



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

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



# **CIRITA-UNIPA**

## ***Role and Responsibilities***

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Anna Geraci

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## Azioni in cui è coinvolto il CIRITA-UNIPA:

### 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 (CNR-IPSP)
- **C4** – Nursery production of improved seedlings of *A. nebrodensis* (CNR-IPSP)
- **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* (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 (CNR)

### Monitoring actions – ACTIONS "D" (D2) (CNR)

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

### Project management – ACTION "F" (F2)(CNR)

## Sub-actions in which the CIRITA is involved:

### ACTIONS "A"



**A1.3** – Set of protocols to investigate biotic and abiotic stresses to seedlings in the nursery

### ACTIONS "C"

**C1.4** – Permanent ground monitoring of the health status of *A. nebrodensis* relic population, mitigation of biotic and abiotic stresses (with special attention to invasive species).



**C2.1** – Enhancement of the genetic diversity of the natural population: promoting the outbreeding through manual cross-pollination



**C2.2** – Selection of outbred seedlings derived from intraspecific crosses to be used in reforestation



**C3** – Establishment of a new clonal orchard for germplasm collection and to boost the genetic variability of the progeny



**C4.2 - Mycorrhization** of *A. nebrodensis* seedlings.



**C4.4** – Application of proper procedures for the control of biotic and abiotic disorders in the nursery

**C7** - Implementation action dedicated to the results' transfer and replication during the project



## ACTION "A1"



**A1.3** – Set of protocols to investigate biotic and abiotic stresses to seedlings in the nursery

- A high mortality of seedlings of *A. nebrodensis* has been observed in the 'Vivaio Piano Noce' nursery.



Foto. 68 - Preparazione della marza a becco di clarino.



**DELIVERABLES** (in charge to CIRITA):

12/2020 - Report on optimized protocols for the reproduction of *A. nebrodensis* trees by seed and grafting propagation



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



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

Excised zygotic embryos: (i) collection of seed samples from selected elite trees at full maturity; (ii) decontamination, 4°C chilling, and zygotic embryo excision under aseptic conditions; (iii) application and comparison of various cryopreservation procedures; (iv) immersion of embryos in LN; (v) rewarming and in vitro germination.

Embryogenic callus lines: isolation of small SE callus clumps, followed by the application of a slow cooling procedure. Rewarmed callus samples will be then plated on proliferation medium and evaluated for characterizing the best cryopreservation procedure.

### **DELIVERABLES** (in charge to CIRITA):

12/2020 - Report of a complete protocol of long-term conservation of *A. nebrodensis* pollens at ultra-low (i.e., cryogenic) temperature

**Action A1 - WHO is involved? = collaborations among partners**

**US** ↔ CNR (IPSP+IBE) ↔ **CIRITA-UNIPA** ↔ DRSRT ↔ EPM



## ***ACTION "C1"***

**C1.4** – Permanent ground monitoring of the health status of *A. nebrodensis* relic population, **mitigation of biotic and abiotic stresses** (with special attention to invasive species).

- **Census and mapping of *Abies nebrodensis* natural regeneration**
- **Collection of dendro-auxometric parameters**

**DELIVERABLES** (in charge to CIRITA-UNIPA):

04/2020 - Report: dendro-auxometric parameters of the trees of the natural population

06/2020 - Report: updated census and mapping of the natural regeneration of *A. nebrodensis*

**Action C1 - WHO is involved?** = **collaborations among partners**

**Beneficiary responsible for implementation:**

**CIRITA-UNIPA**

Collaboration with CNR (IPSP) ↔ DRSRT ↔ EPM





**ACTION "C2"**

**C2.1** – Enhancement of the genetic diversity of the natural population: promoting the outbreeding through *manual cross-pollination*



**C2.3** – Identification and removal of the natural regeneration of alien firs (*Abies cephalonica* and *A. alba*) in reforested areas of the Madonie.



Both the most genetically distant reproductive and the higher outcrossing trees will be identified and a plan of controlled crosses to obtain a good amount of vigorous outcross seeds will be realized.

- Isolation of female cones before pollen dispersal, using special bags to prevent the penetration of pollen grains from outside; Pollen collection and storage from mature trees; Hand pollination between different sexually mature individuals to maximize the number of different combinations between non-relatives



- Phenotypical selection of resulting most vigorous seedlings.



