**Project LIFE18 NAT/IT/164 - LIFE4FIR**

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

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

# *Nov 2024 meeting report of the project*


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- José Carlos **DEL VALLE**
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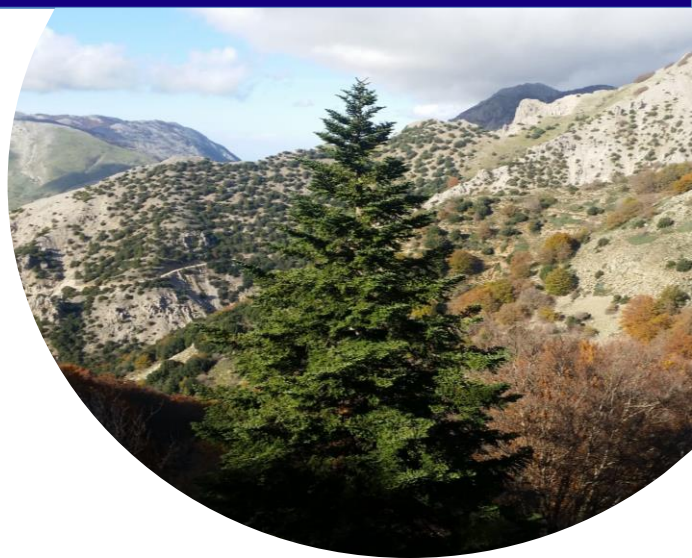

# Our contribution to this project

## A. PREPARATORY ACTIONS

A.1. 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

## C. CONSERVATION ACTIONS

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

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

## E. PUBLIC AWARENESS AND DISSEMINATION OF RESULTS

E2. Tourist visits, workshops, fairs, networking, Institutions and policy makers involvement, awareness rising

## F. PROJECT MANAGEMENT

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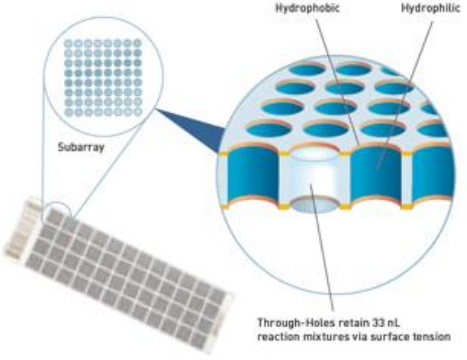
## ACTION A.1

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

1. Sampling and DNA extraction:
   - 30 adult trees + 118 young individuals from the natural population
   - Samples from A. cephalonica & A. alba previously collected
2. RAD-Seq to identify high-quality and information-rich **SNPs** for genotyping. RAD-Seq provided a dataset with 20,824 high-quality SNPs
3. Develop of a panel of 120 SNPs by PCR-based OpenArray Technology (proposed technique Illumina Veracode)
4. Genotyping to assess genetic diversity, infer of pedigree relationships, and detect hybridization with alien congeners

