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SHORT COMMUNICATION

Rooting of *Prosopis alba* mini-cuttings

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Abstract Mesquite (*Prosopis alba* Grisebach), an important species in arid and semiarid regions, is currently commercially propagated by seeds and so there is great interest in developing techniques for species propagation, domestication and improvement. Cloning through mini-cuttings is a new and promising technique for the production of clonal seedlings of forest species. The main objective of this work was to evaluate vegetative propagation using the mini-cutting technique and indolebutyric acid (IBA) at different concentrations (0, 3,000, 4,500, 6,000 and 7,500 mg L^{-1}) on rooting of *P. alba* clones. Rooting was achieved in 98–100 % of the mini-cuttings at all concentrations tested. Increasing IBA concentration resulted in an increase in the number of leaves and leaflets, as well as in fresh matter weight and number and length of roots of clone seedlings, until an optimum point (between 3480 and 4800 mg L^{-1}) was reached. Plants were also vegetatively propagated in the field via conventional clonal garden cuttings under the same rooting conditions to compare the efficiency of the two propagation techniques. Mini-cuttings showed higher rooting and survival percentages than cuttings. A correlation analysis conducted between characteristics of stock plants (height, diameter, number of

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shoots) and shoots (height, diameter) showed a positive correlation between rooting and height of shoots with total root length, leaflet number, and height of clonal seedlings. We propose the mini-cutting technique as a tool for *P. alba* cloning.

Keywords Clonal forestry · IBA · Mesquite · Rooting · Vegetative propagation

Introduction

Prosopis alba Grisebach, a species of the family *Leguminosae*, is of great importance in the arboreal composition of arid and semiarid regions (Felker et al. 2008) and has great potential for the production of forest products (Ewens and Felker 2010). At present, this resource is obtained mainly from natural areas, via the intensive exploitation of native forests (Patch and Felker 1997). Hence, there is a great interest in developing techniques for the domestication, improvement and propagation of this native species (Verzino et al. 2003; Verga et al. 2005).

The species is currently commercially propagated through seeds. Several studies have been conducted to obtain a viable cloning technique for the commercialization of elite clone seedlings of *P. alba* (Klass et al. 1985; De Souza and Felker 1986; Klass et al. 1987; Tabone et al. 1986; Castillo de Meier and Bovo 2000; Felker et al. 2005; Felker 2008; Felker et al. 2008). The use of graftings has proven to be the most promising technique, according to the method described by Wojtusik and Felker (1993) and evaluated by Ewens and Felker (2003), but has not yet been applied at the commercial level (Ewens, personal communication). According to Ewens and Felker (2003), despite the advances in rooting of cuttings (with highly variable results), a clonal vegetative propagation technique viable for the large-scale production of *P. alba* clones has still not been developed. Such technique is necessary given the difficulties in propagule rooting; therefore, Ewens and Felker (2003) recommend the use of grafting for *P. alba* propagation. The development of clonal silviculture is necessary for the propagation of materials of silvicultural importance (Souza et al. 2009), particularly those of high productivity of biomass and pods, and those that are tolerant to high/low temperatures, drought, salinity, and pest resistance (De Souza and Felker 1986; Felker et al. 2005, 2008; Ewens and Felker 2010).

Alfenas et al. (2004) stated that the mini-cutting technique is a viable alternative for commercial-scale cloning of *Eucalyptus* spp. The technique involves the use of sprouts of plants propagated via conventional cuttings or via seedlings produced from seeds and consists of breaking apical dominance by pruning stock plants, which produce new sprouts that are used for rooting and generation of future clonal seedlings. The mini-cutting technique can be considered a variant of the cutting technique, the former being more sensitive to environmental conditions than the latter. Mini-cuttings involve the use of more herbaceous plant material than cuttings, intensive management and more careful treatment of cuttings, especially during collection and acclimation of mini-cuttings (Wendling et al. 2000). The use of the mini-cutting technique has provided rejuvenated material and has promoted a considerable increase in growth and rooting rates in *Eucalyptus* hybrids (Brondani et al. 2010), among other advantages. In addition, mini-cutting associated with breeding programs is responsible for the establishment of homogeneous, highly productive stands in a short time (Souza et al. 2009). The most widely used method of the principal Brazilian forestry companies for the commercial production of *Eucalyptus* clones is the

mini cutting technique (Martínez-Alonso et al. 2012). In the last decade, major forestry companies have used cloning of families for the large-scale production of seedlings. The mother plants used are derived from a mini-garden obtained from seeds of elite families. This practice has provided advantages in terms of time, productivity, quality and uniformity of comercial stands (Andrejow and Higa 2009).

