



## 1<sup>st</sup> Meeting of the ECPGR Cryopreservation Working Group

Concept for development of a rational and efficient way to collaboratively conserve under cryopreservation the relevant European plant genetic resources

3-4 May 2023

*Crop Research Institute, Prague, Czech Republic*

# MAURIZIO LAMBARDI



National Research Council of Italy  
Institute of BioEconomy  
Department of Biology, Agriculture and Food Science

# The activity of preservation of woody plant genetic resources at the CNR-IBE

## IN FIELD CLONAL COLLECTIONS



Experimental Station «Santa Paolina», Follonica (Grosseto)



## CRYOBANK



Micropropagation and cryopreservation laboratory, Sesto Fiorentino (Firenze)



# Experimental Station «Santa Paolina» in Follonica (Grosseto)

Responsible of the Station: Dr. Claudio Cantini



# Center for the *ex situ* conservation of fruit germplasm

Established in the 1967, is a very important and recognized center of fruit species conservation in Italy.

**60 ha of clonal collections and experimental fields**

<u>Species</u>	<u>Accessions</u>	<u>Trees</u>
Peach	538	855
Olive	1,025	1,700
Pear	340	1,232
Cherry	90	360
Kaki	48	192
Plum	42	168
Apple	29	112
Quince	16	64
Grape	13	39
Cypress	34	152
Elm	162	16
	<b>2,337</b>	<b>5,042</b>



1	toscana	207
2	abruzzo	39
3	basilicata	24
4	calabria	37
5	campania	109
6	emilia romagna	23
7	friuli venezia giulia	1
8	lazio	63
9	liguria	50
10	lombardia	60
11	marche	39
12	molise	25
13	puglia	79
14	sardegna	41
15	sicilia	86
16	umbria	325
17	veneto	26

1	algeria	1
2	arabia saudita	6
3	argentina	20
4	australia	2
5	cile	17
6	cipro	8
7	cina	4
8	croazia	6
9	francia	10
10	giordania	3
11	grezia	23
12	iran	24
13	israele	5
14	india	2
15	marocco	10
16	messico	8
17	portogallo	12
18	repub.san marino	11
19	siria	16
20	sud africa	17
21	slovenia	14
22	somalia	2
23	spagna	48
24	turchia	47
25	tunisia	7
26	usa	11

# OLIVE GERmplasm

Collection of 1,025 varieties  
from 17 Italian regions e  
26 Countries in the world



National Research Council ITALY

**Olive Germplasm (*Olea europaea* L.)**  
(cultivars, synonyms, cultivation area, collections, descriptors)  
DOI: 10.7349/OLEA\_databases  
by **Giorgio BARTOLINI**  
Istituto per la Valorizzazione del Legno e delle Specie Arboree (IVALSA)  
Trees and Timber Institute

DataBase design by Stefano CERRETTI  
Area di Ricerca CNR Firenze



[INTRODUCTION](#)

**COLLABORATIONS**

ORGANIZATIONS:  
[FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS](#)  
[FAO \(AGPS\)](#)

PEOPLES:  
Petruccioli Raffaella (IVALSA - CNR)  
Stefani Federico (IVALSA - CNR)  
Briccoli Basi Caterina (CRA - Oli)  
Zelasco Samanta (CRA - Oli)

Insert a Cultivar name or a synonym as you know it.  
You may use also words that are parts of names or synonyms.

[VERIFY IF A NAME IS PRESENT IN OLEADB ARCHIVES](#)

[SEARCH CULTIVARS BY SCIENTIFICS PARAMETERS](#)

[SEARCH COLLECTIONS](#)

[CULTIVAR SHEET BY CULTIVAR AND AUTHOR](#)

[CULTIVAR SHEET BY CULTIVAR AND ARTICLE](#)

[SEARCH HISTORICAL NAMES](#) work in progress!

**BIBLIOGRAPHY**

The information on the cultivars are now updated and corrected in real time. All the descriptive characters will be reported compatibly with the available budget.

Simultaneously it is possible to have information on cultivars, synonyms and unknown names (mentioned) all over the world.

Should you detect any kind of error, according to literature, please inform us as soon as possible in order to correct it.

