

Seed mass variation and *in vitro* embryos culture of *Abies nebrodensis* (Lojac.) Mattei

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Abies nebrodensis (Lojac.) Mattei is an endemic and relict species of 30 individuals scattered in a small area of the Madonie Natural Park in Sicily (Italy); it is listed as a "critically endangered" species (Conti et al. 1997). The germination rate is poor and the presence of seeds lacking in embryos (Scialabba et al. 2007) makes it difficult to evaluate the population genetic variability and to preserve it into a genebank collection. The purposes of this study were to establish: i) the relationship between embryo presence and seed mass to identify those seeds which are suitable for the conservation in a genebank; ii) the best *in vitro* culture conditions for growing embryos that will be used for the *in vitro* germplasm conservation.

The seeds, collected in 2006 from reintroduced plants, were weighted individually, the major and minor axis and the height were measured. On the basis of their mass, seeds were grouped into five classes corresponding to the following fractions: class I, < 30 mg; class II, 31-40 mg; class III, 41-50 mg; class IV, 51-60 mg; class V, 61-70 mg. Seeds were sterilized and imbibed for 48 hours and the embryos were extracted and counted. The isolated embryos were cultured on solidified medium containing salt and vitamins MS (Murashige & Skoog 1962) and 50 g l⁻¹ sucrose as basal medium hormone free (control). Three treatments were used for the isolated embryo growth: basal medium supplemented with indole-3-butyric acid (IBA) in two concentration (5 or 10 µM) or with IBA (2.5 µM) and 6-benzylaminopurine (BAP) (4.4 µM). All explants were incubated for 40 days at 10±1°C under a 16/8 hours photoperiod of light/dark. The embryo growth response was evaluated (in percentage) considering the embryos with roots 2 mm long.

The results showed that seeds had similar sizes but that they were different in mass. The seed belonged to the classes I, II, III, IV and V respectively in percentage of 27.5%, 32.5%, 24.5%, 11.5% and 4%, containing embryos in percentages of 0%, 16.9%, 61.2%, 100% and 100%. The total percentage of seeds containing green embryos was 24%, while albino embryos were present in 6.5%. *In vitro* roots emerged only in the green embryos, but not in the albino ones, and the best culture medium both for the root emergence (75 ± 5%) and for the root elongation was the hormone free one.

This preliminary study permitted to observe that the embryo presence in seeds increases as the mass seed increases. In a genebank the conservation of *A. nebrodensis* germplasm may be carried out collecting seeds with a mass between 40 and 70 mg, preferring the fractions superior to 50 mg in which the embryos are always present. Besides, *in vitro* embryos cultures may be used for *ex situ* germplasm conservation of this endangered species.

Keywords: *Abies nebrodensis*, endangered species, *ex situ* conservation, embryos *in vitro* culture, seed mass.

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