Many works studying the use of mini-cuttings of Eucalyptus spp. and other forest species have involved the application of indolbutyric acid (IBA) to promote rooting (Borges et al. 2011; Brondani et al. 2010; Goulart et al. 2008).

The aim of this work was to evaluate the viability of vegetative propagation using the mini-cutting technique, the effect of IBA at different concentrations on rooting capacity and speed, and the influence of the characteristics of stock plants and propagules used in mini-cutting rooting, as well as to compare the efficiency of this technique with conventional cutting methods.

Materials and methods

Seedlings of *P. alba* were produced using seeds from the seed stand of the locality of Vera (Santa Fe, Argentina). These plants were used to form the clonal garden and mini-garden. The clonal mini-garden was established in a growth chamber using 14-h photoperiod, radiation intensity of 400 μE PAR, day/night temperature of 26/19 °C and 60 % relative ambient humidity. Stock plants were maintained in the chamber for 2 years. Six months before the mini-cutting trial, the 2-year old plants were taken to a greenhouse(26/15 °C day/night temperature and 71.5 % relative ambient humidity) located in the experimental area of the Facultad de Ciencias Agrarias (UNL, Santa Fe, Argentina) (27°29,421S; 58°W).

The clonal mini-garden was established following the method described by Souza et al. (2009), with the following modifications. The pots (276.5 cm^3) contained municipal solid waste compost with 1/4 coarse sand (humidity: 45 %; pH: 7.1; Total N: 0.9 %; P2O5: 0.9 %; K2O: 0.6 %) and an initial density of 40 plants/m^2. To promote the formation of rejuvenated shoots, the plants were cut 20 cm from the stem base and fertilized every 15 days and 1 day before shoot collection with 3 ml of 50 % Hoagland nutrient solution per pot (Hoagland and Arnon 1950). The clonal mini-garden was composed of a total of 55 half sib stock plants.

A clonal garden was formed simultaneously in the field and consisted of 40 stock plants distributed on a 3 × 3 m spacing. Stock plants were individually identified as well as cuttings and mini-cuttings collected from those stock plants for the correlation analysis.

The following parameters were recorded from the stock plants of the clonal garden before mini-cutting collection: diameter, height, area of the plant from which the cuttings were obtained, age of the branch from which the cutting was collected, area of the shoot used for preparing the cuttings, and number, diameter, and height of shoots. The following parameters were recorded before collecting the two-and-a-half year old mini-cuttings: survival percentage, diameter, height and number of stock plants of the clonal mini-garden, and diameter and height of shoots. The biometric data of stock plants from the clonal garden and mini-garden were recorded on the cutting and mini-cutting collection date as well as the shoots that were collected (Table 1.) With the aim of promoting greater etiolation in the clonal mini-garden material used to provide propagules, the density of stock plants was increased to256 plants/m^2. This resulted in partial shading of the apices of the stock plants sprouts (>10 cm in length) and total shading in the remaining shoot areas. The method described by Souza et al. (2009) was modified to prepare the mini-cuttings and

Table 1 Mean values of diameter at stem base, height and number of shoots of stock plants and mean values of diameter at stem base and height of shoots of stock plants from the clonal mini-garden and the clonal garden

Stock plants mini-garden			Shoots mini-garden		Stock plants clonal garden			Shoots clonal garden	
Diameter	Height	Shoots	Diameter	Height	Diameter	Height	Shoots	Diameter	Height
5.85 mm	49.9 cm	5.25	1.55 mm	6.94 cm	40.95 mm	167 cm	18	2.12 mm	10.19 cm

cuttings. Juvenile shoots from the clonal garden and rejuvenated sprouts (via cutting of the stock plant at the stem base—cut stock plant) of the clonal mini-garden were collected to induce rooting. A portion of the spring shoots of the same year was used, including the apical bud and at least three internodes of approximately 6 cm in length. When sprouts were >6 cm in length, the basal portion was discarded. The remaining mini cutting had two or three leaves, that were reduced to 50 % of their total area. Then, the mini-cuttings were treated with the systemic fungicide CHEMCARB® (50 % carbendazim) of the company CHEMIPLANT, at 1 cm^3 L^{-1} dilution.

