†</td>
<td>Bang et al. (2003)</td>
</tr>
</tbody>
</table>

**Citrus**
- *C. amblycarpa (+) Citroncirus webberi* C35: Tolerance to citrus blight, tristeza virus, and *Phytophthora*† for Mexican lime†
  | Medina-Urnutia et al. (2004) |
- *C. limonita (+) C. sunki* cv. Tanaka: Tolerance to citrus blight, tristeza virus, and *Phytophthora*†
  | Costa et al. (2003) |
- *C. reticulata cv. Blanco (+) C. paradisi*: Production of mixoploid plants tolerant to citrus exocortis virus (CEV)†
  | Liu and Deng (2002) |
- *C. reticulata cv. Blanco (+) C. volkameriana*: Resistance to CEV†
  | Guo et al. (2002) |
- *Poncirus trifoliata* cv. Rohde Red (+) *C. volkameriana*: Tolerance to citrus blight, tristeza virus, and *Phytophthora*†
  | Costa et al. (2003) |
- *C. sinensis cv. Ruby Blood (+) C. volkameriana*: Tolerance to citrus blight, tristeza virus, and *Phytophthora*†
  | Costa et al. (2003) |
- *C. sinensis (+) Fortunella crassifolia*: Increased plant vigor*†
  | Cheng et al. (2003) |
- *C. sinensis (+) F. obovata*: Tolerance to citrus blight, tristeza virus, and *Phytophthora*†
  | Costa et al. (2003) |
- *C. sinensis (+) Clausena kansui*: Production of triploid plants*†
  | Fu et al. (2003) |
- *C. unshiu cv. Guoqin No. 1 (+) C. grandis cv. Buntin Pink*: Generation of seedless cybrids†
  | Guo et al. (2004) |
- *C. unshiu cv. Guoqin No. 1 (+) C. reticulata cv. Blanco*: Generation of seedless cybrids†
  | Guo et al. (2004) |
- *C. unshiu cv. Guoqin No. 1 (+) C. reticulata × *C. sinensis*: Generation of seedless cybrids†
  | Guo et al. (2004) |

**Solanum**
- *S. melongena (+) S. aethiopicum*: Resistance to bacterial wilt (*Ralstonia solanacearum*†)
  | Collonnier et al. (2001) |
- *S. melongena (+) S. sicyumbifolium*: Resistance to bacterial and fungal wilts*†
  | Collonnier et al. (2003) |
- *S. tuberosum (+) S. tuberosum*: Resistance to potato virus Y*†
  | Gavrilenko et al. (2003) |
- *S. tuberosum (+) S. nigrum*: Resistance to potato blight (*Phytophthora infestans*)†
  | Szczerekakow et al. (2003) |
- *S. tuberosum (+) S. stenotomum*: Resistance to bacterial wilt (*R. solanacearum*†)
  | Fock et al. (2001) |

*† Trait transfer confirmed.† Trait transfer unconfirmed.