



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

Global review of actions and deliverables

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'Decisive in situ and ex situ conservation strategies to secure the critically endangered Sicilian fir, *Abies nebrodensis*' Lojac.

Duration: 4 years (1.08.2019 – 31.07.2023)

Partners

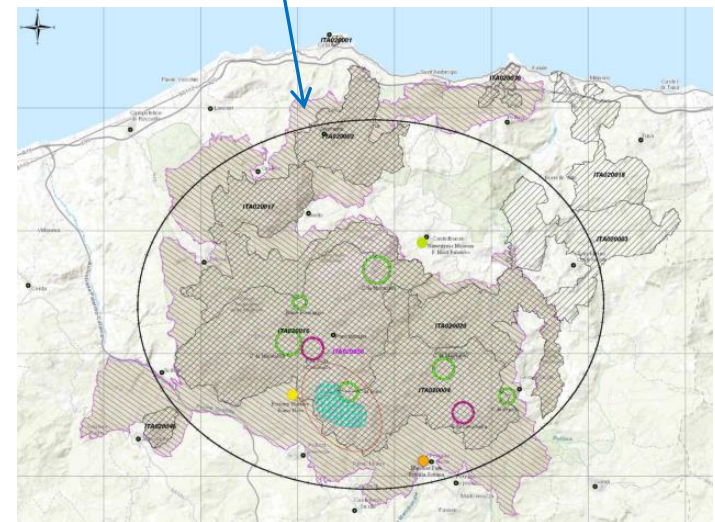
1. CNR: Consiglio Nazionale Delle Ricerche (2 institutes: IPSP and IBE), Sesto Fiorentino, Italy;
2. EPM: Ente Parco delle Madonie, Italy;
3. CIRITA – UNIPA: Centro Interdipartimentale Di Ricerca Sull'interazione Tecnologia-Ambiente - Università Di Palermo, Italy;
4. DRSRT: Assessorato Regionale dell'Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea Dipartimento Regionale dello Sviluppo Rurale e Territoriale, Italy;
5. US: University of Seville (Spain).

Abies nebrodensis is a critically endangered species (IUCN red list), endemic to the north-central part of Sicily.

- Only 30 relic adult trees;
- 165 seedlings of the natural regeneration.

Protection status under European legislation:

- Habitat: 'Apennine beech forests with *Abies alba* and beech forests with *A. nebrodensis* ' is included in the Habitat Directive (Habitat of Community Interest - code 9220*)
- Located in the Madonie Park (Natura 2000, SPA ITA 020050)
- Part of the Natura 2000 SCI ITA 020004



A. nebrodensis: current threats and vulnerability

- Strong genetic erosion;
- Fragmentation;
- Purported self-fertilization;
- Possible hybridization with alien firs;
- Poor natural regeneration;
- High mortality rate in the nursery.



and

- Localized soil erosion;
- Poor and rocky soils;
- Grazing:
 - By wild herbivores
 - By abandoned cattle and goats





Main purpose of the project

Increase (and boost) the genetic diversity of *A. nebrodensis* and improve its state of conservation.

Through implementation of the following technical activities:

- 1. Sustain and protection of the relic population, control of biotic, abiotic and anthropic disturbances (C1)**

By

new system of fences, video surveillance, bioengineering, monitoring of vegetation processes (drone), regular surveys to control pathogens and pests and abiotic stresses.

2. Increase the biodiversity of the progenies (A1, C2, C3)

By

Evaluation of the genetic relatedness among the mature trees, controlled crosses and selection of pure outbred seedlings through paternity tests; implementation of a clonal orchard.

3. Improvement of the growing conditions of seedlings in the nursery (C4)

By

adopting effective techniques to select viable seed, to obtain mycorrhizal seedlings, to control biotic and abiotic disturbances.

Both these activities will led to an increase of the vigour and fitness of saplings that will be used in reforestation.





4. Replanting interventions (C6)

Using 4500 improved seedlings raised in the nursery, in 10 plots suited for reintroduction of *A. nebrodensis*, to create re-diffusion cores.

5. Ex situ conservation (C5)

Ex situ conservation by the development of a highly-efficient propagation technique (somatic embryogenesis), as well as the implementation of a seedbank and a cryobank for the long-term conservation of seeds, pollen, isolated embryos and embryogenic callus lines.

6. Sustain the activities after the end of the project (C7 and F3)

Most actions implemented in the course of the project will need to be continued after its end (After-life plan).



List of the proposed actions

A. Preparatory actions, elaboration of management plans and/or of action plans

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.

A1.1 Evaluation of genetic diversity of adult plants and natural regeneration.