The experimental design consisted of randomized complete blocks with four replications, five IBA treatments and nine clonal seedlings per experimental unit, which was composed of randomly mixed genotypes. The mini-cuttings were submitted to five treatments with IBA dissolved in potassium hydroxide (KOH 1 mol L^{-1}) applied as liquid at the base of each mini-cutting and cutting for 15 s at the following concentrations: 0, 3,000, 4,500, 6,000 and 7,500 mg L^{-1}. The cuttings were subjected to three treatments: 0, 3,000 and 6,000 mg L^{-1}. Cuttings and mini-cuttings were then planted in Dassplastic® plastic tubes (110 cm^3) containing commercial substrate number 2 of Dynamics® purchased from Agri Service, fertilized every 15 days with 2 ml per tube of Bolle Jones nutrient solution (Chaves et al. 2006). The tubes were then transferred to a rooting chamber with intermittent mist and humidity above 80 %, mean maximum temperature of 34.86 °C, mean temperature of 23.57 °C, solar radiation of 20,48 MJ m^{-2} day and mean effective sunshine duration of 9.32 h/day. Survival and percentage of rooted mini-cuttings and cuttings were recorded after 40 days in the rooting chamber. The following vigor parameters were recorded in cuttings, mini-cuttings and emerging clonal seedlings: height, stem diameter, fresh weight of aerial part, number of leaves, number of leaflets, and fresh matter weight, number and total length of roots. The roots were quantified via image digitalization using Image Pro Plus® software.

The Lilliefors and Cochran tests were used to determine normality and homogeneity of variances, respectively. The results obtained were subjected to an ANOVA. Survival and rooting data were arcsine transformed ($\sqrt{X/100}$), data of number of leaves, leaflets and roots were transformed via $\sqrt{(X+1)}$ and data on fresh mass and root length were transformed via $\log_{10}(X+1)$, following Zimmermann (2004). The differences were subjected to regression adjustments. For the quadratic regressions the optimum IBA dose was determined where the derivative was equal to zero. The optimal dose for each variable observed is presented in Results. Data on stock plants from the clonal garden and mini-clonal garden were correlated with data on rooting parameters of cuttings and mini-cuttings to determine which factors of the stock plant and the shoot may have influenced rooting. For this analysis, the Pearson correlation coefficient was used. Statistical analyses were performed using the statistic software InfoStat (Di Rienzo et al. 2010) and ASSISTAT Software (Silva and Azevedo 2009).

Table 2 Percentage of rooting and survival of cuttings and mini-cuttings of *P. alba* treated with different concentrations of IBA

IBA concentration (mg L^{-1})	Vegetative propagation technique			
	Cuttings		Mini-cuttings	
	Rooting (%)	Survival (%)	Rooting (%)	Survival (%)
0	4.54	18.18	100	94.44
3,000	18.18	22.72	100	97.22
4,500	–	–	100	97.22
6,000	0	18.18	100	97.22
7,500	–	–	98	94.44

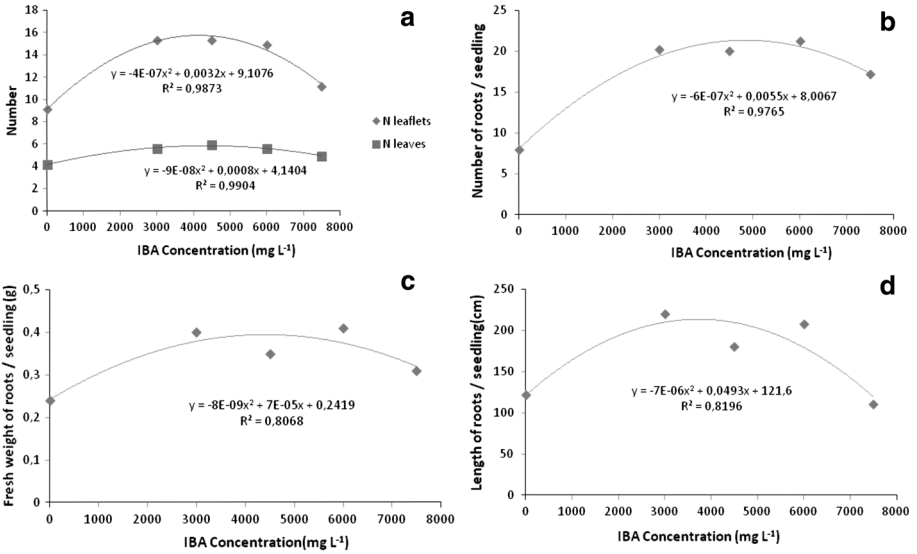

Fig. 1 Effect of indolebutyric acid (*IBA*) concentration on variables of the aerial and root parts of the clonal seedling of *P. alba* 40 days after rooting induction by mini-cuttings

Results and discussion

Survival of stock plants of the clonal mini-garden and garden was 100 %. Rooting and survival percentages of cuttings and mini-cuttings for each IBA concentration are shown in Table 2. Those percentages were higher for mini-cuttings than for cuttings. The high rooting percentage obtained was probably due to the rejuvenating and etiolation treatment as well as other factors that favor rooting via the mini-cutting technique. Previous results from the use of cuttings and mini-cuttings in this species showed very variable rooting rates (Klass et al. 1985; De Souza and Felker 1986; Klass et al. 1987; Ewens and Felker 2003; Felker et al. 2005; Felker 2009; Oberschelp and Marcó 2010). Ewens and Felker (2003) consider that the propagation technique using cuttings is not viable for the commercial-scale vegetative propagation of *P. alba*.