**ACTION "C2"****DELIVERABLES :**

09/2020 - Report: Distribution of natural regeneration of exotic Abies (including a map)

- 09/2023 Recommend actions for conservation programs

**Action C1 - WHO is involved?** **US** ↔ CIRITA-UNIPA ↔ CNR (IPSP)





## ACTION "C3"



**C3** – Establishment of a new clonal orchard for germplasm collection and to boost the genetic variability of the progeny

Establishment of a clonal orchard: clonal plants (ramets) obtained by grafting propagation of the 30 mother plants; 12 replicates of each of the 30 mother plants (ortet), 5 x 5 m spacing (400 trees/ha) to favor the crossing in all possible combinations among the represented genotypes

Surface size: 1.50 ha, protected with a 2 m high fence (partially buried) against damage due to wild animals

**DELIVERABLES** (in charge to IPSP):

04/2023 - Report and map of the newly constituted clonal orchard

**Action C3 - WHO is involved?**

**CNR (IPSP)** ↔ CIRITA-UNIPA

DRSRT ↔ EPM



## ACTION "C4"



### **C4.2** – C4.2 Mycorrhization of *A. nebrodensis* seedlings.



This action will be started in the second year of the project. The most important phases are: a) Preparation of seedlings for artificial mycorrhization, b) inoculation of seedlings with selected ECM (ectomycorrhizal) following the protocol developed in the previous LIFE project, c) periodical observation of the ECM evolution in the seedling roots, d) production of the seedlings for re-planting in the natural habitat (Madonie Mountains).

### **C4.4** – Application of proper procedures for the control of biotic and abiotic disorders in the nursery

Based on A1 (Integrated Pest Management approach):

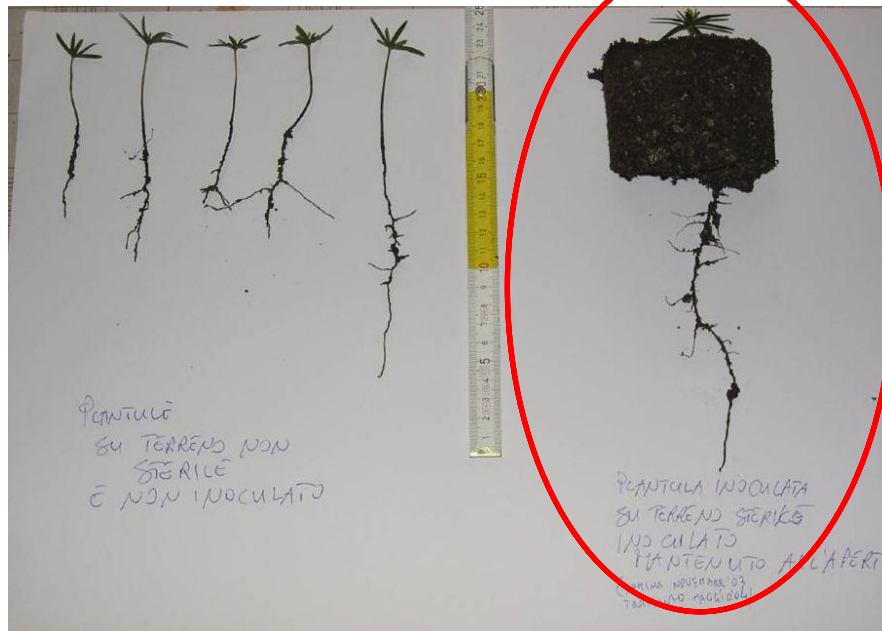
(a) Prevention: Continual monitoring, early diagnosis, to prevent the entry and/or spread of pathogens in the nursery (exclusion).

### **DELIVERABLES** (in charge to IPSP):

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'

### **Action C4 - WHO in involved?**

**CNR (IPSP+IBE)** ↔ CIRITA-UNIPA ↔ DRSRT



## ***C4.2 – Mycorrhization of *A. nebrodensis* seedlings***





## ACTIONS "C"

**C7** - Implementation action dedicated to the results' transfer and replication during the project

Creation of a "replicable model" by means of:

- **Best Practice Handbook** to share technical knowledge with replication partners
- **Replication plan** will detail the technical, economic and social factors in order to replicate the initiative of safeguard carried out with *A. nebrodensis*

The replication partners will be identified and involved through an international network of direct users/stakeholders interested to the experiences and outcomes of the project (e.g. authorities and technicians of Parks, scientists, researchers, foresters, institutions responsible for Parks protection). Some aspect of the project could be replicated to *Abies pinsapo* (in Spain), *Abies borisii-regis* (between Bulgaria and Greece), *Pinus heldreichii* subsp. *heldreichii* (Calabria, Italy), relict populations of *Tetraclinis articulata* (in Spain and Malta).

Dissemination of practices will be based on 2 Replication promotion Events (one kick-off meeting in Palermo in October 2021 and a second event in Seville in the second half of 2022)

### **DELIVERABLES:**

03/2022 - Best Practice Handbook  
09/2022 - Replication Plan

**Action C4 - WHO is involved?**

**All Partners !**

***ACTION C.5:***

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

In the frame of LIFE4FIR, a seed bank and a cryobank will be established for the conservation of tissue and organs from *A. nebrodensis* germplasm. The two gene banks will be established inside building already existing at the host Institution ("Museo Naturalistico Francesco Minà Palumbo" in Castelbuono), in the period June-December, 2020.

***Action C5 - WHO is involved?***

**CNR** ↔ EPM ↔ CIRITA-UNIPA



## ACTIONS "C"

**C 7** - Implementation action dedicated to the results' transfer and replication during the project

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**Action C4 - WHO is involved?**

**All Partners !**



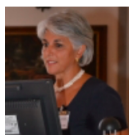




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