A1.2 Genetic characterization of seedlings from the local nursery 'Vivaio Piano Noce' to select intraspecific crosses.

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

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

Responsible: US

Other partners: CNR – IBE, CNR – IPSP, CIRITA – UNIPA, DRSRT, EPM





C. Conservation actions

C1 Support and preserve *Abies nebrodensis* in its natural habitat

C1.1 Installation of a new larger and more **functional protective fence** around groups of plants of the natural population to better protect the natural regeneration.

C1.2 Setting up an **electric fence**.

C1.3 Installation of 5 **camera system** with motion sensor, powered by photovoltaic panel, as grazing prevention of wild herbivore and of abandoned cattles and goats.

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

C1.5 Spatial and 'health' analysis of *A. nebrodensis* natural population using **drone technology**.

C1.6 Erosion protection of *A. nebrodensis* individuals: environmental engineering activity.

Responsible: CIRITA-UNIPA; partners: CNR-IPSP, EPM, DRSRT





C2 Conservation of genetic purity of *Abies nebrodensis* and improvement of its genetic diversity.

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.

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

Responsible: US; partners: CIRITA-UNIPA, CNR-IPSP





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

Responsible: CNR-IPSP; partners: CIRITA-UNIPA, EPS, DRSRT



C4 Nursery production of improved seedlings of *Abies nebrodensis*

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.

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

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.

C4.4 Application of proper procedures for the **control of biotic and abiotic disorders in the nursery**, according to what emerged in the action A1.

Responsible: CNR-IPSP; partners: CIRITA-UNIPA, DRSRT, CNR-IBE





C5 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*.

Responsible: CNR-IBE; partners: CIRITA-UNIPA, EPM



C6 Reforestation with *A. nebrodensis* (4000 improved seedlings) in 10 plots (new diffusion cores) in the Madonie Park in suitable areas for the reintroduction applying innovative planting techniques.

Responsible: CIRITA-UNIPA; partners: EPM, DRSRT



C7 Replication. Implementation action dedicated to the results' transfer and replication during the project

Responsible: CNR; Partners: (All) CIRITA-UNIPA, US, EPM, DRSRT





D. Monitoring of the impact of the project actions (obligatory)

D1 Monitoring of socio-economic assessment and ecosystem services and conditions of LIFE4FIR Project

D1.1 Socio-economic impact

D1.2 Ecosystem services and conditions impact

D1.3 Recommendations to ensure replicability and transferability

Responsible: CNR

D2 LIFE4FIR Impact in *A. nebrodensis* conservation

Responsible: CNR; partner CIRITA-UNIPA



D3 LIFE4FIR (KPI) Performance indicators

Responsible: CNR

E. Public awareness and dissemination of results (obligatory)

Responsible: CNR; Partners: All



F. Project management (obligatory)

F1 Project management by CNR

F2 Life 4Fir Audit

F3 LIFE4FIR After-LIFE plan

Responsible CNR; Partners: All





Deliverables



A1's PROJECT DELIVERABLE PRODUCTS

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. **Responsible: US; partners: CNR-IBE, CNR-IPSP, CIRITA-UNIPA, DRSRT, EPM**

Deliverable name	Deadline
Report on optimized protocols for the reproduction of <i>A. nebrodensis</i> trees by seed and grafting propagation	12/2020
Report of a complete protocol for <i>A. nebrodensis</i> seed and excised zygotic embryo conservation at low (-18°C) and cryogenic (-196°C) temperatures, respectively	12/2020
Samples will be deposited in the DNA-Bank of the University of Seville (Spain)	12/2020
Protocol for disease of <i>A. nebrodensis</i> identification in nursery	07/2020
Report on the genetic variability of the <i>A. nebrodensis</i> population and related results	12/2020
Report of a complete protocol of long-term conservation of <i>A. nebrodensis</i> pollens at ultra-low (i.e., cryogenic) temperature	12/2020



C1's PROJECT DELIVERABLE PRODUCTS

C1 Support and preserve *Abies nebrodensis* in its natural habitat

Responsible: CIRITA-UNIPA; partners: CNR-IPSP, EPM, DRSRT

	Deliverable name	Deadline
➡	Report of both fences installation including the surveillance system	06/2020
	Report of actions related to control and prevention of native and invasive pests and pathogens	06/2023
	Map of final <i>A. nebrodensis</i> population and habitat video/hyperspectral inventory and 'health' state	09/2023
➡	Map of initial <i>A. nebrodensis</i> population and habitat; video/hyperspectral inventory and 'health' state	03/2020
➡	Report: Dendro-auxometric parameters of the trees of the natural population of <i>A. nebrodensis</i>	04/2020
➡	Report: updated census and mapping of the natural regeneration of <i>A. nebrodensis</i>	06/2020
	Report: Description of major potential diseases, pests and their antagonists	08/2021