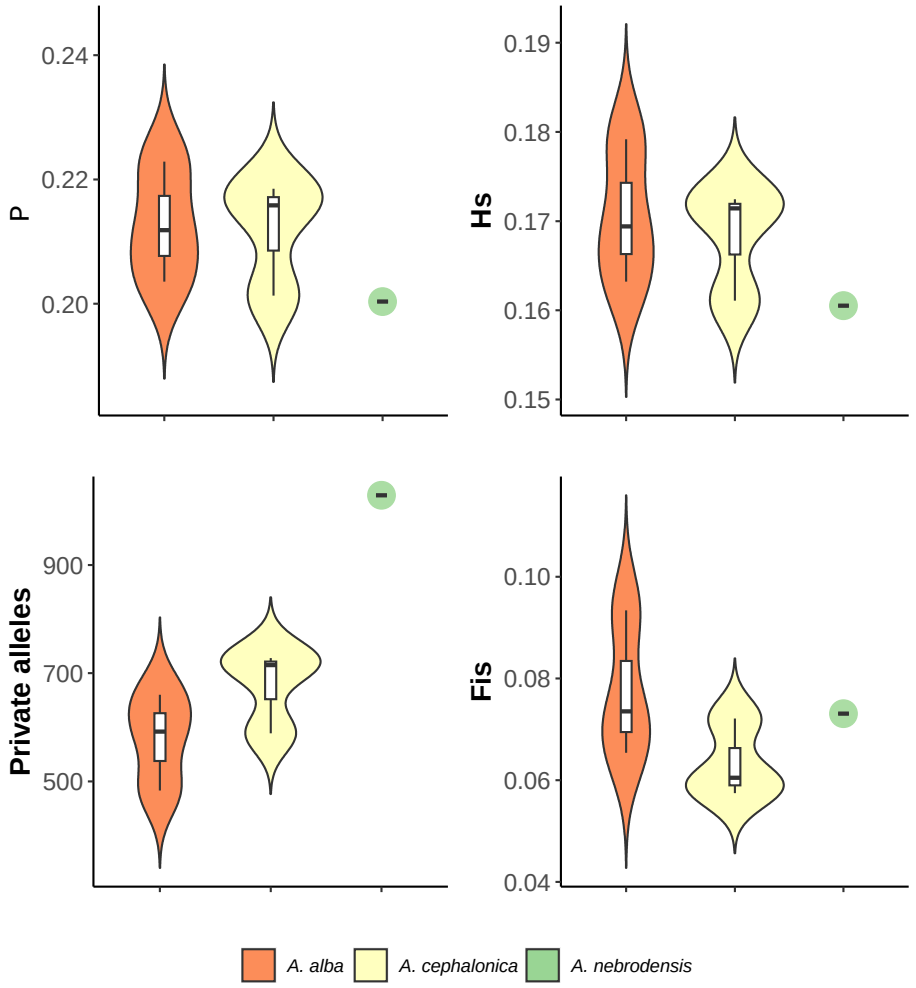



## ACTION A.1

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

Results from the genetic diversity analyses of the **30 adult-trees:**

- Nucleotide diversity ($\pi$), heterozygosity ($H_s$), inbreeding level ($F_{is}$) was notably consistent for *A. alba*, *A. cephalonica*, and *A. nebrodensis*. This latter displayed the highest number of private alleles




Raw available in the NCBI Short Reads Archive (BioProject ID PRJNA563575).

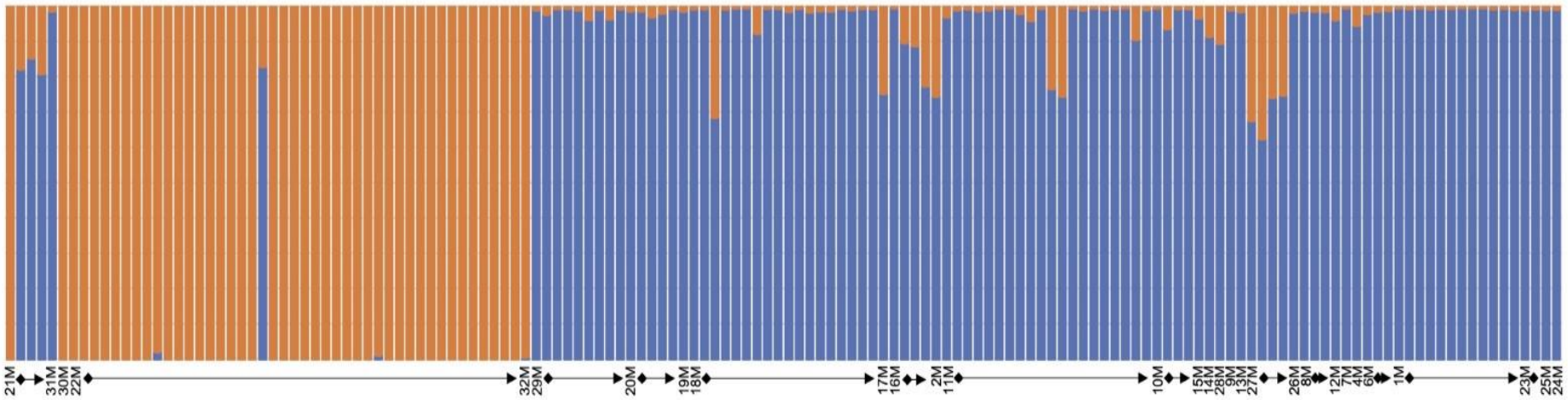
## ACTION A.1

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

Results from **population structuring**:

- Strong impact of **genetic drift** and **inbreeding** on the evolutionary dynamics of this population
- Low population genetic structuring
- Low effective population size ($N_e$ = 6) and moderate inbreeding ($F_{is}$ = 0.373)

Adults + Saplings Natural pop

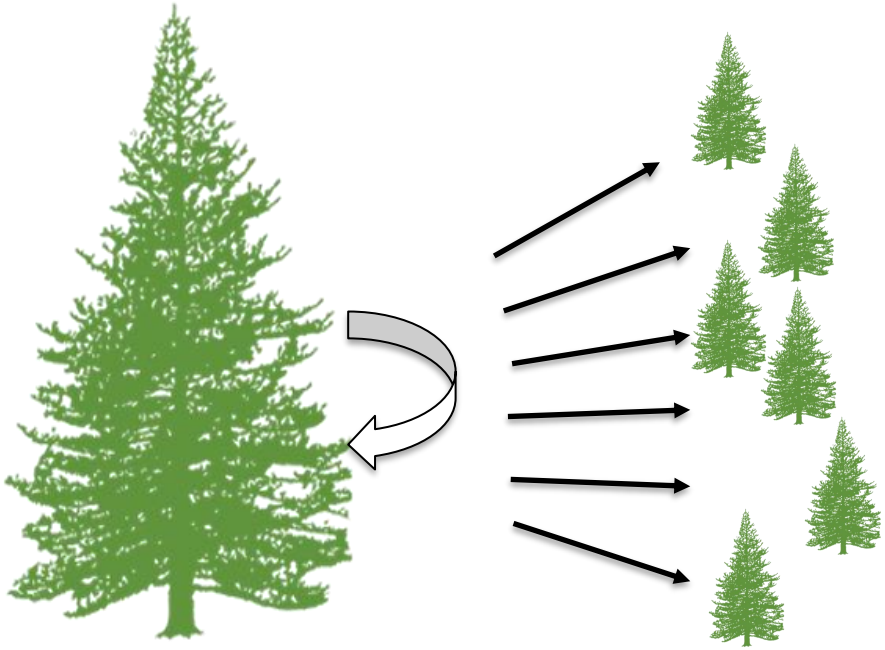


## ACTION A.1

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

Inferences of **pedigree relationships** of seedlings from the natural population:

- Most seedlings originated by **self-fertilization** (94.5% purebred *A. nebrodensis*). **Only 5.5% from outcrossing**
- **Nine** seedlings were putative hybrids (analyzed in the next slide)

