Tissue culture based conservation of fruit germplasm at CREA-OFA, Rome - Italy

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COUNCIL FOR AGRICULTURAL RESEARCH AND ECONOMICS (CREA).

The main **Italian research organization dedicated to the agri-food supply chains**, **supervised by the Ministry of Agriculture, Food Sovereignty and Forests (MASAF)**. **12 Scientific Centers located all around Italy (www.crea.gov.it).**

Scientific activity covers agricultural crops, livestock, fishery, forestry, agro-industry, food science and socio-economics.

Our center: OLIVE, CITRUS AND FRUIT TREE RESEARCH CENTRE (CREA-OFA)

4 STATIONS: Acireale, Caserta, Forlì, Rende, Rome.

CREA–OFA - Rome

- 18 Researches and Technologists
- 20 Technicians

SCIENTIFIC ACTIVITIES of CREA-OFA, Rome

Molecular Breeding

- Genetic and genomic studies applied to fruit breeding
- New Breeding Techniques (NBT) application (cisgenesis, genome editing) (*Prunus* spp.; *Actinidia* spp.) for inducing disease resistance.

Biological agriculture

Food Chemistry

- Application of innovative low impact methods for biological agriculture for defence of abiotic and biotic adversities
- Monitoring of fruit productions quality and Traceability (FT-NIR/MIR; Vis-NIR; Vis; LC; GC-MS) and Post-harvest treatments.

Traditional breeding

- In field breeding of *Drupaceae*, kiwi, pomegranate, small fruits, hazelnut. In 50 years (From 1912 to 2012) of activity of the Experimental Institute of Fruit Growing (now CREA-OFA, Rome – Forli and Caserta), about 180 new varieties have been released, mostly of peaches/nectarines, pears, apples and strawberries.

PLATICARP
PEACHES

Ex situ *in field genetic resources conservation*

National in field, *ex situ* repository of fruit germplasm, financed by **MASAF with 5000 fruit species accessions (Peach, apricot, plum, cherry, hazelnut, walnut..) (40 hectares) in the framework of the RGV-FAO project started in 2004 when Italy signed ITPGRFA**, International Treaty on Plant Genetic Resources for Food and Agricolture).

In field conservation repository

Activities: recovery, conservation, characterization and valorisation of fruit agrobiodiversity.

Other activities in CREA-OFA - Rome

Testing the Distinctiveness, Uniformity and Stability (DUS) for Comunity Plant Variety Office (CPVO):
Union for the Protection of New Varieties of Plants (UPOV)

CPVO
Community Plant Variety Office

Species:
Prunus persica
Prunus salicina
Actinidia spp.
Eucalyptus spp.
Pistacia spp.

In vitro cultures

***In vitro* group partecipants:**

E. Caboni, S. Lucioli, S. Monticelli, A. Gentile, A. Frattarelli

Activities and research studies on:

-Definition of protocol of fruit species micropropagation (*Prunus* spp. cultivars and rootstocks; small fruits; walnut, hazelnut …), started in early 80' thanks to dr. Carmine Damiano, one of the pioneer of micropropagation in Italy and who was leader of our group till 2011.

-**Application of biotechnologies for breeding** adventitious shoot regeneration for genome editing

-**Abiotic stress response** (salinity)

-***In vitro* production of secondary metabolites**

***In vitro* germplasm conservation in culture room, in *slow growth* and cryopreservation**

The objectives for *ex situ in vitro* based fruit agrobiodiversity conservation throughout the years have been:

- **ensuring *in vitro* conservation of Plant Genetic Resources (PGR) by *in vitro* culture, slow growth, through the definition and optimization of protocols;**

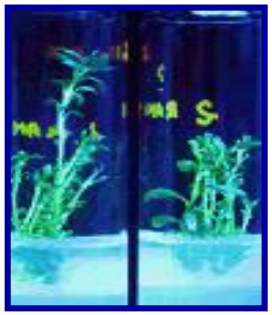
- **defining protocols for cryopreservation of some of these PGRs.**

