An innovative protocol to propagate and preserve the threatened Sicilian fir through somatic embryogenesis technique

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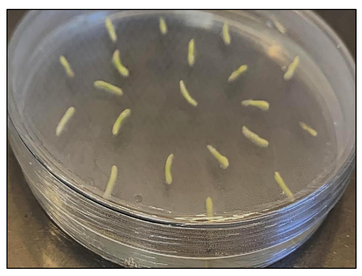
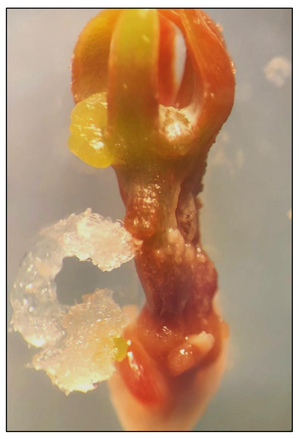
Introduction

Somatic embryogenesis (SE) is a revolutionary biotechnological tool applied to propagate, clone, and even to conserve different plant species. *Abies nebrodensis* is an endangered species endemic to Sicily. Only 30 adult individuals are present all over the world and located in the Madonie Park, Sicily, Italy [1]. This species was always the target of different projects aims to its preservation. Therefore, the development of new approaches, particularly somatic embryogenesis, could be a key step for a large-scale propagation and long-term preservation. The present work illustrates a protocol for SE from the Sicilian fir '*Abies nebrodensis'*.

Materials and methods

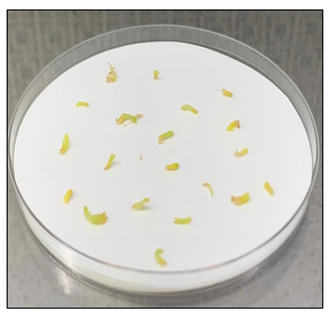
Diverse culture media supplemented with different concentrations of plant growth regulators (PGRs) (6-Benzylaminopurine (BAP) and/or 2,4-Dichlorophenoxyacetic acid (2,4-D) were tested, then the right protocol were established as the following steps :





Cones from *Abies* adult trees with a specific identification number (IN) were collected and full seeds were identified by X-ray. After sterilization under laminar flow, the seed coat was removed, and explants were carefully excised.

The callus induction was established on Schenk and Hilderbrandt (SH) medium supplemented with 4,43 µM of BAP and kept in the dark at 24±1 °C with subcultures every 3-4 weeks .



For maturation, cell lines were transferred onto SH basal salt medium, supplemented with 4,27 µM abscisic acid (ABA), 8% polyethylene glycol (PEG-4000) and 4% of maltose [2].

Somatic embryos with a full cotyledonary shape were used for germination trial after a partial desiccation.

Results

Our results, showed that SH medium was the best medium for callus induction when supplemented with 4,43 µM BAP, for both mature and immature embryos. Callus initiation rates were recorded 0% in all the other basal salt media used (DKW, WPM and MS). Moreover, the effect of PGRs, donor trees and the evolution of callus induction were studied and the results are presented below (Table.1, Fig.1 and Fig.2).

Initiation of SE from mature zygotic embryos

Table.1 Effect of PGRs on the type of callus and on the induction rate from mature zygotic embryos (%)

Medium	Embryogenic callus	Non embryogenic callus	Globular green callus
SH hormone free	0.0 [b]	0.0 [b]	0.0 [b]
SH+ BAP (4,43 µM)	4.6 [a]	80.1 [a]	30.6 [a]
SH+ 2.4D (4,52 µM)	0 [b]	76.7 [a]	0.0 [b]
SH + 2.4D (4,52 µM) + BAP (4,43 µM)	3.5 [ab]	77.8 [a]	21.7 [a]
Significance	***	***	***

