# Propagation of the endangered Azorean cherry *Prunus azorica* using stem cuttings and air layering

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*Prunus azorica* (Hort. ex Mouillef.) Rivas Mart., Lousã, Fern. Prieto, E. Dias, J.C. Costa & C. Aguiar is an endangered tree endemic to the Azores, with an ecological and ornamental interest. The objective of this study was to determine the conditions necessary for the successful propagation of *P. azorica* by stem cuttings and air-layering. Stem cuttings collected in March with two apical leaf pairs pruned to 1/3 of their leaf area were submitted to different treatments, including a basal split wound, two rooting mixtures, namely, perlite and peat (1:1) or perlite and natural soil (1:1), and dipping of the base in indole-3-butyric acid (IBA) solution at four concentrations (0, 2500, 5000 or 7000 mg/L). After eight weeks 75% rooting was achieved with 75 to 88% survival, without addition of IBA and with split wound using both substrate mixtures. Air layering was conducted in June in branches of adult trees with the addition of 0, 2500 or 3000 mg/L of IBA. After 12 months 100% rooting and survival was recorded in all treatments. Our study thus indicates that *P. azorica* is a taxon amenable for vegetative propagation by stem cuttings and air layering without requiring addition of IBA to induce rooting. Both methods should be used in order to recover natural populations, when seeds are not available or are available in reduced amounts.

Key words: endemic, IBA, population recover, rooting, vegetative propagation

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## INTRODUCTION

*Prunus azorica* (Hort. ex Mouillef.) Rivas Mart., Lousã, Fern. Prieto, E. Dias, J. C. Costa & C. Aguiar, commonly named Azorean cherry or "ginja-do-mato", is an endangered tree endemic to the Azores, only found in São Miguel, Terceira, São Jorge, Pico and Faial Islands (Schäfer 2005; Silva et al. 2009). It is usually a small tree or shrub, rarely over 4 m tall. However, in sheltered locations (i.e. water stream margins), it is able to grow up to more than 10 m high. It is usually found at altitudes above 500 m, mainly in craters and deep narrow ravines, or disperse in undisturbed hyper-humid native forest. Only scattered individuals were found in São Miguel and Pico, and the plant might have been recently become extinct in Flores. It is considered as one of the top 100 priority taxa for conservation in Macaronesia (Cardoso et al. 2008; Silva et al. 2009).

Although it is presently propagated by seed germination, with germination rates of ca. 50% (Fagundo & Isidoro 2004), due to the scarceness of populations and thus low availability of seeds, the development of alternative propagation methods is a priority. In this context, the use of technically simple and inexpensive vegetative propagation methods will be of utmost importance, e.g. cuttings and air layering. These methods are especially useful to make true-to-type copies of relict individuals, and therefore avoid the extinction of small local populations and loss of their genetic diversity.

Research with other species of *Prunus* showed that stem cuttings and air layering are suitable propagation methods. For these species, the addition of indole-3-butyric acid (IBA) was deemed effective for promoting rooting of cuttings (Dirr & Heuser 1989; Dehgan et al. 1990; Ribeiro & Antunes 1997; Tchoundjeu et al. 2002; Tofanelli et al. 2002) and of air layered branches (Castro & Silveira 2003; Castro & Medeiros 2007).

Semihardwood cuttings were efficient to propagate *P. serotina* (Dehgan et al. 1990) and some cultivars of *P. persica* (Tofanelli et al. 2002). Apical cuttings with 1/3 of leaf area cut were appropriate for *P. lusitanica*. Rooting percentage and root number increased with leaf area of *P. africana* juvenile cuttings while leafless cuttings did not root (Tchoundjeu et al. 2002).

Season had a significant effect on rooting success of some species, with positive results occurring only in the hot season for *P. africana* (Tchoundjeu et al. 2002) and March or April for *P. serotina* and *P. umbellata*, respectively (Dehgan et al. 1990). Furthermore, little or no rooting occurred with cuttings of three growth habits of *P. persica* collected in August or October (Tworkoski & Takeda 2007).

Beneficial effects of arbuscular mycorrizal fungi inoculation have also been reported for the rooting and growth of *P. maritime* hardwood cuttings (Zai et al. 2007) and several *Prunus* rootstock cultivars, particularly those from *P. insitia* (Calvet et al. 2004).

