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Seed germination in *Abies nebrodensis* (*Pinaceae*)

Abstract

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Abies nebrodensis is a strict Sicilian endemic species, listed as "Critically Endangered" in the IUCN Red List of Threatened Species. Seed production highly varies annually and among specimens. The species is affected by a very low seed germination rate, mainly depending on the large number of empty seeds (from 68 to 96%). The embryo presence in seeds increases with increasing seed size and weight. Optimal germination rate (61%) was achieved at constant 5°C in the darkness. The low proportion of embryos in seeds is a relevant threat for *A. nebrodensis* conservation, as it affects the intrapopulation genetic diversity and reduces the potential genetic variability of seed accessions preserved in a gene bank collection.

Keywords: endangered species, germplasm conservation, Sicily.

Introduction

Abies nebrodensis (Lojac.) Mattei (Nebrodi fir or Sicilian fir) is a Sicilian critically endangered species (Thomas 2011), restricted to small areas (overall c. 150 ha) located in the Vallone Madonna degli Angeli, Mt. Scalone, Mt. dei Pini and Mt. Cavallo (Madonie Natural Park, NW Sicily), at 1360-1700 m of elevation.

The natural population is currently restricted to 30 mature individuals, each labelled by a single ID number (Virgilio & al. 2000), with a very fluctuating number of juveniles derived from natural regeneration. Actually, only 24 specimens produce cones, giving low germinating seeds (Scialabba & al. 2007).

Though conservation and restoration programs have been carried out since 1992 (Venturella & al. 1997), the low sexual performance of mature individuals and the low survival rate of seedlings and saplings involve specific actions of *ex-situ* conservation supporting the *in-situ* protection strategies (Ducci 2014). To this regard, the study of seed germination behaviour and embryo growth capability are pivotal activities to ensure appropriate conservation of this relict species.

1. *Abies nebrodensis* (Lojac.) Mattei (*Pinaceae*)

Accession data

Si: Polizzi Generosa (Palermo), loc. Madonie, Vallone Madonna degli Angeli (WGS84: 37.845512°N, 14.020996°E), Specimen ID number 22, 1390 m a.s.l., 20 Oct 2004, *Corpo Forestale dello Stato & A. Scialabba* (SPGR/PA – Acc.1403, Sicilian Plant Germplasm Repository of Palermo University SGPR/PA, formerly HBP Bank).

Germination data

Pre-treatments: Manual removal of wings; sterilization with a solution of 30% sodium hypochlorite and Tween 20 for 5 minutes, followed by 3 rinses in sterile water; imbibition for 48 hours in distilled water.

Germination medium: 4 sheets of sterilized filter paper (Whatman 40), imbibed with 10 ml distilled water under sterile conditions.

Sample size: 75 seeds for each test (15 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
61.0%	constant 5°C	0/24h	63.0	70.0	110.0	75.24

Observations

Mature cones were collected in October from wild trees with the identification number (ID) 13, 16, 17, 22, 25, 27. The seeds were individually evaluated, measuring both the major axis and weight. These morphological parameters are useful to distinguish well-developed seeds (Fig. 1a) from empty ones (Fig. 1b), which can affect the germination rate.

As far as the seed size is concerned (Scialabba & al. 2007), there are big seeds (9-12 mm), small seeds (< 9 mm), and small flattened seeds which are always empty. Based on seed weight (Scialabba & al. 2009), 3 main classes can be distinguished: seeds < 30 mg were always empty; the highest amount of seeds (mean value of 58.4%) belonged to the 31-50 mg weight class, with a very low embryo presence, ranging from 6% (specimen ID 27) to 39.9% (specimen ID 16); while 17.3% of seeds belonged to the weight class of 51-70 mg, where the embryo presence increased but with different values among the specimens, ranging from 22% (specimen ID 13) to ca. 98% (specimens ID 16 and 22). A fourth weight class > 71 mg was only found in plant ID 13 with a high embryo presence (93.3%). Therefore, the embryo presence increases both seed size and weight, and weight higher than 40 mg represents the most suitable fraction for valuable seed bank collections. Unfortunately, seed production in *A. nebrodensis* varies annually very much among different specimens (4% ID 25 to 32% ID 22), so that it is not possible to predict the number of vigorous seeds available per year.

Different germination tests were carried out 6 months after seed harvesting, under different thermoperiod conditions, i.e. constant temperatures (5°C and 15°C) and alternating temperature (5/15°C), in full darkness, applying in the medium pure distilled water or 10^{-3} M gibberellic acid (GA_3) water solution. Not germinated seeds were checked to verify the embryo presence or absence and the endosperm presence or not within empty seeds.

