LIFE4FIR – Project LIFE18 NAT/IT/000164

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

Report: Samples deposited in the DNA-bank of the University of Seville.
Action A1.1

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REPUBBLICA ITALIANA
REGIONE SICILIANA
ASSESSORATO REGIONALE
DELL'AGRICOLTURA, DELLO SVILUPPO RURALE
E DELLA PESCA MEDITERRANEA

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ACTION 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

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

1. Introduction

This deliverable concerns the activity conducted in the Action A1.1 on the population genetics of the species *Abies nebrodensis*.

The general aim of this action is the evaluation of the genetic variability of the natural population of *Abies nebrodensis* and the genetic relationships among the 30-adult trees and 118 plantlets sampled from the natural regeneration of 13 mother trees. The genetic diversity and structure of the natural population of *A. nebrodensis* was studied. SNPs genotyping was used to assess the genotype of these individuals. Then, paternity tests were carried out on the seedlings to determine the rate of outcrossing (cross between unrelated individuals), inbreeding and self-fertilization and to assess the rate of introgression (eventual hybridization) due to fertilization of female cones with pollen coming from alien firs (*Abies alba* and *Abies cephalonica*).

To attain this goal, an adequate amount of leaves (needles) were collected from each sampled specimen of the population. Part of the collected samples were used for DNA extraction and employed for PCR amplification, sequencing and genotyping. The remaining part was stored in the DNA bank of the University of Seville. The table reported below, include the complete list of accessions of the samples stored in the DNA bank.

Table 1. List of accession numbers of the vegetal material sampled from the adult trees and 118 plantlets of the natural regeneration of *Abies nebrodensis*, deposited in the DNA bank of the University of Seville. Identification of field individuals correspond to original nomenclature also followed in the previous Life project (2000). Identification of the plants of the natural regeneration followed the nomenclature used in the deliverable concerning the 'Updated census and mapping of the natural regeneration of *Abies nebrodensis*': the Id code (left column) was composed by two numbers, the first representing the respective mother tree, the second representing the single plant of a same group derived from the same mother (es: 11.3 indicates the plant nr. 3 derived from the mother plant nr. 11).

ID number of adult trees and young plants of the natural regeneration	Accession number assigned in the DNA Bank of the University of Seville
1M	599
2M	600
4M	601
6M	602
7M	603
8M	604
9M	605
10M	606
11M	607
12M	608
13M	609
14M	610
15M	611
16M	612
17M	613
18M	614
19M	615
20M	616
21M	617
22M	618
23M	619
24M	620
25M	621
26M	622
27M	623
28M	624
29M	625
30M	626
31M	627
32M	628
1.1.P	629
1.2.P	630
1.3.P	631

ID number of adult trees and young plants of the natural regeneration	Accession number assigned in the DNA Bank of the University of Seville
1.4.P	632
1.5.P	633
1.6.P	634
1.7.P	635
1.8.P	636
1.9.P	637
1.10.P	638
1.11.P	639
6.1.P	640
6.2.P	641
8.1.P	642
8.2.P	643
10.1.P	644
10.2.P	645
10.3.P	646
11.1.P	647
11.2.P	648
11.3.P	649
11.4.P	650
11.5.P	651
11.6.P	652
11.7.P	653
11.8.P	654
11.9.P	655
11.10.P	656
11.11.P	657
11.12.P	658
11.13.P	659
11.14.P	660
11.15.P	661
11.16.P	662
11.17.P	663
11.18.P	664
11.19.P	665
16.1.P	666
16.2.P	667
16.3.P	668
18.1.P	669
18.2.P	670
18.3.P	671
18.4.P	672
18.5.P	673
18.6.P	674
18.7.P	675

ID number of adult trees and young plants of the natural regeneration	Accession number assigned in the DNA Bank of the University of Seville
18.8.P	676
18.9.P	677
18.10.P	678
18.11.P	679
18.12.P	680
18.13.P	681
18.14.P	682
18.15.P	683
18.16.P	684
18.17.P	685
20.1.P	686
20.2.P	687
20.3.P	688
20.4.P	689
21.3.P	690
21.4.P	691
21.5.P	692
22.1.P	693
22.2.P	694
22.3.P	695
22.4.P	696
22.5.P	697
22.6.P	698
22.7.P	699
22.8.P	700
22.9.P	701
22.10.P	702
22.11.P	703
22.12.P	704
22.13.P	705
22.14.P	706
22.15.P	707
22.16.P	708
22.17.P	709
22.18.P	710
22.19.P	711
22.20.P	712
22.21.P	713
22.22.P	714
22.23.P	715
22.24.P	716
22.25.P	717
22.26.P	718
22.27.P	719

ID number of adult trees and young plants of the natural regeneration	Accession number assigned in the DNA Bank of the University of Seville
22.28.P	720
22.29.P	721
22.30.P	722
22.31.P	723
22.32.P	724
22.33.P	725
22.34.P	726
22.35.P	727
22.36.P	728
22.37.P	729
22.38.P	730
22.39.P	731
22.40.P	732
22.41.P	733
22.42.P	734
23.1.P	735
27.1.P	736
27.2.P	737
27.3.P	738
29.1.P	739
29.2.P	740
29.3.P	741
29.4.P	742
29.5.P	743
29.6.P	744
29.7.P	745
29.8.P	746

2. Conclusions

The table reported below includes a comprehensive list of both the sampled adults and the young individuals of the natural regeneration, included in the gene bank. Samples were stored in the gene bank as vegetal material in silica gel, as done in most DNA banks.
For each sample in the list, DNA was extracted, but the DNA samples are still at the Lab in Barcelona where they were used for sequencing. When the extracted DNA samples will be delivered back to the University of Seville they will be added to the database of the gene bank. So, *Abies nebrodensis* samples will be stored in the gene bank in two forms: silica gel and extracted DNA (stored at -80°C). The DNA bank of the University of Seville will be able to provide extracted DNA and/or genotypes originated from 120 SNP-array for further studies.