This research aims to test stem cuttings and air layering as feasible and efficient methods for *P. azorica* vegetative propagation, in view of the urgency to develop measures for *ex situ* conservation of the depleted natural populations and the growing demand of saplings by the nursery industry.

## MATERIAL AND METHODS

### PLANT MATERIAL

Plant material was obtained from mature, flowering and seed producing individuals, belonging to two populations located at Tronqueira, São Miguel Island. The populations were composed of 30 to 80 individuals with a size range of 2.5 to 6.5 m and a basal perimeter of 20-75 cm.

### STEM CUTTINGS

Semihardwood stems (i.e. with visible lignification at the base) were obtained 7 March 2008. Stems were surface cleaned with water and a few drops of a mild household detergent, and then cut into 10-15 cm cuttings, with two apical leaf pairs which were pruned to 1/3 of their leaf area. The cuttings were immersed in an antifungal solution of Benlate at 6% (w/v), for 5 min. In half of the cuttings a split wound of ca. 1 cm was made at the base. Cuttings were basally dipped up to 2.5 cm in different solutions of IBA at 0, 2500, 5000 and 7000 mg/L for about one second. The cuttings were planted 4 cm deep in polystyrene trays composed of 216 square cells, and each cell measured 5 cm side and 12 cm height. Two different substrates were tested: a perlite and peat mixture (1:1), and a perlite and soil mixture (1:1). The soil (ando soil), collected at the natural habitat was a clay loam with pumice of volcanic origin. The soil was left untreated since mycorrhizae effect was being tested. Whenever weeds appeared were manually removed. The total number of treatments were 16, with three replicates per treatment and 23 cuttings per replicate, in a total of 1104 stem cuttings. The experiment took place inside a glass ceiling nursery room with a mean temperature of 19 ºC and the natural photoperiod occurring at Ponta Delgada between March and May 2008. The cuttings were kept moist and weekly sprayed with antifungal solution (200 mg/L of Benlate or 1.5 ml of Previcur® and 1 ml of Derosal® per litre) and rooting was evaluated after eight weeks.

### AIR LAYERING

Air-layering was applied in mid-June 2008 to 41 trees. For each tree, three branches were randomly selected. Lateral branches were removed up to 15-20 cm of the apical bud and a 1-1.5 cm ring of bark was removed at ca. 40 cm from the branch apex. The diameter of the branches at the place where the air layering was done was approximately 6-10 mm. IBA talc in concentrations of 0, 2500 or 3000 mg/L was applied directly to the wounds with a brush. The layering site was covered with wet *Sphagnum* spp. and wrapped in a black polyethylene sheet. Rooting was evaluated after 12 months.

### STATISTICAL ANALYSIS

Percentage of rooting and survival were recorded for each air layering treatment and the degree of development (considering both root number and length) of the adventitious root system was visually estimated on a scale of 1 (poor: with few, short roots) to 3 (good: many, long roots). For each treatment with cuttings, percentages of rooting and survival, root number and root length were recorded. Root number was log transformed in order to normalize its distribution. The effect of each factor (IBA concentration, split wound and addition of soil to the substrate) on the recorded depended variables was evaluated using ANOVA, and whenever multiple comparisons were needed Tukey test was used. Error bar charts were edited to represent results obtained with each treatment. When interactions between factors were significant, the results were represented using interaction plots. All calculations were performed using SPSS version 15.

## RESULTS

### STEM CUTTINGS

Regarding rooting percentage (Table 1), ANOVA showed a significant interaction between IBA concentration and split wound ($F=3.282$; $p=0.033$) in addition to a significant singular effect of IBA concentration ($F=10.319$; $p < 0.001$). There was a clear tendency for a reduction in rooting percentage with an increase in IBA concentration (Fig. 1). The negative effect of IBA increased with the presence of a split wound and the only case where the wound improved rooting was in the absence of the growth regulator.

Root number (log transformed) was significantly affected by IBA concentration ($F=4.099$; $p=0.014$) and by split wound ($F=6.948$; $p=0.013$). The best IBA concentration (Table 1) was 2500 mg/L (1.03 ± 0.21), although this result was only significantly different from that obtained with the highest IBA concentration used in the study, 7000 mg/L (0.34 ± 0.13). Considering all treatments, applying a split wound to the cuttings significantly decreased the number of roots from 0.86 (± 0.12) to 0.48 (± 0.10).