In order to keep the DB updated, the Authors of research on olive germplasm are kindly invited to send copies of their publications to the following addresses:

e-mail [bartolini@ivalsa.cnr.it](mailto:bartolini@ivalsa.cnr.it)

FAX 39 055 5225656

post address:  
Giorgio Bartolini  
IVALSA CNR  
via Madonna del piano 10  
50019 Sesto Fiorentino (FI)  
ITALY

Thank you for cooperation.  
[Giorgio Bartolini](#)

**DataBase**  
**www.oleadb.it**





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# «Santa Paolina» Experimental Station

OLEA databases

[www.oleadb.it](http://www.oleadb.it)



**Informatization**

**Morphological and molecular characterization**

**Recovery of pathogen-free plants**

**Micropropagation and *in vitro* conservation**

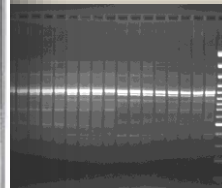
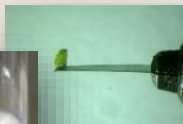
**Germplasm**

**CLONAL COLLECTIONS**

- Production of high-quality certified trees
- valorization of products (fruits, oil, wood)

**Propagation (cutting, grafting)**

**Cryopreservation**



# CRYOBANK of CNR-IBE

IBE, Istituto per la BioEconomia

## CRIOBANCA

Conservazione di germoplasma vegetale  
 in azoto liquido (-196° C)



## What is presently preserved in the IBE cryobank:

- ✓ Ancient apple varieties (19 accessions - DBT)
- ✓ Ancient citrus germplasm (24 accessions - PES)
- ✓ Plum (8 accessions - DBT)



# ANCIENT ITALIAN APPLE VARIETIES: cryopreservation of dormant buds



-5°C

Winter collection of scions and cold hardening at -5°C (8 wks)



Uni-nodal sections, cold hardened at 4°C and dehydrated up to a MC of 35-26% (6-8 wks)



Slow cooling up to -30°C (-1°C/hour)



-196°C

Thawing and rehydration in damp peat at 2°C (2 wks)