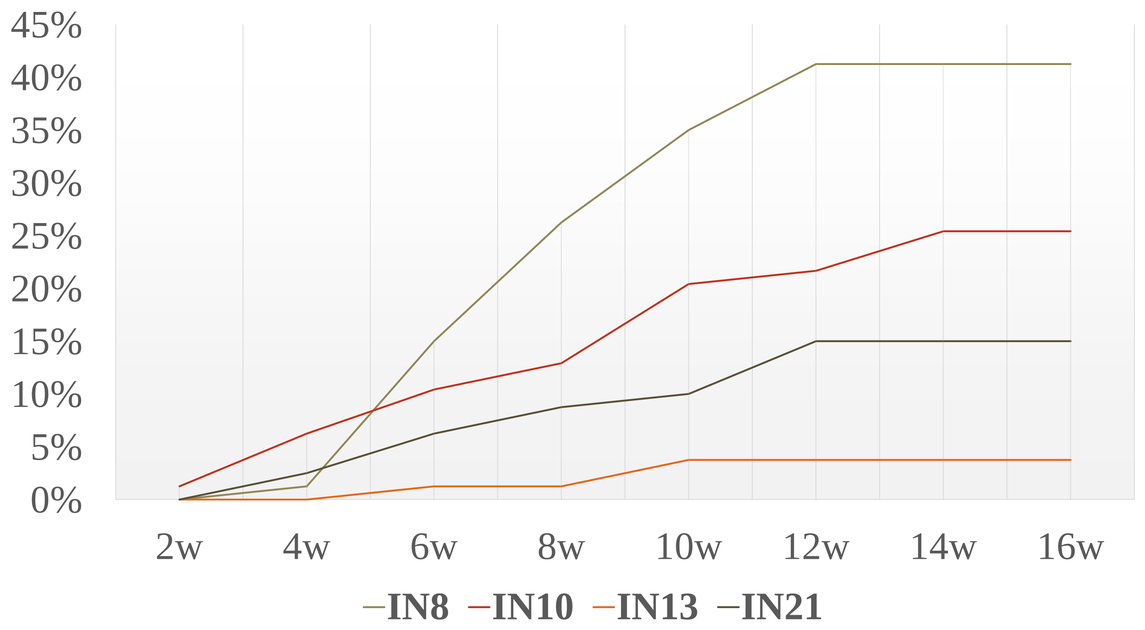


Fig.1 Callus induction rates for embryogenic tissue among four donor trees (IN8, IN10, IN13 and IN21) in SH medium supplemented with 4,43 µM of BAP.
Callus induction rates was recorded every two weeks; Axis (X: period in weeks (W); Y: Callus induction rates).

Initiation of SE from Immature zygotic embryos

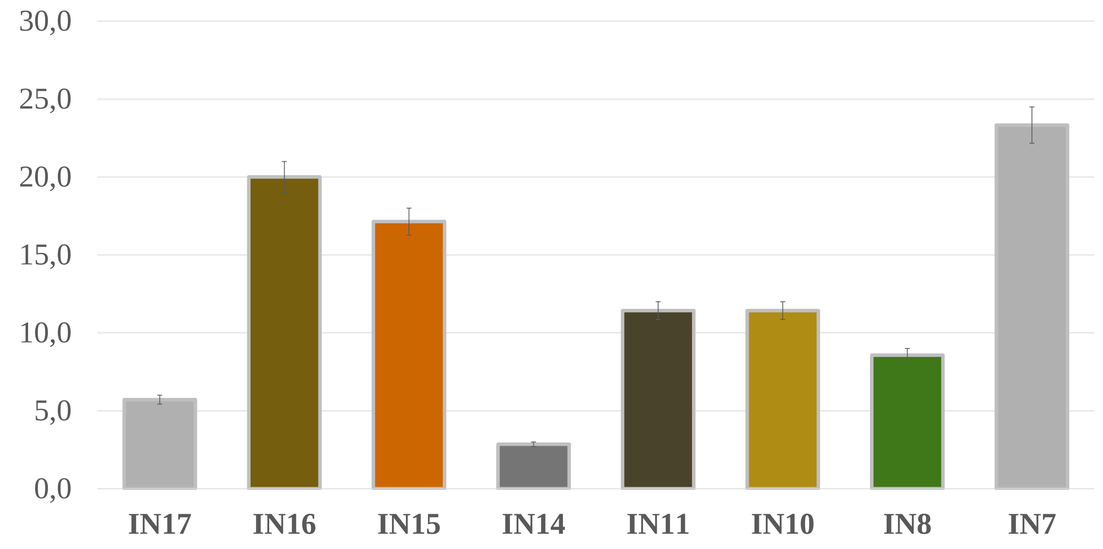


Fig.2 Callus induction rates among donor trees (IN7, IN8, IN10, IN14, IN15, IN16 and IN17) from **Immature zygotic** embryos in SH medium supplemented with 4,43 µM of BAP.
Callus induction rates recorded after 10 weeks; Axis (X: IN of donor trees ; Y: Callus induction rates).

Conclusion

In the presented work, a successful protocol for somatic embryogenesis of the critically endangered *Abies nebrodensis*, from mature and immature zygotic embryos was established. The induction and proliferation of embryogenic callus together with the maturation and germination of somatic embryos are key steps for large-scale propagation and long-term preservation of coniferous germplasm. The reported findings suggest that the use of SE in combination with cryo-conservation and other biotechnological tools could be a crucial way to conserve this threatened species.

References: [1]Pasta S, Sala G, La Mantia T, Bondì C, Tinner W (2020) The past distribution of *Abies nebrodensis* (Lojac.) Mattei: results of a multi-disciplinary study. Veg Hist Archaeobotany 29:357, [2] Salajová T, Salaj J (2003) Somatic embryo formation on mature *Abies alba* × *Abies cephalonica* zygotic embryo explants. Biol Plant 47:7–11.

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