Table 1. Rooting percentages, number of roots (log transformed), root length and survival of *Prunus azorica* stem cuttings after eight weeks, with different IBA concentrations (0-7000 mg/L), with or without the application of a split wound or the addition of natural soil to the rooting mixture.

| IBA (mg/L) | Split wound | Soil | Rooting (%) | | Root # (log) | | Root length (cm) | | Survival (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | ±SE | Mean | ±SE | Mean | ±SE | Mean | ±SE |
| 0 | - | - | 46.4 | 10.1 | 0.7 | 0.2 | 1.2 | 0.2 | 76.8 | 8.8 |
| | | Soil | 49.3 | 13.8 | 0.8 | 0.2 | 1.3 | 0.4 | 81.2 | 8.1 |
| | Wound | - | 75.4 | 18.2 | 0.8 | 0.4 | 1.4 | 0.7 | 75.4 | 18.2 |
| | | Soil | 75.4 | 10.5 | 0.7 | 0.3 | 1.3 | 0.5 | 88.4 | 9.5 |
| 2500 | - | - | 42.0 | 16.7 | 1.0 | 0.4 | 1.0 | 0.5 | 56.5 | 0.0 |
| | | Soil | 69.6 | 6.6 | 1.8 | 0.3 | 1.1 | 0.2 | 46.4 | 11.9 |
| | Wound | - | 47.8 | 27.2 | 0.7 | 0.6 | 0.7 | 0.5 | 26.1 | 19.9 |
| | | Soil | 30.4 | 0.0 | 0.6 | 0.1 | 0.4 | 0.0 | 8.7 | 5.0 |
| 5000 | - | - | 31.9 | 3.8 | 0.7 | 0.1 | 0.6 | 0.1 | 20.3 | 3.8 |
| | | Soil | 42.0 | 10.1 | 0.9 | 0.3 | 0.7 | 0.2 | 21.7 | 7.5 |
| | Wound | - | 18.8 | 12.9 | 0.4 | 0.4 | 0.3 | 0.3 | 10.1 | 10.1 |
| | | Soil | 8.7 | 6.6 | 0.2 | 0.2 | 0.1 | 0.1 | 2.9 | 2.9 |
| 7000 | - | - | 20.3 | 13.8 | 0.4 | 0.3 | 0.3 | 0.2 | 13.0 | 8.7 |
| | | Soil | 27.5 | 16.3 | 0.6 | 0.4 | 0.4 | 0.3 | 11.6 | 11.6 |
| | Wound | - | 14.5 | 2.9 | 0.3 | 0.0 | 0.2 | 0.0 | 2.9 | 1.4 |
| | | Soil | 4.3 | 4.3 | 0.1 | 0.1 | 0.1 | 0.1 | 4.3 | 4.3 |

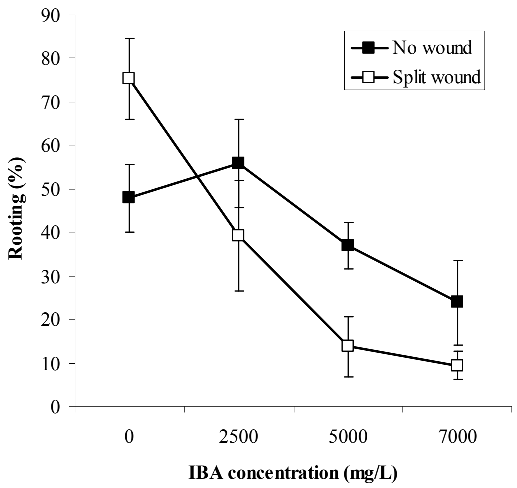


Fig. 1. Rooting percentages of *Prunus azorica* stem cuttings after eight weeks. Interaction between IBA concentrations (0-7000 mg/L) and the presence or absence of a split wound.

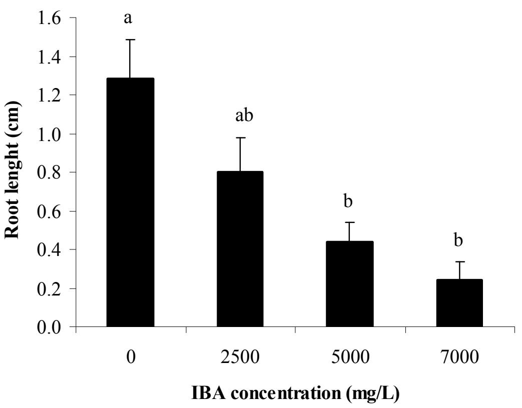


Fig. 2. Length of roots formed on *Prunus azorica* stem cuttings, after eight weeks, with different IBA concentrations (0-7000 mg/L). Different letters indicate significant differences (Tukey test applied after ANOVA, α=0.05).