Chip budding



Melo Rosa gentile  
 GM 58  
 26% LN

After 1-2 wks



Melo San Piero  
 GM 37  
 35% LN

After 6 wks



# 2008 at Veneto Agricoltura.....

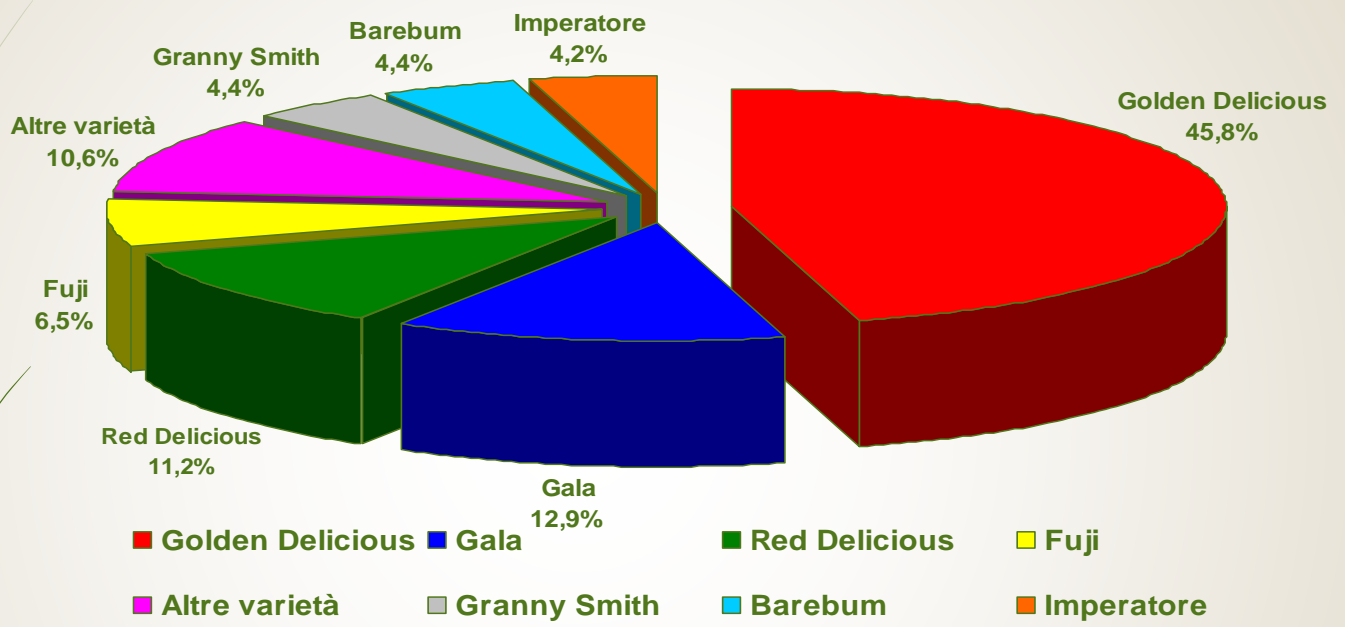



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 Institute of BioEconomy  
 Department of Biology, Agriculture and Food Science

**VENETO**  
**AGRICOLTURA**
  
 Azienda Regionale per i settori Agricolo, Forestale e Agro-Alimentare

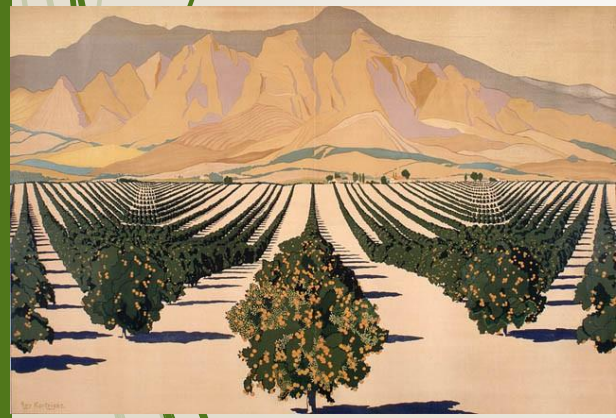


# Apple production in Italy: 2,080 tons (year 2022)



*..... 70% of the production from only 3 cultivars !!!*

*Italian apple germplasm in the early 20<sup>th</sup> Century included hundreds of varieties !!!*





# Cryo-banking of ancient Italian apple germplasm by the dormant-bud technique



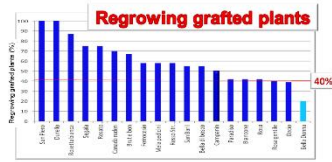
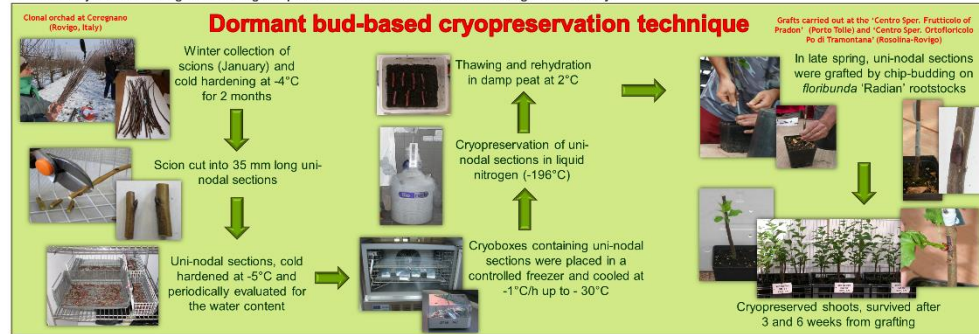
Carla Benelli<sup>(1)</sup>, Francesco Da Re<sup>(2)</sup>, Anna De Carlo<sup>(1)</sup>, Elif Aylin Ozudogru<sup>(1)</sup>,

Simone Serra<sup>(2)</sup>, Maurizio Lambardi<sup>(1)\*</sup>

1) IVALSA/Istituto per la Valorizzazione del Legno e delle Specie Arboree - National Research Council (CNR), 50019 Sesto Fiorentino (Florence), Italy.  
\*lambardi@ivalsa.cnr.it

2) VENETO AGRICOLTURA, Agripolis, 35020 Legnaro (Padova), Italy.

