Acclimatization of Micropropagated plants of *Arbutus unedo* L. (strawberry tree)

Fig. 1 – *Arbutus unedo L.* (strawberry tree)

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INTRODUCTION

The genus *Arbutus* includes about 20 species from which *A. unedo* is the most interesting. The production of a spirit represents the main income. The plant is resistant to forestry fires and grows in poor as well as in water deficient soils, making it an ideal species to recover degraded lands and to prevent forestry fires. In this work adult plants were selected (Fig. 1) for its potential for fruit production. Micropropagation has been achieved by axillary shoot proliferation. Following root development, the plantlets were transferred to containers and placed in a greenhouse. Different substrates were evaluated for plant acclimatization.

MATERIALS AND METHODS

Adult plants were selected for its potential for fruit production. Branches (30 – 40 cm length) were collected in the field and maintained in the greenhouse or in a culture chamber until epicormic shoots start to develop (Fig. 2). Following sterilisation, shoot tips (< 2 mm) and nodal segments (10-20 mm) were tested for *in vitro* plant establishment .

Shoot proliferation was achieved on a basal De Fossard (De Fossard *et al*., 1974) medium containing 9 µM BA. MS micro-nutrients, FS organics and 3 % sucrose were also added. Rooting induction was assayed in darkness. Shoots were inoculated on a basal medium containing Knop macronutrients (Gautheret, 1959) and 3 IBA treatments (9.8 or 24.7 µM IBA for 6 days; and 9.8 x10^3 µM IBA for 15 sec) were tested and compared with the control (no IBA). Following root induction shoots were subcultured (5 weeks) on the same medium culture without growth regulators and containing charcoal (1.5 %).

Rooted plantlets (600) were transferred to containers (covered with plastic bags) and placed in the greenhouse. Five substrate were tested according to Table 1.

Table 1 - Substrates tested (composition)

Substrate	Composition	% (Volume)
I	perlite; peat (1)	70:30
II	perlite; peat (1)	50:50
III	perlite (1)	100
IV	perlite	100
V	sand; composted pine bark, peat (1)	50:35:15

(1) supplemented with slow release fertilizer (0.6 g/plantlet)

When 100% perlite (IV) was used the plantlets were sprayed with Knop solution after the first month of culture. The levels of humidity were gradually decreased by raising the covertures, after 3-4 weeks. The covertures were removed after 1.5 month. Plants were transferred to individual 220 cm^3 containers (peat, vermiculite, perlite: 1:1:1.5), after 2 months. Plant survival rate was recorded after 2 and 3 months.

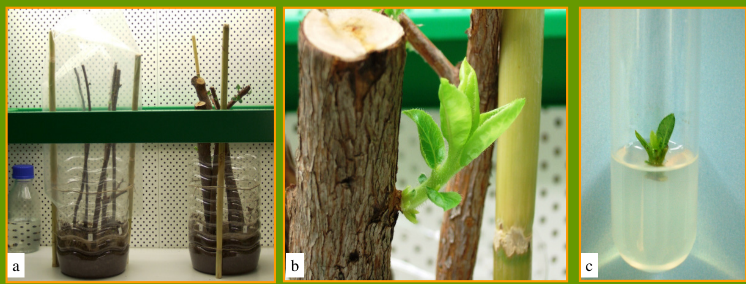

Fig. 2 - Woody branches, from adult selected plants at culture chamber (a), showed epicormic shoots (b). At establishment, best results (survival rate of 38.65 ± 9.78 %) were achieved with shoot tips < 2 mm (c).

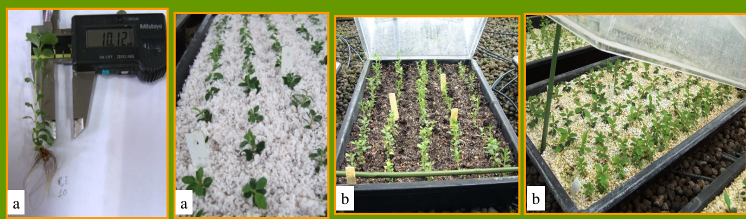

Fig. 3 –Acclimatization: after 5 weeks on root development medium (a) and 1.5 month in the green-house (b)

RESULTS

•The survival and necrosis rates showed significantly differences (P ≤ 0.05) according to the explants size/type. Best results (survival rate of 38.65 ± 9.78 %) were achieved with shoot tips (< 2 mm; Fig. 2).
•The highest rooting rate (93.3 %) were achieved when shoots were inoculated in root induction medium containing 24.7 µM IBA (during 6 days) or dipped on 9.8 x103 µM IBA (for 15 sec), and followed by its subculture on the same medium without growth regulators and containing charcoal (P ≤ 0.05).
•After 5 weeks on root development medium, shoots were healthy and showed neither callus formation at the shoot base nor apical necrosis and were acclimatized (Fig. 3).
•After 2 months, when micropropagated plants were transferred to individual containers (Fig. 4), the survival rate was recorded. According to the substrates different root systems were developed, when fertilizer was added to perlite 100%, the plantlets showed necrosis due to high nutrient levels. However, the same substrate without fertilizer showed the best root development (Tab. 2, Fig. 4). This observation was confirmed when survival rate was recorded after 3 months (Tab. 3). Best results were achieved with perlite 100% without fertilizer (98.3%). The obtained plants are now being used for cutting production and for clonal trails (Fig. 5).

Table 2 – Effect of substrate treatment at acclimatization on survival rate (%) after 2 months

Substrate Acclimatization	Survival rate (%) (mean ± std)	P< 0,05 Tukey HSD test
I	90,89 ± 4,7	ab
II	95,00 ± 7,0	ab
III	0,00 ± 0	c
IV	98,66 ± 5,2	a
V	84,67 ± 22,7	b
Mean	87,73 ± 25,4	

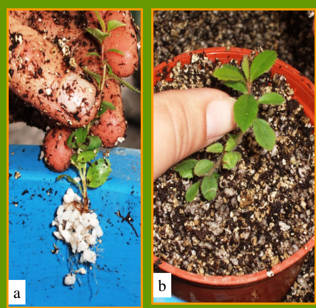

Fig. 4 –After 2 months, when micropropagated plants (a) were transferred to individual containers (b)

Table 3 – Effect of substrate treatment at acclimatization on survival rate (%) after 3 months

Substrate Acclimatization	Survival rate (%) (mean ± std)	P< 0,05 Tukey HSD test
I	69,91 ± 21,9	b
II	74,24 ± 10,7	b
III	0,00 ± 0	c
IV	98,30 ± 5,4	a
Mean	85,54 ± 28,9	

CONCLUSIONS

The results so far obtained indicate that shoot tips are more efficient for in vitro establishment than nodal segments. Moreover, an auxin, in this case IBA, is absolutely necessary for root induction. Concerning plant acclimatization, the data show that 100% perlite without fertilizer is the best substrate to obtain a large percentage (98.3) of acclimatized plants.
The micropropagation and acclimatization of adult selected plants, based on fruit quality and fruit production or in other characteristics that could be judged important, is determinant to 1) the improvement of farmers economic resources due to better incomes, 2) the increase of forested areas with *A. unedo* and the consequent decrease of fire impacts in the regions where the species is cultured and 3) the conservation of genetic resources that can be further used in programs of genetic improvement.

Fig. 5 - Plants were established as mother plants for cutting production (a) and for clonal trails (b)

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