Root length was only significantly affected by growth regulator concentration (F=7.744; $p < 0.001$), with a tendency for the development of shorter root systems with the increase in IBA concentration (Table 1, Fig. 2).

Survival (Table 1) was significantly affected by split wound (F=7.674; $p$=0.009) and by IBA concentration (F=44.938; $p < 0.001$). Percentages of survival above 75% were only obtained on treatments without IBA and these values were significantly higher than those obtained with all other treatments (Fig. 3). Globally, the effect of split wound originated a significant decrease in survival from 41% (± 6.05), without split wound, to 27.4% (± 7.50), with split wound.

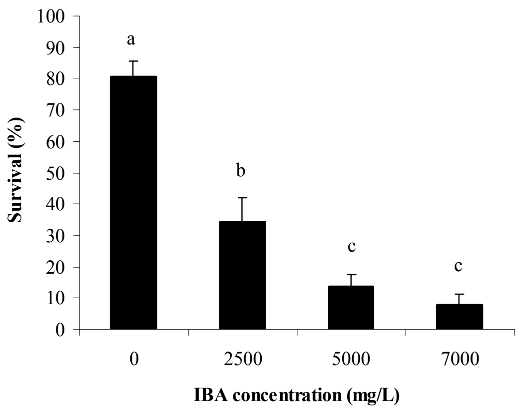


Fig. 3. Survival of stem cuttings from *Prunus azorica*, after eight weeks, with different IBA concentrations (0-7000 mg/L). Different letters indicate significant differences (Tukey test applied after ANOVA, α=0.05).

### AIR LAYERING

There were no significant differences among IBA concentrations experimented since 100% rooting and survival were recorded in all treatments after 12 months. Visual estimation of the degree of development of the adventitious root system indicated a good (3) development on all treatments with the formation of many, long roots (Fig. 4).

## DISCUSSION

There was not a clear beneficial effect of IBA on rooting of *P. azorica* cuttings, similarly to what has been reported for Gisela 5 dwarfing cherry rootstock (Stefancic et al. 2005) and for some *P. salicina* cultivars (Neto 2006). It has been suggested that the endogenous concentration of auxins influence the effect of exogenously supplied IBA on rooting of *Prunnus persica*

Fig. 4. Rooting on three air layered branches from a single tree of *Prunus azorica*, obtained with the application of three IBA concentrations (0, 2500 or 3000 mg/L, from left to right), after 12 months.

(Tworkoski & Takeda 2007). Furthermore, it is known that exogenously applied auxins, in seasons when endogenous levels are high due to high meristematic activity (i.e. spring), can bring the total auxin concentration to supra optimal levels of other tree genus, e.g. *Populus* (Nanda & Anand 1970), *Olea* (Ansar et al. 2009), which can explain the low survival percentages of *P. azorica* *c*uttings obtained in the higher IBA concentrations.

Contrarily to the findings of Blazich & Bonamino (1983), split wound promoted rooting without the need of IBA addition and resulted in rooting percentages approximate to those obtained for the closely related *P. lusitanica*, using this methodology and an IBA supplement (Ribeiro & Antunes 1997). When in the presence of IBA, the split wound always resulted in lower percentages of survival and was not deemed beneficial. Wounding increases sensitivity to an exogenous source of auxin and may only be effective when in presence of a correct concentration of this growth regulator (see Ribeiro & Antunes 1997, for a review). The negative effect of split wound when in presence of IBA agrees with the hypothesis of high levels of IBA occurring endogenously in the *P. azorica* cuttings used in this experiment.

There was no significant beneficial effect of using natural soil in the rooting mixture which suggests that *P. azorica* does not require mycorrhizae inoculation for developing a fully functional adventitious root system.

## CONCLUDING REMARKS

In summary, after eight weeks, 75% of cuttings developed a functional root system, with 75 to 88% survival, without addition of IBA and with split wound using both substrate mixtures. For all air layering treatments, 100% rooting and survival was recorded after 12 months. Our study thus indicates that *P. azorica* is a taxon amenable for vegetative propagation by stem cuttings and air layering without requiring addition of IBA to induce rooting. Both methods should be used in order to recover natural populations, when seeds are not available or are available in reduced amounts.

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