In 2000, Veneto Agricoltura, the agricultural agency of the Veneto region (Italy), has started a program aimed at conserving ancient apple cultivars. This program is focused on the documentation of specimen trees from old cultivars and their propagation and maintenance in in-field collection. Today the clonal orchard contains about 200 cultivars. In order to guarantee a safe duplication of this valuable germplasm, a cryobank of dormant buds has been recently established at the CNR-IVALSA Institute of Florence and annually enriched with additional cultivars. The use of dormant-bud technique has the advantage of allowing the direct transfer of accessions from the field to the tank and vice versa, i.e., with no passage in tissue culture, which is necessary with all other cryogenic techniques before and after their storage in liquid nitrogen. For this reason, the cryopreservation of apple dormant buds allows to ensure a cryobank for long-term storage of plant material with low cost and safe genetic fidelity.



Percentage of graft survival after cryopreservation of 19 apple cultivars of Veneto region. Graft is considered survived when at least one of the two buds grafted has developed.

Procedure is considered optimized when the graft survival after cryopreservation is higher than the 40%

**Post-conservation pre-grafting tests of viability**

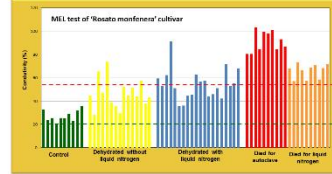
' CUT TEST '

Check immediately before graft

' TTC TEST '

(0.5% TTC, 1 day at 30°C and darkness)

Alive buds / Dead buds



' MEL TEST ' - Electrolyte Leakage Test

The conductivity is measured by EC-Meter GLP 31-Crison using vials filled with 50 ml distilled water ( $C_{H_2O}$ : 1.2 µS, approximately). Uni-nodal sections (each section was previously weighed) were incubated in water for 24 h before measuring the conductivity. Then, the samples were autoclaved and the conductivity was measured again after 4 h. The MEL-value is calculated as follows (Verleyen *et al.*, 2004):

$$C_{24h} = 100 (C_x - C_0) / (C_x - C_0)$$

$C_x$ : conductivity measured after incubation for 24 h;  $C_0$ : water conductivity;  $C_x$ : conductivity measured after autoclave

**Apple cultivars preserved in cryobank:**  
Bella di bosco, Biancone, Brut e bon, Campanin, Canada ruden, Decio, Durello, Ferrocasio, Mela pedicini, Paradiso, Rosa, Rosa gentile, Rosato monfenera, Rosetta bianca, Rosso Str. Francesco, San Baril, San Piero, Segala.



Project is carried out partially with the support of Ente Cassa di Risparmio di Firenze

At present, in the CRYOBANK of CNR-IVALSA, dormant buds from 18 cultivars of apple germplasm of Veneto region are preserved. This cryobank is the first example in Italy.

# Ancient citrus germplasm

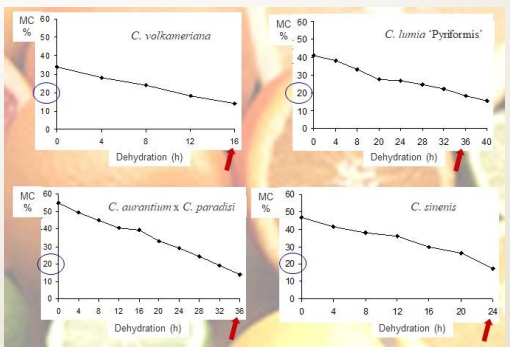
## Cryopreservation of polyembryonic seeds for the duplication of the Medicean collection of the Villa Reale Medicea di Castello in Firenze

**Branchi del seme**  
**Citroni in acqua**  
**CROBANCA**  
 La crioconservazione è una tecnica innovativa che permette di congelare le collezioni, avendo modo in stazionario liquido (-196 °C) e, grazie ai "tubi vegetali".

La crioconservazione (conservazione in azoto liquido) apre oggi importanti prospettive alla salvaguardia della biodiversità vegetale. A -196°C, infatti, le cellule vegetali entrano in uno stato di "quiescenza assoluta", in quanto tutte le reazioni fisiche e biochimiche sono praticamente arrestate; peraltro, se le cellule sono portate in questa condizione di ultra-raffreddamento seguendo opportune procedure "preparatorie", la vitalità non ne risulta compromessa e, al ritorno a condizioni standard di coltura, queste possono riassumere la loro piena funzionalità. Obiettivo del presente progetto è la realizzazione di una CROBANCA di semi a tutela dell'antico germoplasma di agrumi della 'Villa Medicea di Castello'.