The present results suggest that the juvenility of the material may have influenced the rooting. Xavier et al. (2009) stated that although most juvenile materials have higher rooting capacity, they require greater control of environmental conditions during the entire process. Another factor that may have been involved in rooting success was etiolation produced in the explants, which is directly related to a lower degree of lignification and to physiological alterations of vegetative material (Maynard and Bassuk 1988). Bassuk et al. (1985) demonstrated that etiolation in stock plants of *Fagus sylvatica*, *Carpinus betulus* and *Pinus strobus* significantly increased rooting of cuttings of those species. In work involving mini-cuttings of *Eucalyptus globulus* hybrids, Borges et al. (2011) observed that reduced lignification of tissues promoted higher rooting rates.

The use of IBA influenced the number of leaves and leaflets (Fig. 1a), number (Fig. 1b), weight of fresh matter (Fig. 1c) and total length of roots (Fig. 1d). IBA application increased mini-cutting rooting 40 days after cutting and possibly accelerated the formation and development of the root system. However, rooting showed a quadratic behavior as a function of concentration, with a reduction at higher concentrations. Some clones of forest species require the hormone stimulus to strengthen rooting, as demonstrated by Brondani et al. (2010); Goulart et al. (2008) and Titon et al. (2003) for clones of *Eucalyptus benthamii* × *E. dunnii*, *E. grandis* × *E. urophylla* and *E. grandis*, respectively. However, Oberschelp and Marcó (2010) did not find significant differences among IBA concentrations used in rooting and height of *P. alba* seedlings in semi-woody cuttings and height of herbaceous cuttings. The use of IBA did not affect rooting in hybrids of *E. globulus* (Borges et al. 2011) or in *Toona ciliata* (Souza et al. 2009). This lack of effects of IBA may be due to the endogenous auxin levels of the plant material, whose juvenility was sufficient to induce rooting with no need for auxin application. The variation in the number of leaves of seedlings after 40 days under different IBA concentrations showed that the number increased with increasing IBA concentration up to the optimum value of 4,306.7 mg L^{-1} after which the number of leaves started to decrease. The number of leaflets also increased with increasing IBA concentration up to its optimum value of 4,113.5 mg L^{-1}, subsequently decreasing with increasing IBA concentration.

The same phenomenon was observed in relation to the variables analyzed in the root system. Number, fresh matter weight and length of seedling roots exhibited the best responses at *optimum point* concentrations of 4,840, 3,480 and 3,726 mg L^{-1}, respectively, after which those parameters started to show a negative effect. A similar behavior was observed in clones of *Eucalyptus* (Goulart et al. 2008). Higher IBA concentrations also had a negative effect on rooting of herbaceous cuttings of *P. alba* (Oberschelp and Marcó 2010). The IBA concentration did not affect seedling diameter. The correlation analysis showed a positive relationship between number of leaflets and fresh matter ($r = 0.64$), number ($r = 0.62$ and length ($r = 0.61$) of roots (p value <0.001). The presence of leaves on a cutting stimulates rooting because leaves provide carbohydrates and hormones (Xavier et al. 2003). Statistically significant correlations were observed between shoots used to prepare the mini-cuttings and the clonal seedlings they produced with all the characteristics evaluated. All the correlations were positive, with the most outstanding ones being those of shoot height with: total root length ($r = 0.62 < 0.001$), number of leaflets ($r = 0.56$) and height of clonal seedlings ($r = 0.56$) with a p value <0.001. These results suggest that, regardless of the standardization of mini-cutting size, the use of the most developed sprouts allowed a faster root growth in clonal seedlings, probably related to reserve levels in shoots. The same phenomenon was observed by Ferreira et al. (2012) and Souza et al. (2009) in rooting of *T. ciliata*, and by Freitas et al. (2010) in *E. urophylla*. According to Gomes (1987), reserves are essential for propagule survival until rooting and

further development because they facilitate root emergence and increase photosynthesis. With higher reserves and C/N ratio, propagule rooting is higher and seedling growth is faster, as also reported by Paiva and Gomes (1995).

Conclusions

Development of clonal silviculture using the minicutting technique offers a viable alternative for commercial production of clonal seedlings of *P. alba.* Further research is necessary to validate the mini-cutting technique in other species of silvicultural interest.

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