**LIFE4FIR – Project LIFE18 NAT/IT/000164**

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

## Report on 'Molecular data derived from this project will be exploited by the participants and will be deposited in the free-access public genetic databases'

**Action C2**


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## 1. Summary of the activity carried out in recent months for this action.

From the controlled hand-pollinations made on 2020 between *Abies nebrodensis* individuals from the natural population, we selected 10 crosses with a high number of seeds and 50-60 seeds/cross (Table 1). In *Abies*, it is common to find empty seeds, so in order to optimize the selection process of seeds with embryo, we proceeded to weigh all the seeds. We then sowed some seeds in Petri dishes to germinate them and obtain larger seedlings for DNA extraction. But after several weeks without success, we decided to extract the embryos directly from the seeds. Given the small size of the embryos, we needed several weeks to optimize the DNA extraction method. Once obtained, we sent the DNA for sequencing and are currently proceeding with the bioinformatics analysis.

Additionally**,** SNP sequences for *Abies nebrodensis* obtained in the action A.1 were uploaded to an open virtual repository.

## 2. Main results achieved

Ten crosses were selected (♀17x ♂24, ♀14x ♂10, ♀24x ♂14, ♀25x ♂13, ♀1x ♂13, ♀22x ♂24, ♀6x ♂2, ♀7x ♂17, ♀27x ♂1, ♀2x ♂1), for which we had a total of 522 seeds. After weighing all of them, we observed that only seeds larger than 0.05g had developed embryos (Fig. 1). Therefore, only 174 of the seeds had embryos and the number of embryos obtained from each cross was highly variable (Table 1). DNA extraction required the optimization of the process, where it was essential to use liquid nitrogen in the lysis step, and to increase the incubation times proposed in the extraction kit protocol (DNeasy Plant Minikit Qiagen). Finally, we successfully obtained DNA from 144 samples, in different proportions from each cross (Fig. 2), which has been sequenced using the 124 SNPs previously developed for *Abies nebrodensis* (Action A), and bioinformatics analysis is underway.

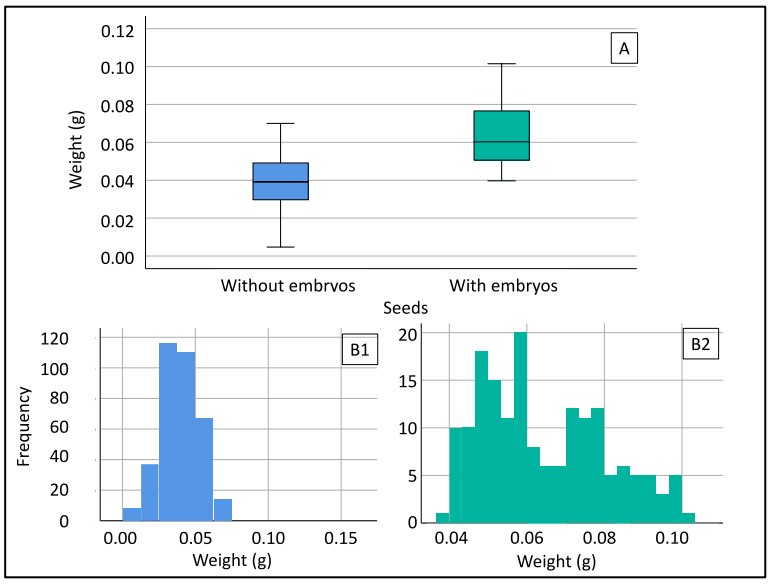


**Figure 1.** Mean weight (A) and frequency distribution (B) of *Abies nebrodensis* seeds with (green) or without (blue) embryo.

**Table 1**. Total number of seeds available for each selected cross after controlled hand pollination in individuals of *Abies nebrodensis*, and number of seeds with and without embryo by cross.

| Cross | N seeds | Without embryos | With embryos |
|---|---|---|---|
| ♀17x ♂24 | 60 | 30 | 30 |
| ♀14x ♂10 | 52 | 24 | 28 |
| ♀24x ♂14 | 52 | 49 | 4 |
| ♀25x ♂13 | 53 | 52 | 1 |
| ♀1x ♂13 | 52 | 31 | 21 |
| ♀22x ♂24 | 50 | 20 | 30 |
| ♀6x ♂2 | 52 | 41 | 11 |
| ♀7x ♂17 | 51 | 31 | 20 |
| ♀27x ♂1 | 50 | 45 | 5 |
| ♀2x ♂1 | 50 | 26 | 24 |

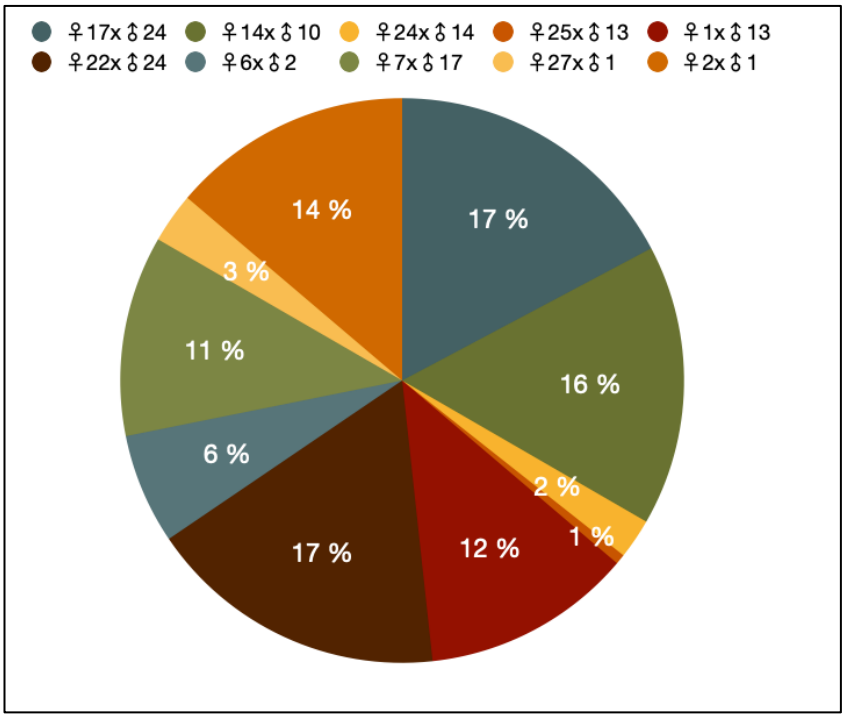


**Figure 2**. Percentage of seeds for each *Abies nebrodensis* cross with embryo whose DNA was correctly extracted and sent to sequencing.

## 3. Uploading molecular data from this project in public genetic databases

Molecular data related to 124 SNPs sequences derived from this project have been deposited in the free-access public genetic databases from University of Seville (idUS, https://idus.us.es/handle/11441/133288). We gave GenBank as an example of a repository, but it does not support the type of data (SNP) we have generated. So, we deposited it in the free repository of the University of Seville.

## 4. Conclusions concerning this action so far.

Despite the apparently formed seeds, many are empty. We have successfully obtained DNA from 144 samples. They have been sent for sequencing. We have just received the sequencing data and they will be analyzed as soon as possible. We are waiting for more seeds from a second pollination event to be included in the analysis. But in addition to seed formation the weight should be considered (best >0.06g).