La collezione è costituita da circa 500 esemplari di agrumi in grandi conche di terracotta, di svariate dimensioni (alcune imponenti) ed età, la cui origine risale al 1544 ad opera di Cosimo I<sup>o</sup> de' Medici. Le accessioni conservate nella Villa costituiscono un importantissimo patrimonio agricolo, comprendente specie e varietà ornamentali di alto valore storico, difficilmente reperibili in altri contesti e, quindi, di interesse internazionale. Gli esemplari sono sotto costante "rischio" di perdita della biodiversità di cui sono depositari, in quanto le piante soggiacciono alle insidie di eventi climatici straordinari (quali le gelate tardive), atti vandalici o possibili infezioni di patogeni.

La collezione comprende accessioni di *Citrus* di importanza storica e varietà rare, caratterizzate da frutti grandi o bizzarri, quali il *Citrus medica* 'Florentina' (a), il *C. limon* 'Digitata' (b), il *C. aurantium* 'Turonicum salicifolia' (c), il *C. aurantium* 'Canaliculata' (d), il *C. limon* 'Peretta', il *C. aurantium* 'Bizzarria'.



## Villa Medicea di Castello in Florence



One of the villas of Florence which belonged to the Medici Family

The collection was initiated by **COSIMO I<sup>o</sup> de' MEDICI** in the XVI<sup>th</sup> century



It accounts for over **600 ACCESSIONS** some of them unique



**Shoot proliferation**



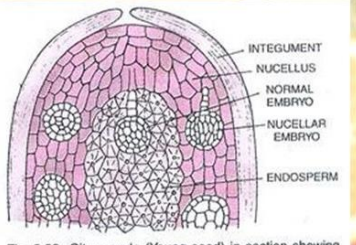
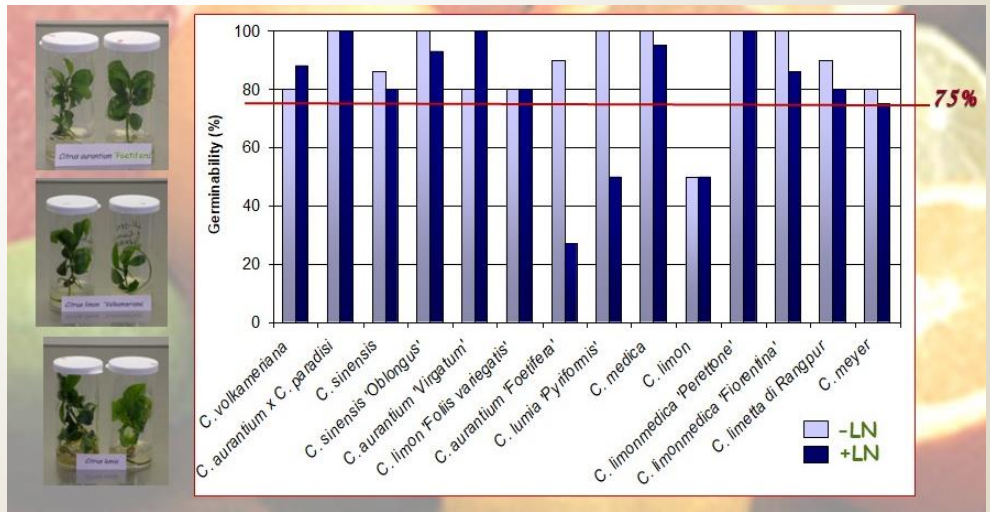




Fig. 2.33. *Citrus ovule* (Young seed) in section showing normal and nucellar (adventive) embryos.



