# Strawberry tree (*Arbutus unedo* L.) micropropagation: shoot proliferation, organogenesis and somatic embryogenesis

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Strawberry tree (*Arbutus unedo*) is a shrub or small tree growing around the Mediterranean basin. It is well adapted to stress conditions, showing a large potential for fruit production. However, this potential has not been fully exploited and the species is even considered a Neglected or Underutilized Crop (NUC). Trying to make this species more attractive for producers and stakeholders an intensive propagation and breeding program is being carried out.

Several genotypes with interesting characteristics have been selected and micropropagation protocols were developed. A shoot proliferation protocol was established, using a culture medium supplemented with 2 mg/L BAP. Using this protocol a maximum of 10.8±1.3 shoots per explant were obtained. In order to reduce production costs, a very efficient and easy to apply micropropagation protocol in liquid medium was also developed. Moreover, a protocol for organogenesis induction was established using 1 mg/LTDZ. When leaves were used as explant, the maximum induction rate obtained was 60%, while a 100% induction rate was achieved with shoots. When compared to others, this method is very effective in terms of the number of plantlets produced. However, the plantlets obtained from organogenesis tend to be smaller than those obtained from shoot proliferation, due to the high number of shoots formed in the calli. The rooting and acclimatization rates of the plants obtained from the three methods were compared, and no significant differences were found. In most cases induction rates higher than 90% were achieved.

Regarding somatic embryogenesis, a one-step induction protocol was established. Although strawberry tree is a very recalcitrant species, calli formation was found in some of the tested genotypes when the leaves were cultured on a medium containing 2 mg/L BAP and 5 mg/L NAA. The induction rates and number of formed embryos per explant showed to be genotype-dependent. Both the induction and formation of somatic embryos occurred on the same medium and embryo development was not synchronized. When the quality of the somatic embryos was accessed, several types of abnormal embryos were found. However, some of those embryos were able to germinate and originate phenotypically normal plants. An anatomical analysis was carried out to compare tissue organization between somatic and zygotic embryos. The anatomy of the somatic and zygotic embryos is quite similar. However, the size of the somatic embryos is quite variable, depending on the number of embryos formed per explant. The amount of reserve substances increased progressively with the maturation of the embryos, and a large amount of proteins and starch could be found at the cotyledonary stage. Nevertheless, the amount of these substances is considerably high on zygotic embryos than in somatic embryos.

The propagation protocols that have been developed are very effective especially shoot proliferation in liquid medium, that allows the production of a large amount of plants, consuming less resources and time than other techniques. Although the results obtained on the induction and conversion of somatic embryos are very promising, the all processes must be optimized in order to apply it for mass propagation purposes.

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