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Definition and application of protocols for *in vitro* cultures (establishment, multiplication and rooting)

Main Critical Factors:
- **medium composition,**
- **type and concentration of growth regulators**

→ **"Slow growth" at 4-5°C**



Accessions conserved *in vitro* and in slow growth in the frame of the Italian program RGV-FAO (MASAF) in *in vitro* laboratory of CREA-OFA, Rome

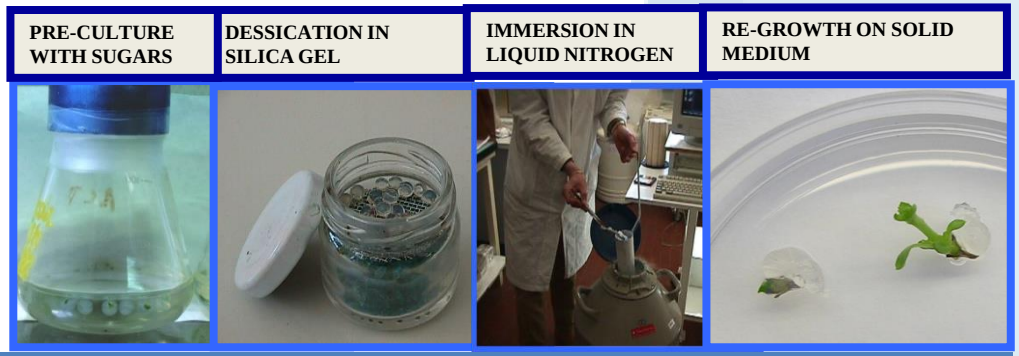
Peach (n. 2)
Plum (n. 10)
Apple (n. 9)
Pear (n. 9)
Cherry (n. 6)
Apricot (n. 4)
Walnut (n. 3)
Mulberry (n. 1)
Raspberry (n. 9)
Almond (n. 3)
Pomegranate (n. 5)
Hazelnut (n. 7)
***C. colurna* (n. 2)**
Grape (n. 4)
Fig (n. 4)

<u>Main factors to define protocols</u>: type and concentration of sugar and of growth regulators, according to the species and to the genotype

Definition of protocols for cryopreservation

Cryopreservation by encapsulation-dehydration

Explants are encapsulated in calcium alginate beads (Fabre and Dereudre, 1990), precultured with sugars and dehydrated in silica-gel before immersion in LN



Activity started (in 90' years) thanks to cooperation of dr. Carmine DAMIANO with dr. Florent ENGELMANN of International Plant Genetic Resources Institute (IPGRI), Rome, Italy.

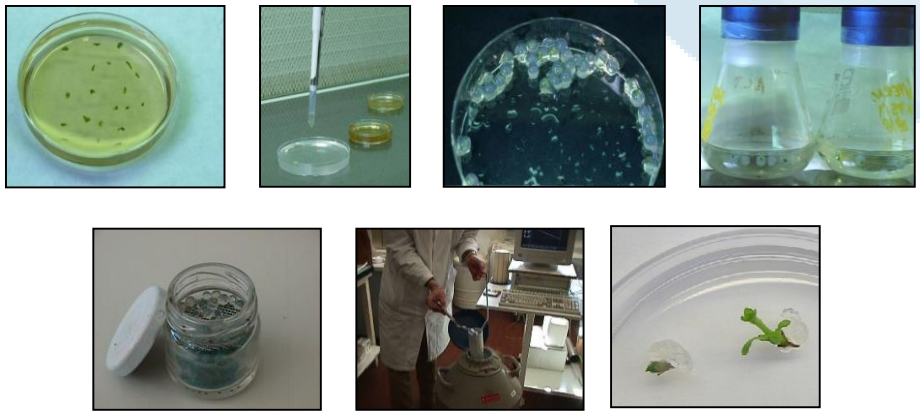
The protocols were established acting on various factors such as:

- **sugar type (glucose or sucrose) in the solution, concentration (0.5, 0.75 or 1M) and duration of application (from 1 to 5 days) in the dehydration phase;**
- **duration of desiccation in silica (4 to 20 hours).**

Studies performed on cryopreservation by <u>encapsulation-dehydration</u> apical tips or axillary buds excised from *in vitro* growing shoots of:

- **pear (*Pyrus communis* L.),**
- **apple (*Malus domestica* Borkh.),**
- **strawberry (*Fragraria* x *ananassa* Duch.),**
- **almond (*Prunus dulcis* Mill.),**
- **peach (*Prunus persica* (L.) Batsch),**
- **mulberry (*Morus alba* L.),**
- **blackberry (*Rubus fruticosus* L.) and raspberry (*Rubus idaeus* L.),**
- **hazelnut (*Corylus avellana* L.).**

Best factors combinations: concentration and duration of application of 0.5, 0.75 or 1M sucrose (from 1 to 3 days) in the dehydration phase of beads and silica desiccation of 8 - 10 h to obtain around 20% moisture content



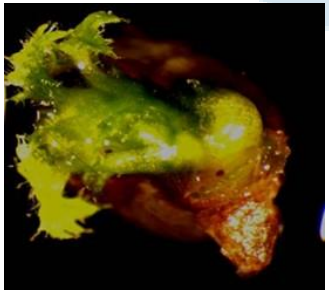
Main Results

Pear: in **wild pear** (*Pyrus pyraster*), **dehydration** of Na-alginate beads **for 2 days** in **0.75 M sucrose** and **desiccation** beads desiccation for **10 h** with **silica gel** to 20% moisture content gave **60% recovery** after exposure to LN.

In **peach** (**Cvs. San Giorgio, Summer Grand, Babygold)** the **best regrowth (from 33 to 46%)** was obtained with **dehydration** of Na-alginate beads for **3 day in 0.5 M sucrose** and **desiccation** for **9 h in silica** to 20% moisture content.

In **mulberry** (*Morus alba* L.), the highest regrowth (**62%)** was obtained with **dehydration** with **0.75 M sucrose** for **3 days** and **9 days** of **silica treatment** of beads**.**

In **hazelnut** (*Corylus avellana* L.), Italian cultivars "Tonda Gentile Romana" and "Montebello" the **highest regrowth** (**40%**) was obtained dehydrating alginate beads with **0.75 M sucrose** applied for **1 day** and with beads desiccation for **8 h** with **silica gel**.



cv. Tonda Gentile Romana encapsulation-dehydration. Regrowth after LN (5 weeks).

Genetic stability of wild pear (*Pyrus pyraster*, Burgsd) after cryopreservation by encapsulation dehydration
Condello, E., Palombi, M.A., Tonelli, M.G., Damiano, C., Caboni, E.
***Agricultural and Food Science*, 2009, 18(2), pp. 136–143.**

Cryopreservation of peach shoot tips by encapsulation dehydration
Damiano, C., Sgueglia, A., Arias, M., ...Condello, E., Caboni, E.
***Acta Horticulturae*, 2011, 918, pp. 121–124.**

Cryopreservation of fruit tree species through encapsulation-dehydration at the CRA - Fruit research centre of Rome
Damiano, C., Caboni, E., Frattarelli, A., ...Arias, M., Engelmann, F.
***Acta Horticulturae*, 2011, 908, pp. 187–19**

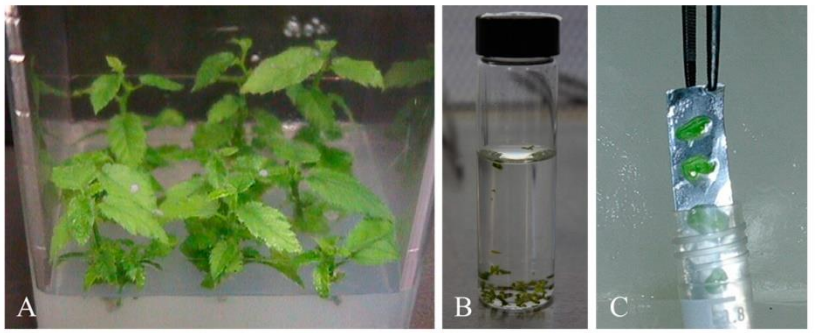
Cryopreservation of white mulberry (*Morus alba* L.) by encapsulation-dehydration and vitrification
Arias Padrò, M.D., Frattarelli, A., Sgueglia, A., ...Damiano, C., Caboni, E.
***Plant Cell, Tissue and Organ Culture*, 2012, 108(1), pp. 167–172.**