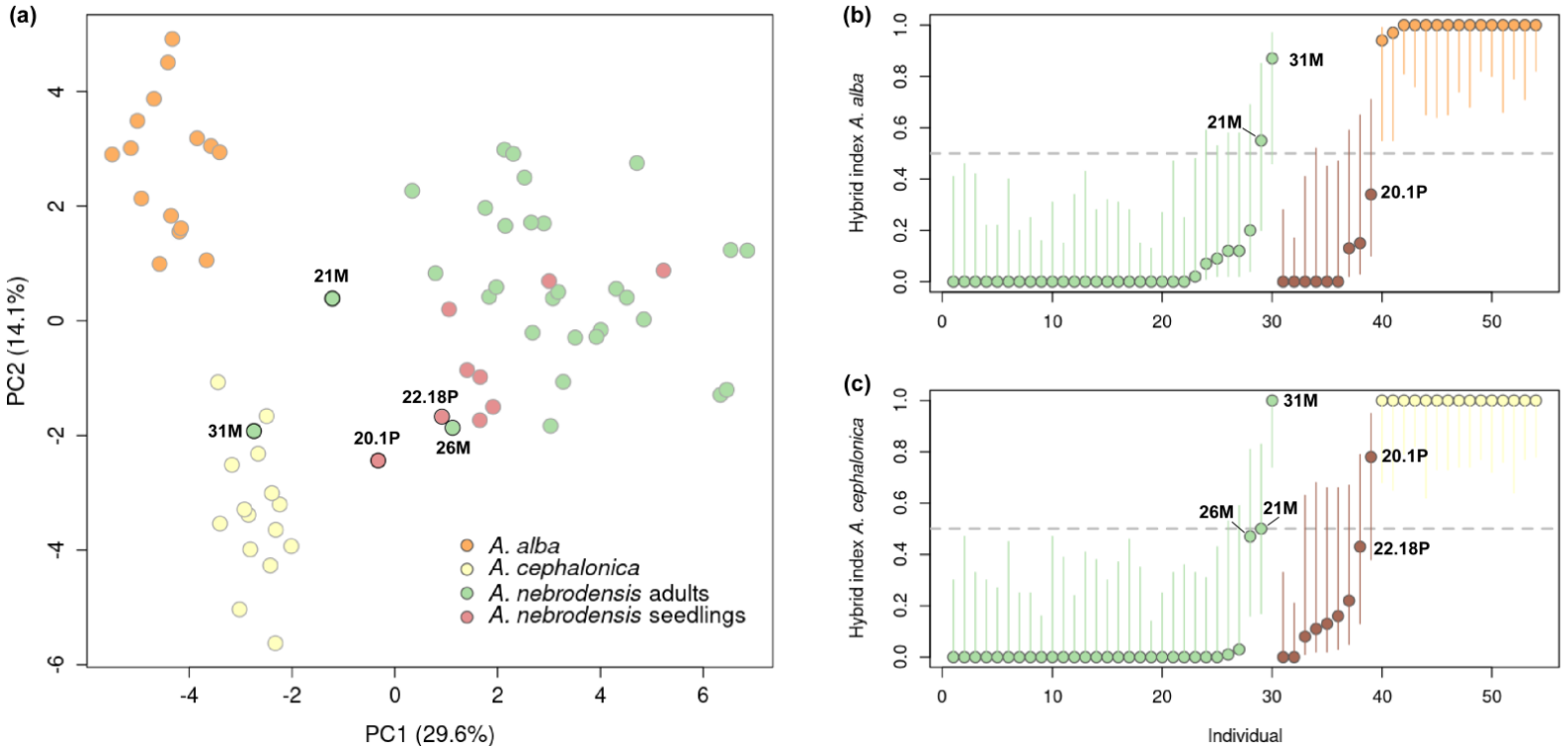


## ACTION A.1

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

Hybridization analyses:

- 31M is probably a Greek fir (*A. cephalonica*), 21M and 26M show signs of ancient introgression. Only two seedlings (20.1P and 22.10P) have a hybrid origin



## ACTION A.1

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

1. Sampling and DNA extraction:
   - 2064 seedlings

Pedigree relationships of seedlings from the **nursery**:

- Low effective population size (***$N_e$* = 12**) and moderate inbreeding (***$F_{is}$* = 0.354**)
- **897 (43%) purebred *A. nebrodensis*** seedlings with high autogamy rates (97.9%). **Only 19 seedlings resulted from outcrossing**
- **879 (42,5%)** seedlings were identified as **putative hybrid**s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### 2. LIST OF SEEDLINGS THAT ARE RECOMMENDED TO BE ELIMINATED