The germination rate is deeply affected by big quantity of empty seeds. The individuals of *A. nebrodensis* actually produce high percentages of seeds without embryo and even without endosperm. For this reason, seedlings can be obtained only by selecting seeds to germinate by their size and weight.

In fact, optimal results were reached only on selected seeds with embryo at 5°C (Fig. 1c), but germination tests carried out at 15°C in GA_3 10^{-3} M provided similar germination percentages. Seed germination proceeded very gradually; the specimen ID 22 showed the highest germination capability and the highest production of full developed seeds, with G = 25% using randomly selected seeds. Integument rupture (IR) was also observed in not germinated seeds (IR = 80% in plant ID 25 after 23 days incubation at 5°C in pure water). Seeds not containing embryo often also lacked endosperm (63.95%) (Fig. 1b). Embryos showed five cotyledons, they were albino (mainly in ID 16, 17 and 22 respectively for 83.0, 79.7 and 75.5%), and partly green (Fig. 1d) or fully green (Fig. 1e) (mainly in ID 13 and 27 respectively for 87.1 and 83.3%).

It must be emphasized that proteomic analyses carried out on embryos and endosperm of not germinated seeds, at different incubation times (30, 60 and 130 days), revealed specific protein patterns for both embryo at different developmental stages and endosperm at different mobilization of seed storage reserves (data not shown) and indicated that a metabolic active phase occurred also in non-germinating seeds.

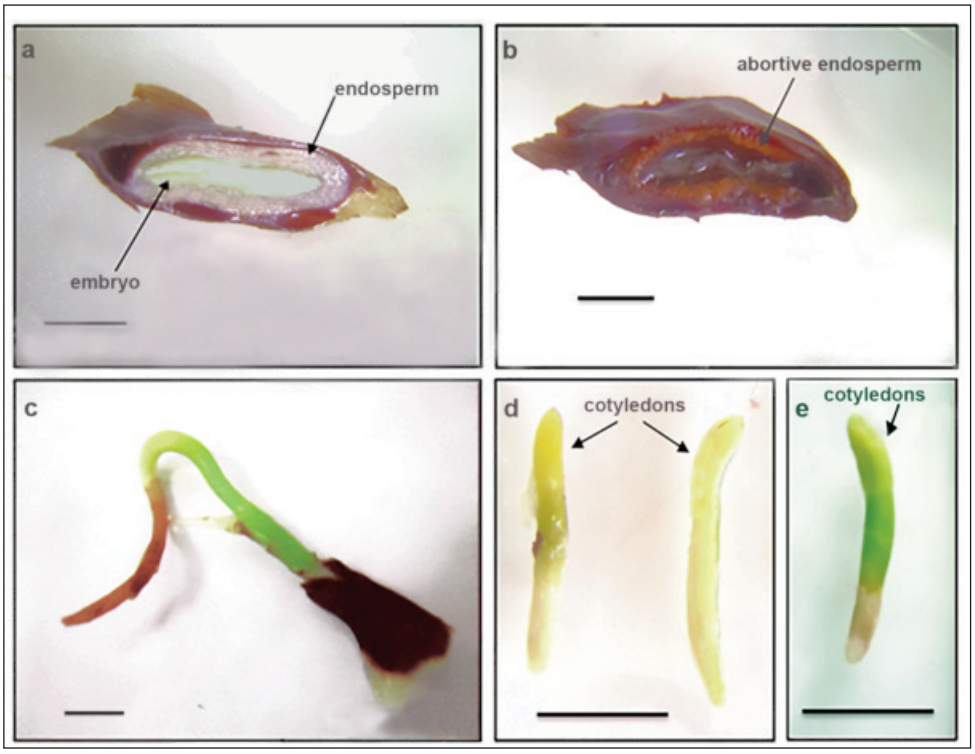

Fig. 1. Seeds of *Abies nebrodensis*: **a,** longitudinal section of fully developed seed; **b**, longitudinal section of empty seed. **c**, seedling with cotyledons covered by integument; **d**, albino embryos; **e**, green embryo. Bars: 5 mm.

