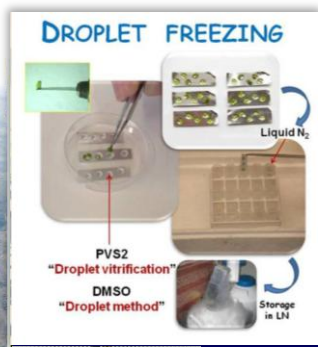




**LIFE4FIR:** Decisive in situ and ex situ conservation strategies to secure the critically endangered Sicilian fir, *Abies nebrodensis*

LIFE18/NAT/IT/000164 **LIFE4FIR**



# **IBE-CNR** **Role and** **Responsibilities**

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Consiglio Nazionale delle Ricerche

**IBE**  
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## Actions in which CNR-IBE is involved:

### Preparatory actions – ACTIONS "A"

- **A1** – Protocol setup to define genetic traits of *Abies nebrodensis* population and to improve its propagation and conservation at low and cryogenic temperatures of selected tissues and organs (US)

### Implementation actions – ACTIONS "C"

- **C1** – Support and preserve *A. nebrodensis* in its natural habitat (CIRITA-UNIPA)
- **C2** – Conservation of genetic purity of *A. nebrodensis* and improvement of its genetic diversity (US)
- **C3** – Establishment of a new clonal orchard for germplasm collection and to boost the genetic variability of the progeny
- **C4** – Nursery production of improved seedlings of *A. nebrodensis*
- **C5** – Constitution of a seed bank and a cryobank for the long-term conservation of seeds, pollen, isolated embryos and embryogenic callus lines of *A. nebrodensis* (Responsible **CNR-IBE**)
- **C6** – Reforestation with *A. nebrodensis* (4000 seedlings) in 10 plots in the Madonie Park in suitable areas for the reintroduction applying innovative planting techniques (CIRITA-UNIPA)
- **C7** – Replication. Implementation action dedicated to the results' transfer and replication during the project

### Monitoring actions – ACTIONS "D" (D1, D2, D3) (CNR)

### Communication and Dissemination – ACTIONS "E" (E1, E2) (CNR)

### Project management – ACTION "F" (CNR)



## Sub-actions in which the *IPSP* is involved:

### *ACTIONS "A"*



**A1.4** – *Seed conservation at low temperature (-18°C), application of cryopreservation protocols*



### *ACTIONS "C"*



#### **C4**

**C4.1** – *Application of an improved seed propagation protocol, implementation of a system for the selection of viable seed through the use of a "ballistic" machinery*



**C4.3** - *Nursery propagation of (i) 4500 *A. nebrodensis* seedlings for the reforestation program, and (ii) 360 grafted plants for the establishment of the clonal orchard*



#### **C5**

*Constitution of a seed bank and cryobank for the long-term conservation of seeds, pollen, isolated embryos and embryogenic callus lines of *Abies nebrodensis**



#### **C7**

*Replication. Implementation action dedicated to the results' transfer and replication during the project*





## ACTION "A1"

### **A1.4** – Seed conservation at low temperature (-18°C), application of cryopreservation protocols



Seed conservation at -18°C and cryopreservation of excised zygotic embryo (ZE). Actions:

- collection of seeds at full maturity from elite trees (DSRT, EPM, collaboration CNR-IBE)
- determination of moisture content and desiccation in silica gel to reach 10% MC, storage for 6, 12, 24, 36 months (CNR-IBE, collaboration with UNIPA, EPM)
- determination seed viability after each storage period (CNR-IBE, collaboration UNIPA)
- decontamination, chilling, ZE excision, cryopreservation, rewarming, post-cryo in vitro germination (CNR-IBE, collaboration UNIPA)

Somatic embryogenesis and cryopreservation of selected SE callus lines. Actions:

- cone harvesting and seed extraction (UNIPA, collaboration with EPM, CNR-IBE)
- production of at least 10 embryogenic callus lines (UNIPA, collaboration CNR-IBE)
- Development of a cryopreservation procedure by slow cooling technique (CNR-IBE)

Pollen. Actions:

- harvest of shoots, collection of pollen (CNR-IBE, collaboration DRSRT, EPM)
- cryopreservation and post-cryo viability test (CNR-IBE)





## ***ACTION "A1"***



**A1.4** – *Seed conservation at low temperature (-18°C), application of cryopreservation protocols*



### ***DELIVERABLES***

12/2020

1. Report on protocol for pollen cryopreservation (CNR-IBE)
2. Report on protocols for seed and grafting propagation (EPM, collaboration CNR-IBE)
3. Report on protocols for (i) seed conservation at low temperature, (ii) excised ZE at -196°C, (iii) pollen at -196°C, SE callus lines at -196°C (CNR-IBE)

### ***MILESTONES***

06/2020

Collection of the male flowers to obtain pollen and cones to obtain immature ZE for SE (EPM, collaboration UNIPA, CNR-IBE)

**Action A1.4 - WHO is involved? Collaborations among partners**

**CNR-IBE** ↔ CIRITA-UNIPA ↔ DRSRT ↔ EPM





## *ACTION "C4"*

***C4.1*** – *Application of an improved seed propagation protocol. Implementation of a system for the selection of viable seed through the use of a "ballistic" machinery*

- application and development of the "ballistic" procedure, through collaboration with the "Centro Nazionale per lo Studio e la Cons. della Biodiv. For." dei Carabinieri di Peri (CNR-IBE)

