# *In vitro* germination of mature zygotic embryos from seeds of *Abies nebrodensis*, an endangered species in Sicily

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## Introduction

*Abies nebrodensis* is the rarest conifer in the European Flora. The present population is limited to 30 mature trees located in the Madonie Park (Northern Sicily). Sicilian Fir was listed actually as “Critically Endangered” in the IUCN Red List of Threatened Species, due to its small distribution range and the low number of reproductive individuals (Thomas 2017; Pasta et al. 2020). The previous researches on seed germination have recorded very low rates, due to the lack of embryo in seeds and the existence of albino embryos (Scialabba et al. 2009). *In vitro* culture could be a crucial method to develop new tools for the conservation and propagation of this species. Therefore, in the present project ‘LIFE4FIR’, new biotechnological approaches are applied to create a protocol for the preservation of this species. This study aims to investigate the *in vitro* germination of zygotic embryos as a preliminary step for somatic embryogenesis initiation.

## Materials and methods

Cones of *Abies nebrodensis* from mature trees were collected in the last week of September 2020. Seeds were collected from 10 adult individuals with the following identification numbers (IN): 6, 7, 8, 10, 12, 13, 19, 21, 22 and 29 and conserved under controlled temperature at 4 °C. On the basis of their weight, 300 seeds (100 for every replication) were selected and grouped into two classes : Class I > 30 mg and Class II < 30 mg (Tab.1). After sterilization, seeds are opened under sterile condition to test the presence of embryos. Three media were tested for in vitro germination. C: MS (Murashige and Skoog, 1962) medium hormone free; T1: MS + $GA_3$(0.5 mg $L^{-1}$) and T2: MS +2,4-D (0.5 mg $L^{-1}$). Moreover, germination from full seeds on filter paper imbibed with sterilized water was tested.

Germination rates were calculated after 2 weeks in *in vitro* culture

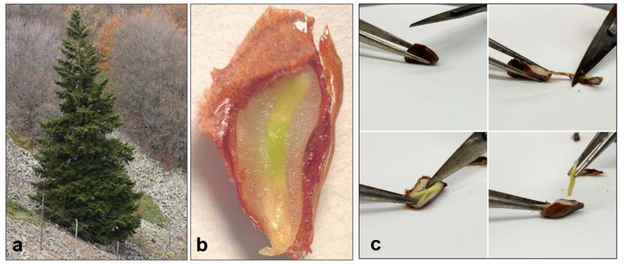



**Fig.1:**
**a. *Abies nebrodensis* mature tree ;**
**b. Full mature seed with embryo;**
**c. Excision of zygotic embryo under laminar flow.**

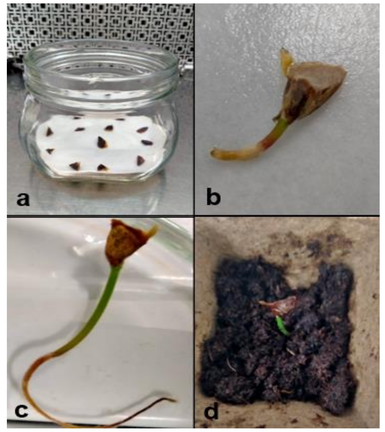
## Results

Seed selection revealed a different percentage of full seeds (number of seeds full of embryos among all the seeds tested) among donor trees. Seeds from IN21 and IN10 had the highest percentages of full seeds respectively 35,1% and 30.0%. While seeds collected from IN19 tree had not full seeds. The size of seeds i was different among genotypes, but this has no effect on the presence of zygotic embryo.

**Tab. 1 Results of seed weight from different trees**

| Tree (IN) | Full seeds >30 mg (%) | Full seeds < 30 mg (%) | Total of Full seeds (%) |
|---|---|---|---|
| **6** | 24.5 | 0.01 | 10.1 |
| **7** | 50.3 | 0.1 | 27.6 |
| **8** | 36.1 | 0.0 | 26.8 |
| **10** | 84.2 | 0.06 | 30.0 |
| **12** | 8.9 | 0.0 | 5.0 |
| **13** | 13.6 | 0.0 | 9.7 |
| **19** | 0.0 | 0.0 | 0.0 |
| **21** | 41.3 | 0.0 | 35.1 |
| **22** | 46.4 | 0.0 | 12.3 |
| **27** | 7.1 | 0.01 | 3.8 |