# Current projects

- **Agreement CNR/IBE-Ferrero-Battistini Vivai**
  - ↳ Development of cryopreservation of *Corylus* spp.  
👉 **Doaa Elazab Elkassas**
- **Agreement with CAV-Center for Nursery Activity of Faenza, Italy**
  - ↳ Development of **dormant-bud protocols** for the duplication of obsolete fruit germplasm
- **LIFE4FIR Project: preservation of the endangered species *Abies nebrodensis* from the Madonie Park, Sicily**
  - ↳ Establishment of a **seed-bank** and a **cryobank** for the conservation of *Abies nebrodensis*

# Development of dormant-bud protocols for the duplication of obsolete fruit germplasm



Protocols of DBT developed for accessions of:

- ✓ Apple
- ✓ Plum
- ✓ Cherry

*(no results with pear and peach)*



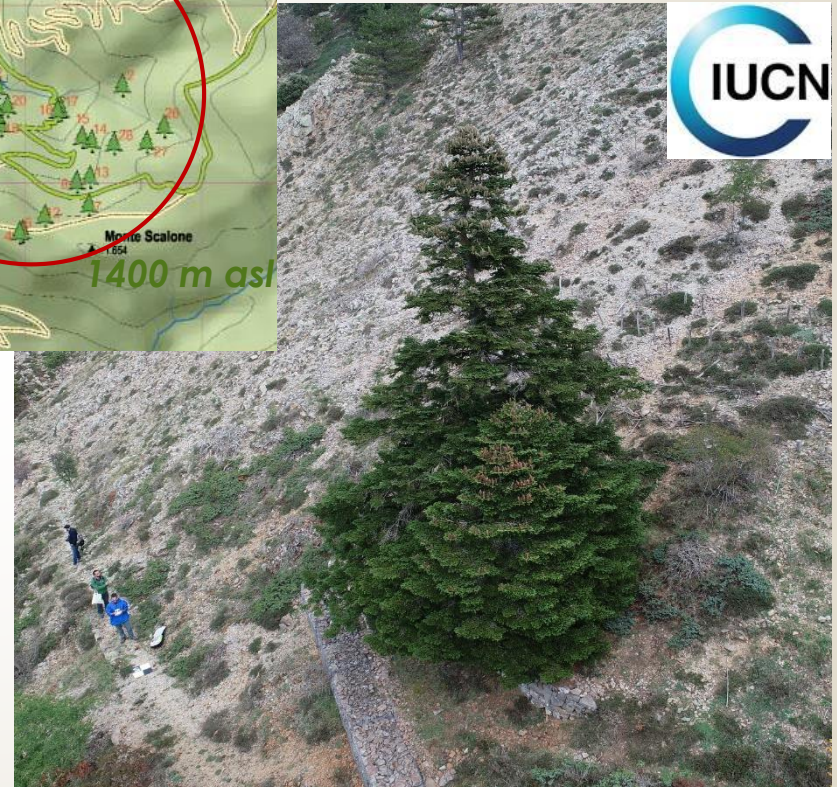
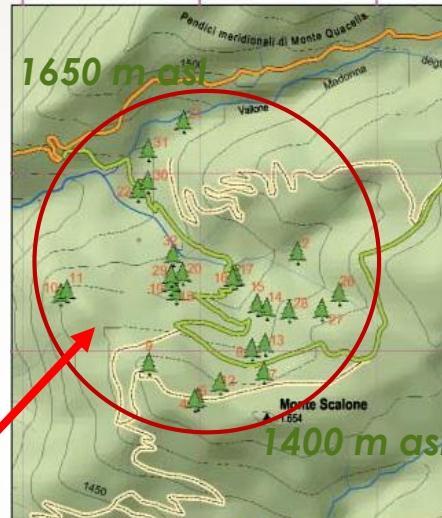
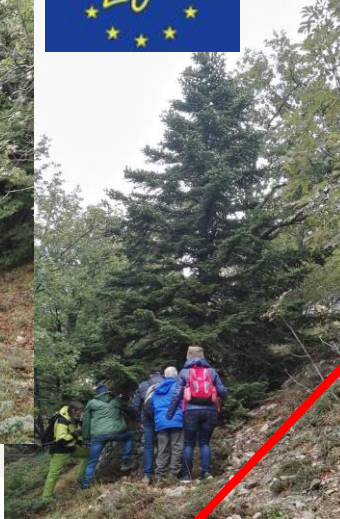
➔ **Protocols effective with buds from screenhouse, i.e., subjected to mild winter temperatures (never < 0°C)**

Decisive in situ and ex situ strategies to secure the critically endangered Sicilian fir, *Abies nebrodensis* LIFE18 NAT/IT/000164 'LIFE4FIR'



***Abies nebrodensis***: it is an endemic conifer, presently **30 trees** in a small area of the Sicily region, in Italy

An highly endangered species, included in the IUCN red list







Today, the residual population of the Sicilian Fir consists of only 30 adult trees and is highly threatened by:



Genetic erosion due to...