C2's PROJECT DELIVERABLE PRODUCTS

C2 Conservation of genetic purity of *Abies nebrodensis* and improvement of its genetic diversity. **Responsible: US; partners: CIRITA-UNIPA, CNR-IPSP**

Deliverable name	Deadline
Recommend actions for conservation programs	09/2023
Report: Implemented procedure to quickly determine the genetic origin of seedlings. Dissemination our results to Natural Park Managers at the end of the Project	09/2022
Report: Distribution of natural regeneration of exotic <i>Abies</i> (including a map)	09/2020
List of the seedlings growing in nursery indicating their genetic origin will be delivered	09/2021
Molecular data derived from this project will be exploited by the participants and will be deposited in the free-access public genetic databases (e.g. GenBank)	10/2021
List of all the hybrid seedlings to be eliminated	10/2022



C3's PROJECT DELIVERABLE PRODUCTS

C3 Establishment of a new clonal orchard for germplasm collection and to boost the genetic variability of the progeny. **Responsible: CNR-IPSP;**
partners: CIRITA-UNIPA, EPS, DRSRT

Deliverable name	Deadline
Report and map of the newly constituted clonal orchard	04/2023



C4's PROJECT DELIVERABLE PRODUCTS

C4 Nursery production of improved seedlings of *Abies nebrodensis*

Responsible: CNR-IPSP; partners: CIRITA-UNIPA, DRSRT

Deliverable name	Deadline
Report on the effective production of mycorrhizal <i>A. nebrodensis</i> seedlings and on the improved health status of the seedlings of the local nursery of 'Vivaio Piano Noce'.	06/2023



C5's PROJECT DELIVERABLE PRODUCTS

C5 Constitution of a seedbank and a cryobank for the long-term conservation of seeds, pollen, isolated embryos and embryogenic callus lines of *Abies nebrodensis*. **Responsible: CNR-IBE; partners: CIRITA-UNIPA, EPM**

Deliverable name	Deadline
Report on the seed- and cryobanks constitution	07/2021
Database of the pollen/excised embryos/somatic embryogenesis samples in the cryobanks reported in the website	01/2023
E-manual on the website for downloading, containing practical information on the constitution and management of seed and cryobank for the long-term conservation of fir genetic resources	04/2022
Report of a complete protocol of somatic embryogenesis and cryopreservation of proembryonic masses of <i>A. nebrodensis</i>	12/2021
Database of the seed samples in the bank reported in the website	03/2023



C6's PROJECT DELIVERABLE PRODUCTS

C6 Reforestation with *A. nebrodensis* (4000 improved seedlings) in 10 plots (new diffusion cores) in the Madonie Park in suitable areas for the reintroduction applying innovative planting techniques.

Responsible: CIRITA-UNIPA; partners: EPM, DRSRT

Deliverable name	Deadline
List of reforested plots explaining the characteristics of each areas, plantingtechnics adopted including maps of the reforestation in GIS	09/2020



C7's PROJECT DELIVERABLE PRODUCTS

C7 Replication. Implementation action dedicated to the results' transfer and replication during the project. **Responsible: CNR-IPSP;**
Partners: (All) CIRITA-UNIPA, US, EPM, DRSRT

Deliverable name	Deadline
Best Practice Handbook	03/2022
Replication Plan	09/2022



D1 and D2 PROJECT DELIVERABLE PRODUCTS

D1 Monitoring of socio-economic assessment and ecosystem services and conditions of LIFE4FIR Project. **Responsible: CNR-IPSP, partners: all**

Deliverable name	Deadline
Report on the socio-economic impact of LIFE4FIR project	07/2023
Report on the LIFE4FIR impact on ecosystem services and conditions	07/2023

D2 LIFE4FIR impact in A. nebrodensis conservation
Responsible: CNR, partners: all

Deliverable name	Deadline
Impact in A. nebrodensis conservation report	07/2023



Focus immediate actions

LIST OF DELIVERABLE PRODUCTS OF THE PROJECT

Name of the Deliverable	Number of the associated action	Who (partners involved)	Deadline
▪ Map of initial A. nebrodensis population and habitat video/hyperspectral inventory and 'health' state	C 1	CIRITA-UNIPA, CNR-IPSP, EPM, DRSRT	31/03/2020
▪ Report: Dendro-auxometric parameters of the trees of the natural population of A. nebrodensis	C 1	CIRITA-UNIPA, CNR-IPSP, EPM, DRSRT	30/04/2020
▪ Report of both fences installation including the surveillance system	C 1	CIRITA-UNIPA, CNR-IPSP, EPM, DRSRT	30/06/2020
▪ Report: updated census and mapping of the natural regeneration of A. nebrodensis	C 1	CIRITA-UNIPA, CNR-IPSP, EPM, DRSRT	30/06/2020