The high presence of empty seeds in *A. nebrodensis* seems to be related with pro-embryo degeneration due to self-pollination or hybridization with *A. cephalonica* Loudon and *A. alba* Mill., which also occur in the area (Scialabba & al. 2005). *A. nebrodensis* appears intermixed with the other *Abies* species, supporting the hypothesis of remarkable hybridization processes within the genus *Abies* (Conte & Cristofolini 2003). The quotient of inbreeding depression (Q. I.), expressed as the ratio between developed and empty seeds and the total amount of seeds was very high in the whole population (80.24% ± 5.22) and single specimens (from 64.80% to 95.26%) of *A. nebrodensis*. These data are consistent with the low population density, as recorded in other conifers (Restoux & al. 2008), and the presence of the same haplotype, detected by cpSSRs, in at least 7 individuals (Parducci & al. 2001).

Conclusion

The low reproductive rate of the seeds in *A. nebrodensis*, mainly depending on the high amount of empty seeds, represents a relevant threat for its conservation, as previously hypothesized by Messeri (1958). The low percentage of embryos inside the seeds affects the results from germination tests, when carried out on randomly selected seeds, and decrease the genetic diversity of seed accessions. This, however, can be improved by manually selecting seeds containing embryo (based on their size/weight), thus avoiding overestimation of the genetic diversity of seed bank collections and improving the qualitative and quantitative potentiality of seed accessions.

References

Conte, L. & Cristofolini, G. 2003: Assessment of RAPD variation in *Abies nebrodensis* (Lojac.) Mattei (*Pinaceae*) using haploid tissue analysis. – Isr. J. Plant Sci. **51(3):** 199-206.

Ducci, F. 2014: 15.1. Species restoration through dynamic *ex situ* conservation: *Abies nebrodensis* as a model. – Pp. 225-232 in: Bozzano, M., Jalonen, R., Thomas, E., Boshier, D., Gallo, L., Cavers, S., Bordács, S., Smith, P. & Loo, J. (eds), Genetic considerations in ecosystem restoration using native tree species. State of the World's Forest Genetic Resources – Thematic Study. 1st Ed. – Roma.

Messeri, A. 1958: Nuovi dati sulla sistematica dell'*Abies nebrodensis* (Lojac.) Mattei. – Atti Reale Accad. Naz. Lincei, Mem. Cl. Sci. Fis., Sez. Reale Bot. **25(6):** 547-556.

Parducci, L., Szmidt, A. E., Madaghiele, A., Anzidei, M. & Vendramin G. G. 2001: Genetic variation at chloroplast microsatellites (cpSSRs) in *Abies nebrodensis* (Lojac.) Mattei and three neighboring *Abies* species. – Theor. Appl. Genet. **102:** 733-740.

Restoux, G., Silva, D. E., Sagnard, F., Torre, F., Klein, E. & Fady, B. 2008: Life at the margin: the mating system of Mediterranean conifers. – Web Ecol. **8:** 94-102.

Scialabba, A., Lombardo, G. & Schicchi, R. 2009: Seed mass variation and *in vitro* embryos culture of *Abies nebrodensis* (Lojac.) Mattei. – P. 307 in: Bacchetta, G. (ed), Book of abstract "Biodiversity Hotspots in the Mediterranean Area". 45th International Congress of SISV & FIP. – Cagliari.

—, Schicchi, R. & Cordì, R. 2005: Monitoraggio e analisi del polline. – Pp. 94-98 in: Raimondo, F. M., Schicchi, R. (eds), Conservazione *in situ* ed *ex situ* di *Abies nebrodensis* (Lojac.) Mattei. Rendiconto Progetto Life Natura n° LIFE2000NAT/IT/7228. – Palermo.

—, — & — 2007: Potenziale germinativo dei semi di *Abies nebrodensis*. – P. 237 in: Venturella, G. & Raimondo, F. M. (eds), Atti 102° Congresso Società Botanica Italiana. – Palermo.

Thomas, P. 2017: *Abies nebrodensis*. The IUCN Red List of Threatened Species 2017: e.T30478A91164876. – http://dx.doi.org/10.2305/IUCN.UK.2017-2.RLTS.T30478A91164876.en. [Last Accessed 29.07.2019].

Venturella, G., Mazzola, P. & Raimondo, F. M. 1997: Strategies for the conservation and restoration of the relict population of *Abies nebrodensis* (Lojac.) Mattei. – Bocconea **7:** 417-425.

Virgilio, F., Schicchi, R. & La Mela Veca, S. 2000: Aggiornamento dell'inventario della popolazione relitta di *Abies nebrodensis* (Lojac.) Mattei. – Naturalista Sicil., s. 4, **24(1-2):** 13-54.

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