***C4.3*** – Nursery propagation of (i) 4500 *A. nebrodensis* seedlings for the reforestation program, and (ii) 360 grafted plants for the establishment of the clonal orchard

- Propagation by seeds and grafting (DSRST, collaboration EPM, CNR-IBE)





## ACTION "C4"

### **DELIVERABLES**



06/2023

Report on the effective production of mycorrhizal *A. nebrodensis* seedlings and on the improved health status of the seedlings of the local nursery of 'Vivaio Piano Noce' (CNR-IPSP, collaboration DSRST, CNR-IBE)

### **MILESTONES**

12/2019

Viable seeds selection with the ballistic machinery (CNR-IBE, collaboration DSRST, EPM)

10/2020

2-day training course for nursery employees on (i) prevention and control pathogens, (ii) propagation by seeds and graftings (CNR-IPSP, collaboration UNIPA, CNR-IBE, EPM)

#### **Action C4.1 - WHO is involved?**

**CNR-IBE**

#### **Action C4.3 - WHO is involved?**

**DSRST** ↔ CNR-IBE ↔ EPM





## ***ACTION "C5"***

*Constitution of a seed bank and cryobank for the long-term conservation of seeds, pollen, isolated embryos and embryogenic callus lines of *Abies nebrodensis**

Establishment of a seed bank for conservation at -18°C. Actions:

- acquisition and establishment of a climatic chamber at the "Museo Naturalistico Francesco Minà Palumbo in Castelbuono (CNR-IBE, collaboration EPM)
- selection and conservation of seeds from *A. nebrodensis* trees (EPM, collaboration CNR-IBE)

Establishment of a cryobank. Actions:

- acquisition of equipments and establishment of a cryobank at the "Museo Naturalistico Francesco Minà Palumbo in Castelbuono (CNR-IBE, collaboration EPM)
- selection and conservation of embryonic axes, pollen and SE callus lines from *A. nebrodensis* trees (CNR-IBE, collaboration UNIPA, EPM)







## ACTION "C5"

### **DELIVERABLES**

07/2021

Report on the seed- and cryobank constitution

12/2021

Report on a complete protocol of SE and cryopreservation of proembryonic masses

04/2022

E-manual on website, containing practical information on the constitution and management of seed- and cryobanks

01/2023

Database of the pollen/excised embryos/somatic embryogenesis samples in cryobanks

03/2023

Database of the seed samples in the cryobank



### **MILESTONES**

05/2020 - Constitution of the structures for the seed bank and cryobank (CNR-IBE & EPM)

05/2021 - Workshop at the University of Palermo on the production and cryopreservation of SE cell lines (UNIPA, collaboration CNR-IBE)

06/2021 - Optimization of the best conservation conditions of seeds at low temperature (CNR-IBE)

11/2021 - Cryopreservation of pollen samples (CNR-IBE)

12/2021 - Optimization of the best cryopreservation protocol for SE cell lines and embryonic axes (CNR-IBE)

11/2022 - Conservation of seeds in the seed bank (EPM and CNR-IBE)

03/2023 - Conservation of the SE cell lines in the cryobank (CNR-IBE, collaboration EPM, UNIPA)

**Action C5 - WHO is involved?** CNR-IBE ↔ UNIPA ↔ EPM



## ACTIONS "C7"

*Replication. Implementation action dedicated to the results' transfer and replication during the project*

### **DELIVERABLES**

03/2022

Best Practice Handbook

09/2022

Replication Plan

### **MILESTONES**

10/2021

Replication kick-off meeting

10/2022

Replication event (???) in Seville

**Action C7 - WHO is involved?**

**All Partners !**





## Summary of scheduled DELIVERABLES:

### 12/2020

1. Report on protocol for pollen cryopreservation (A1.4)
2. Report on protocols for seed and grafting propagation (A1.4)
3. Report on protocols for (i) seed conservation at low temperature, (ii) excised ZE at  $-196^{\circ}\text{C}$ , (iii) pollen at  $-196^{\circ}\text{C}$ , SE callus lines at  $-196^{\circ}\text{C}$  (A1.4)

### 07/2021

Report on the seed- and cryobank constitution (C5)

### 12/2021

Report on a complete protocol of SE and cryopreservation of proembryonic masses (C5)

### 04/2022

E-manual on website, containing practical information on the constitution and management of seed- and cryobanks (C5)

### 01/2023

Database of the pollen/excised embryos/somatic embryogenesis samples in cryobanks (C5)

### 03/2023

Database of the seed samples in the cryobank (C5)

### 06/2023

Report on the effective production of mycorrhizal *A. nebrodensis* seedlings and on the improved health status of the seedlings of the local nursery of 'Vivaio Piano Noce' (C4)



## Summary of scheduled MILESTONES:

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Viable seeds selection with the ballistic machinery (C4)

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Constitution of the structures for the seed bank and cryobank (C5)

06/2020

Collection of the male flowers to obtain pollen and cones to obtain immature ZE for SE (A1.4)

10/2020

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12/2021

Optimization of the best cryopreservation protocol for SE cell lines and embryonic axes (C5)

11/2022

Conservation of seeds in the seed bank (C5)

03/2023

Conservation of the SE cell lines in the cryobank (C5)



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*THANK YOU*  
*GRACIAS*  
*BACIAMO LE MANI, AH!*

**Maurizio Lambardi – Carla Benelli**