Cryopreservation of Italian cultivars of hazelnut by the encapsulation-dehydration technique
Sgueglia, A., Gentile, A., Frattarelli, A., Germanà, M.A., Caboni, E.
***Acta Horticulturae*, 2021, 1307, pp. 159–162.**

Definition of protocols for cryopreservation

Cryopreservation by droplet vitrification

Explants were immersed in individual micro-droplets of plant vitrification solution (PVS) placed on small pieces of aluminum foils and then immersed in LN (Panis et al. 2005).



Collaboration with dr. BART PANIS
Bioversity International, c/o KU Leuven, Be
COST action 871 – CRYOPLANET 2006 - 2010.

Studies performed on apple, raspberry and hazelnut

The droplet vitrification protocols were established optimizing:

- age of mother-plants and of type of PVS

- duration of application of PVS

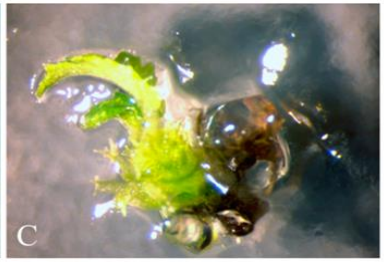
Results

Apple. Cvs. Pinova and Jonagold. The **highest shoot regrowth (47% and 40%)** was obtained with **60 min PVS2** treatment to **nodal segments** with **axillary buds** excised **from 4-months old *in vitro* shoots** and using a **recovery medium containing 4.5 µM BA and 0.5 µM IBA** (activity started in the lab. of dr. Bart Panis, in Cryoplanet Project).

Raspberry. In **cv. Latham**, **regrowth (18%)** was obtained with buds treated with the **PVS2 for 30 min** (In cooperation with dr. Djurdjina RUŽIĆ Fruit Research Institute, Kralja Petra I/9, 32000 Čačak, Republic of Serbia).

Hazelnut. Using **nodal segments** with **axillary buds** of the **Italian cultivar "Tonda Gentile Romana"**, the **highest regrowth** percentage (**56.7%**) was obtained applying **PVS3** for **60 min**, while the application of **PVS2 reduced regrowth to 41.5%**. Increasing the exposure to **PVSs** to **90 min reduced regrowth**.

Hazelnut (*Corylus avellana*), cv. Tonda Gentile Romana.), regrowing shoot 8 weeks after immersion in LN.



Cryopreservation of apple *in vitro* axillary buds using droplet-vitrification
Condello, E., Caboni, E., Andrè, E., ...Swennen, R., Panis, B.
***Cryo-Letters*, 2011, 32(2), pp. 175–185.**

Raspberry cryopreservation by droplet vitrification technique
Condello, E., Ruzic, D., Panis, B., Caboni, E.
***Acta Horticulturae*, 2011, 918, pp. 965–969.**

Cryopreservation of hazelnut (*Corylus avellana* L.) axillary buds from *in vitro* shoots using the droplet vitrification method
Sgueglia, A., Frattarelli, A., Gentile, A., ...Germanà, M.A., Caboni, E.
Horticulturae,. 2021, 7(11), 494.

Hypothesis for next steps in fruit species cryopreservation:

-To develop protocol for plum (*Prunus domestica*, L.) Italian varieties (in progress).

-Starting stable cryopreservation for apple, plum (?) and hazelnut varieties with the protocols developed.

Thanks for the attention