**Table 8.** List of seedlings growing at the nursery Piano Noce showing their genetic origin.

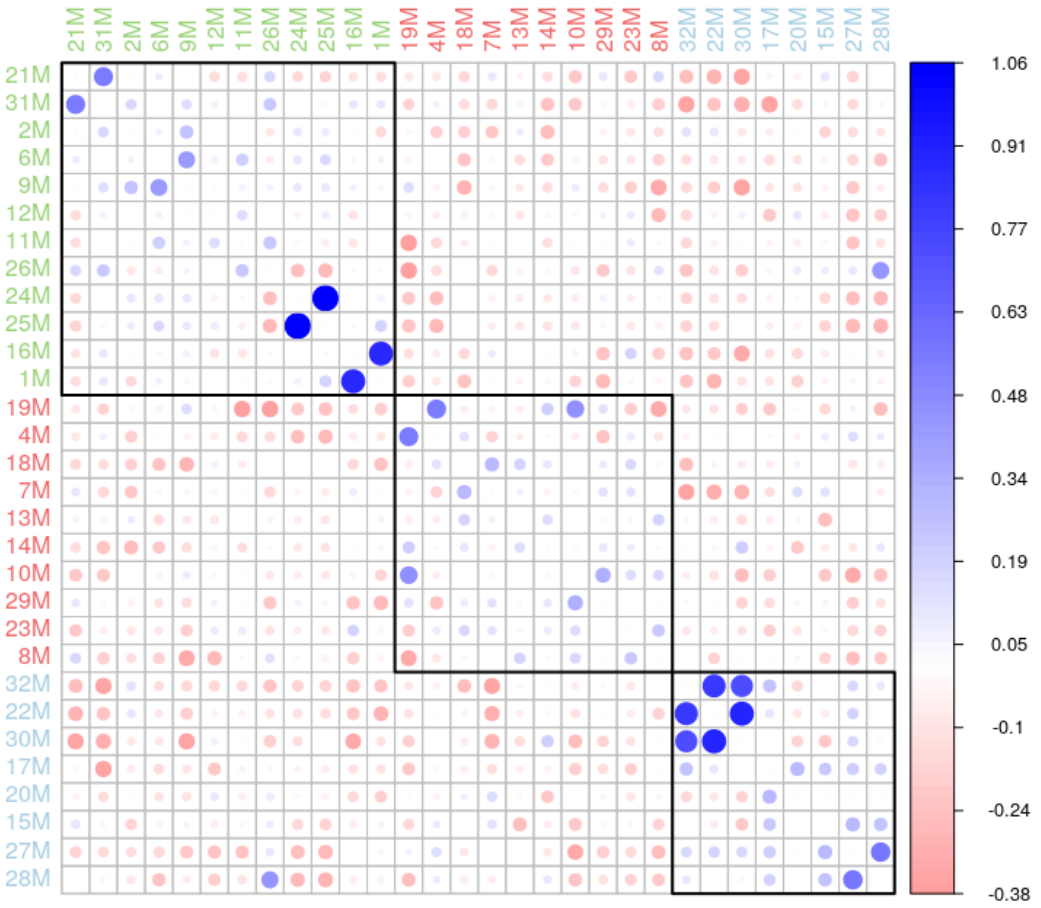
| Offspring ID | Pure *A. nebrodensis* (100%) | Conservation Action |
|---|---|---|
| 08_2013_0001 | Yes | |
| 08_2013_0002 | Yes | |
| 08_2013_0003 | Yes | |
| 08_2013_0004 | Yes | |
| 08_2013_0005 | Yes | |
| 08_2013_0006 | Yes | |
| 08_2013_0007 | Hybrid | ELIMINATE |
| 08_2013_0008 | Yes | |
| 08_2013_0009 | Hybrid | ELIMINATE |
| 08_2013_0010 | Yes | |
| 08_2013_0011 | Yes | |

## ACTION C2

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

**AGF**(assisted gene flow) **conservation program**

- Active management of *A. nebrodensis* population through outcrossing of those adults genetically more different to increase the genetic diversity



## ACTION C2

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

**Table 2.** List of 30 recommended crosses between mature adult trees of *A. nebrodensis* ordered by more distant co-ancestry and, therefore, more convenient crosses to increase genetic diversity. Plants with a suspicious origin were highlighted with red font. Please note some of the recommended outcrossing may involve non-reproductive individuals.