**Fig.2 Germination of seeds from tree with IN10 on filter paper.**




Optimal results of zygotic embryos germination (100%), from trees IN 7, 10, 12, 21 and 27, were obtained on MS media hormone free or supplemented with gibberellic acid. For IN6 and IN13 trees the lowest percentage were obtained on MS hormone free with respectively 66.6% and 75.0%. On the contrary, the zygotic embryos from IN8 tree recorded a higher germination percentage on MS hormone free (70.0%), while on medium with GA3 showed the lowest value (60.0%). In MS medium supplemented with auxin, the hypocotyl elongated, then swelled and callus induction was noted. No germination was achieved on this medium.

The experiment of germination on filter paper revealed a very low germination rate, about 10.0%, for each tree.

**Germination rate (%) of zygotic embryos from different donor trees on diverse media**

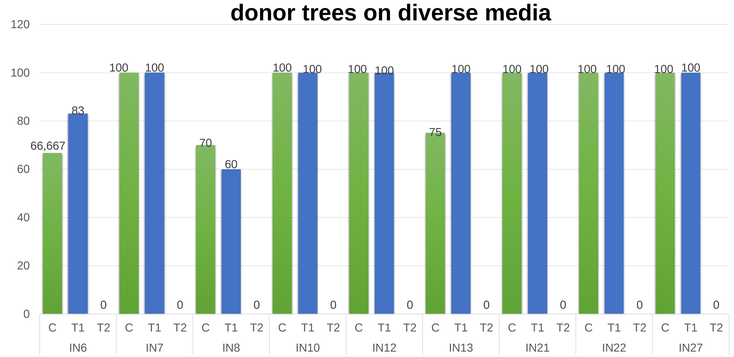
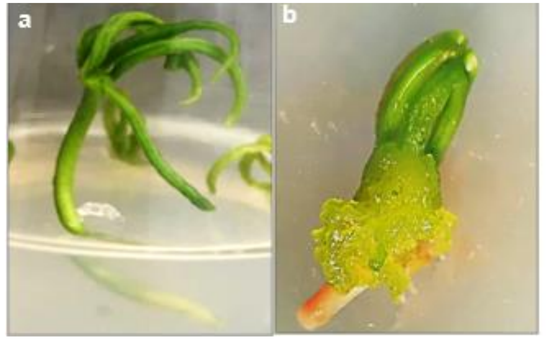



**Fig. 3 Embryo germination on different media. C: MS hormone free; T1: MS + $GA_3$ 0.5 mg $L^{-1}$ and T2: MS +2,4-D 0.5 mg $L^{-1}$**

**Fig. 4 *In vitro* culture of zygotic embryos**

**a. Germination of zygotic embryo from tree IN10 on MS hormone free medium after 4 weeks in culture;**
**b. Response of zygotic embryo on medium containing auxin.**




## Conclusion

**The high percentage of empty seeds in the *A. nebrodensis* makes difficult the propagation and the conservation of this species. A protocol for *in vitro* germination and development with the following acclimatization *in vivo* could be a major step forward for the safeguard of this species. This study confirm the variation of percentage of full seeds among donor trees, as well as the germination frequencies (Scialabba, 2009). The application of new biotechnological tools to preserve the Sicilian Fir, such as micropropagation, somatic embryogensis and cryopreservation are our principal challenges in this project.**

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**References:** Pasta, S., Sala, G., La Mantia, T., Bondì, C., & Tinner, W. (2020) *Vegetation History and Archaeobotany*, *29*(3), 357-371; Scialabba, A. (2007) Fl. Medit. 29: 272-276; Scialabba, A., Lombardo, G. & Schicchi, R. (2009) 45th International Congress of SISV & FIP. – Cagliari. P. 307: Thomas, P. (2017) The IUCN Red List of Threatened Species;

**Acknowledgement:** This study was financially supported by **LIFE project No.LIFE18 NAT/IT/000164 LIFE4FIR ‘Decisive in situ and ex situ conservation strategies to secure the critically endangered Sicilian Fir, Abies nebrodensis’, Coordinator: Dr. Roberto Danti.** We thank Prof. Rosario Schicchi (University of Palermo) and Dr. Giuseppe Di Noto (University of Palermo) for collecting the cones of *Abies nebrodensis.*