(i) a very limited natural renewal



(ii) superficial and soil



(iii) grazing of wild herbivorous animals



# Main objective of the project LIFE4FIR:

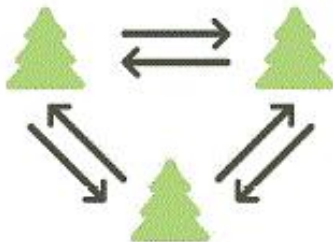
① To limit the risk of extinction by improving conservation and developing a model of protection of the species.

## Specific actions:

① to protect the remaining trees directly in their natural habitat



② to increase the genetic diversity of the progeny by promoting the breeding between individuals



③ to promote the ex situ conservation by means of the creation of a SEED BANK....



...the long-term conservation of the species

....and a CRYOBANK for....



## The Seedbank and Cryobank of the MAN (Museum of *Abies nebrodensis*) in Polizzi Generosa, Sicily



### In Cryobank:

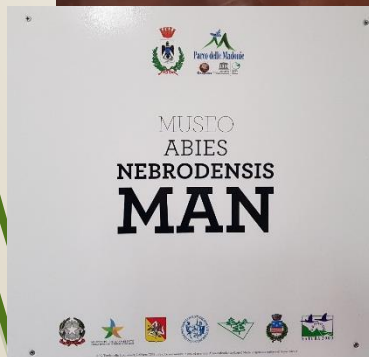
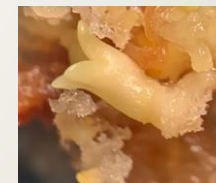
✓ Excised embryos



✓ Pollen

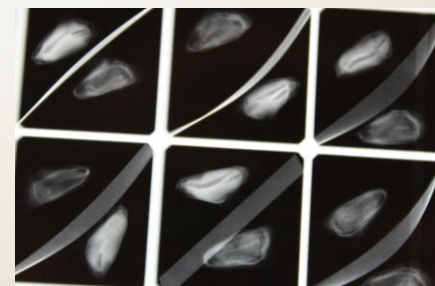


✓ Embryogenic callus



### In Seedbank:

✓ Seeds



# Final considerations

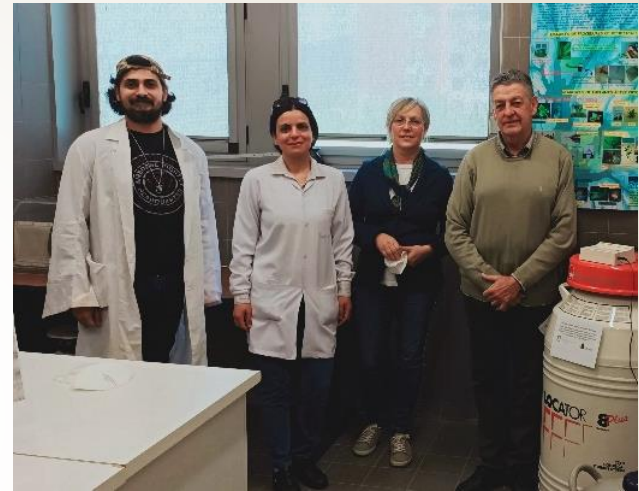
- The dormant-bud technique is the best approach for the cryobanking of fruit species, when chip-budding is the used grafting technique of the species (e.g., cannot be applied to olive).
- Nodal microcuttings can be collected also from trees that have been subjected to mild winter temperatures.
- The LIFE4FIR European Project is a good example of application of cryobanking to a critically endangered species, *Abies nebrodensis*.
- Networking is strategic for cryobanking!

# The CRYOBANK at the CNR-IBE, Institute of BioEconomy of Sesto Fiorentino (Firenze)

IBE, Istituto per la BioEconomia

## CRIOBANCA

Conservazione di germoplasma vegetale  
 in azoto liquido (-196° C)



**Tolga Izgu (Turchia) Carla Benelli  
 Waed Tarraf (Siria) Maurizio Lambardi**



**Anna De Carlo**



**Doaa Elazab  
 (Egitto)**