| | | |
|---|---|---|
| Cross 1 | 19M | 26M |
| Cross 2 | 11M | 19M |
| Cross 3 | 17M | 31M |
| Cross 4 | 9M | 30M |
| Cross 5 | 31M | 32M |

## ACTION C2

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

**3,600 seeds resulting from 24 manual cross-pollination**

- No relationship between phenotype and genetic diversity
- DNA extractions directly from embryos for pedigree analyses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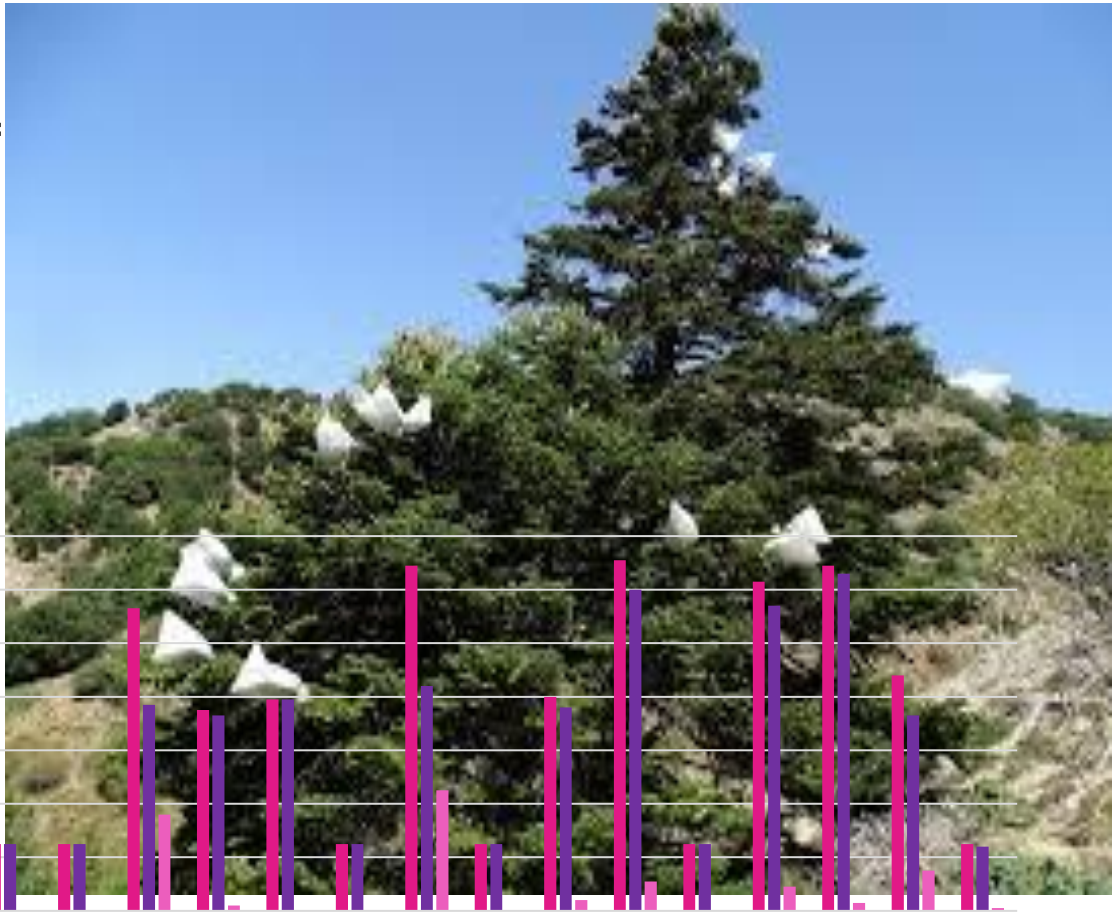
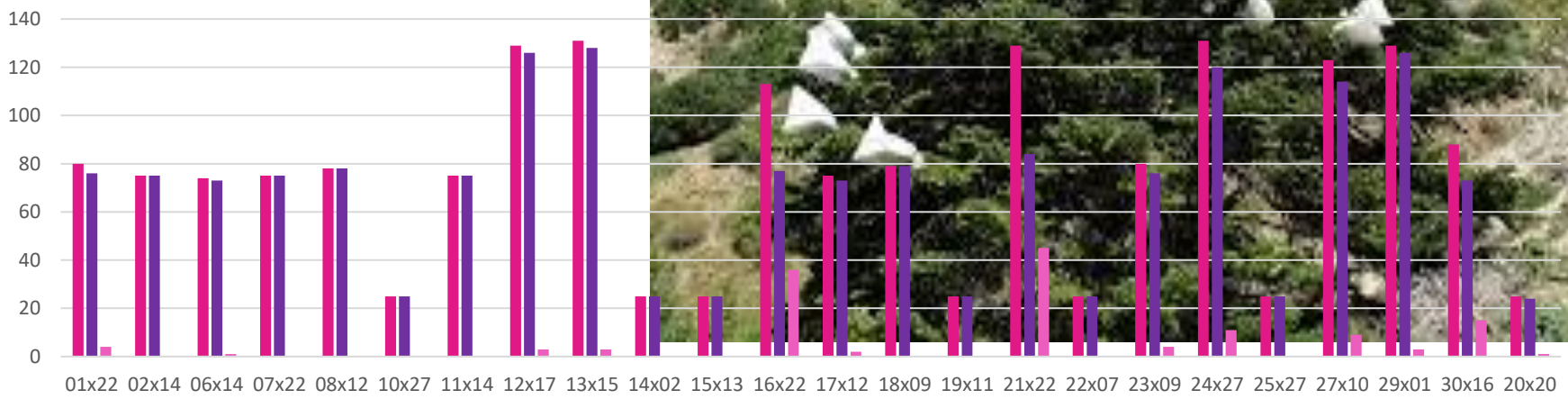
## ACTION C2