Focus immediate actions

LIST OF DELIVERABLE PRODUCTS OF THE PROJECT

Name of the Deliverable	Number of the associated action	Who (partners involved)	Deadline
<ul style="list-style-type: none"> Protocol for disease of A. nebrodensis identification in nursery 	A 1	US, CNR – IBE, CNR – IPSP, CIRITA – UNIPA, DRSRT, EPM	31/07/2020
<ul style="list-style-type: none"> List of reforested plots explaining the characteristics of each areas, planting techniques adopted including maps of the reforestation in GIS 	C 6	CIRITA-UNIPA, EPM, DRSRT	30/09/2020
<ul style="list-style-type: none"> Report: Distribution of natural regeneration of exotic Abies (including a map) 	C 2	US, CIRITA-UNIPA, CNR-IPSP	30/09/2020





LIST OF DELIVERABLE PRODUCTS OF THE PROJECT

Name of the Deliverable	Number of the associated action	Who (partners involved)	Deadline
▪ Report of a complete protocol for A. nebrodensis seed and excised zygotic embryo conservation at low (-18°C) and cryogenic (-196°C) temperatures, respectively	A 1	US, CNR – IBE, CNR – IPSP, CIRITA – UNIPA, DRSRT, EPM	31/12/2020
▪ Report of a complete protocol of long-term conservation of A. nebrodensis pollens at ultra- low (i.e., cryogenic) temperature	A 1	US, CNR – IBE, CNR – IPSP, CIRITA – UNIPA, DRSRT, EPM	31/12/2020
▪ Report on optimized protocols for the reproduction of A. nebrodensis trees by seed and grafting propagation	A 1	US, CNR – IBE, CNR – IPSP, CIRITA – UNIPA, DRSRT, EPM	31/12/2020
▪ Report on the genetic variability of the A. nebrodensis population and related results	A 1	US, CNR – IBE, CNR – IPSP, CIRITA – UNIPA, DRSRT, EPM	31/12/2020
▪ Samples will be deposited in the DNA-Bank of the University of Seville (Spain)	A 1	US, CNR – IBE, CNR – IPSP, CIRITA – UNIPA, DRSRT, EPM	31/12/2020



Focus immediate actions

MILESTONES OF THE PROJECT

Name of the Milestone	Number of the associated action	Deadline
LIFE4FIR website	E 1	31/10/2019
<ul style="list-style-type: none"> Beginning of the action: Collection of samples (seeds and scions) 	A 1	31/12/2019
<ul style="list-style-type: none"> Definition of the monitoring criteria and preparation of proper evaluation spread sheet for the field activity. Start of the field monitoring (C1.4) 	C 1	31/12/2019
<ul style="list-style-type: none"> Nomination of the team of expert involved in the action and scheduling of the field monitoring activity (C1.4) 	C 1	31/12/2019
<ul style="list-style-type: none"> Start of the elimination of the natural regeneration of exotic firs (C2.3) 	C 2	31/03/2020
<ul style="list-style-type: none"> Viable seeds selection with the ballistic machinery 	C 4	31/03/2020
<ul style="list-style-type: none"> Beginning of the nursery production of grafted plants of <i>A. nebrodensis</i> following optimized protocol developed during the preparatory action A1 	C 4	30/04/2020
<ul style="list-style-type: none"> Hand-pollinations between genetically distant trees to obtain high quality seeds (C2.1) 	C 2	30/04/2020
<ul style="list-style-type: none"> Installation of the new protective fence around groups of plants of the natural population (including natural regeneration) (C1.1) 	C 1	30/04/2020
<ul style="list-style-type: none"> Setting up of an additional electric fence (24V) (C1.2) 	C 1	30/04/2020





Focus immediate actions

MILESTONES OF THE PROJECT

Constitution of the structures for the seed bank and the cryobank (i.e., designation of the rooms / spaces dedicated to aforesaid banks, assurances of the continuous energy and liquid nitrogen supply)	C 5	31/05/2020
End of the census of exotic firs (C2.3)	C 2	31/05/2020
Collection of the male flowers to obtain pollens and cones to obtain immature zygotic embryos for SE	A 1	30/06/2020
Installation of a camera system with a motion sensor as deterrent to vandalism and abusive grazing (C1.3)	C 1	30/06/2020