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

**Results of the genetic analysis verifying the proper execution of controlled crosses:**

- **86.1% are correctly executed**
- **13.9% are not, due to self-pollination**





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

**TECHNICAL AND MONITORING MEETING, AND REPLICATION EVENT**

| | |
|---|---|
| Date: | 7th November 2022 |
| Venue: | Ronda, Convento de Santo Domingo, C/ Armiñan 1 (Málaga) |
| Invited partners: | All partners are invited to attend this meeting |

**Attendees:**

**-Andalusian Government Biodiversity Conservation Officer**
**-Head of Abies pinsapo management and conservation**
**-Technical managers of Botanical Gardens**
**-Forestry agents assigned to the Spanish fir forests**

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**Articles:**

Preprint uploaded to **BioRxiv** titled: "*Genomic-guided conservation actions to restore the most endangered confiner in the Mediterranean Basin*" (doi: 10.1101/2023.11.24.568549).

Current status: **minor revision in Molecular Ecology** (Q1).



1 **Genomic-guided conservation actions to restore the most**
2 **endangered conifer in the Mediterranean Basin**

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4 Luis Ortiz[1], Francisco J. Jiménez-López[2], Anass Terrab[1], Francisco Balao[1]

bioRxiv
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## ACTION E

**E.2** Tourists visits, workshops, fairs, networking, Institutions and policy makers involvement, awareness rising

**Attendace at Scientific Meetings:**

- Development of SNP markers to monitor genetic relationship and hybridisation in natural population of *Abies nebrodensis*. **SESBE VII 2020** (Spain).
- Conservation genetics of the endangered Nebrodi fir: estimating the effective population size, inbreeding and hybridization. **SESBE IX 2022** (Spain)
- Conservation of Mediterranean forest species" organized within the dissemination activity of the project **LIFE4FIR** "Innovative strategies for *in situ* and *ex situ* conservation of *Abies*". Oral contribution. **2022** (Spain)
- Conservation genomics for challenging the extinction of Abies nebrodensis. Oral contribution. **SEBICOP 2023** (Spain)
- Genomic-guided conservation actions to restore the most endangered conifer in the Mediterranean Basin. Oral contribution. **IBC 2024** (Spain)

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# Grazie per la vostra attenzione