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# Rooting and establishment of *Limoniastrum monopetalum* (L.) Boiss stem-tip cuttings

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Rooting of *Limoniastrum monopetalum* stem-tip cuttings and establishment of produced plantlets were investigated in order to facilitate the use of the species in urban and suburban areas, and historical Mediterranean landscapes as an ornamental plant. Cuttings collected in winter or spring rooted at higher percentages than those collected in summer or autumn. Water-ethanol solutions containing 1000 to 3000 mg L<sup>-1</sup> indol-3-butyric acid (IBA) were more effective in rooting induction than the controls that did not contain IBA or powder IBA for softwood cuttings. Dipping for 1 min in an IBA solution was more effective than dipping for 5 min. Ethanol used in the IBA-solutions inhibited rooting depending on dipping time. All plantlets survived after transplantation. Plantlets transplanted on a peat-perlite (2 : 1, v/v) mixture and fertilized once a month, with 2 or 4 g L<sup>-1</sup> water-soluble complete fertilizer, had bigger elongation and produced more axillary shoots than those transplanted on a mixture amended with grape marc compost or enriched peat. Pinching of the main shoot one month after transplantation promoted axillary shoots production and a more compact plant shape.

**Key words:** *Statice monopetala* L., asexual propagation, rooting hormone indol-3-butyric acid (IBA), substrate, fertilization, ethanol rooting inhibition.

# INTRODUCTION

*Limoniastrum monopetalum* (L.) Boiss (*Statice monopetala* L., Plumbaginaceae) is a small, evergreen shrub, with much-branched, leafy stems, native in coastal sands and salt marshes in southern Greece and other Mediterranean countries (Blamey and Grey-Wilson, 1993). Due to its fleshy, silvery blue-green leaves and its

impressive bright pink, drying violet, inflorescences during summer, it is used as an ornamental plant recently. Its adaptation to a variety of environmental stresses like salinity, water deficit, intense radiation or high temperatures (Neves et al., 2008) and its growth on soil poor in organic matter content (Salama, 2007), make

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Author(s) agree that this article remains permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> *L. monopetalum* an ideal plant for xeriscaping and landscape architecture in semi-arid Mediterranean areas, especially in poor, saline, neglected or degraded soils. Its ecological value, as sand accumulator, salt tolerant, windbreak (Salama, 2007) and inhibitor of soil erosion should not be ignored, while it can grow in oil-contaminated soils (Hussein and Terry, 2002) and has the potential of phytoremediation of heavy metals from polluted sites (Cambrollé et al., 2013; Manousaki et al., 2014).

*L. monopetalum* is rich in nutritive values and thus mass production of its vegetative yield could be raw material for fodder industries (Neves et al., 2007; Zahran and El-Amier, 2013). Moreover it is rich in phenolics, so it could constitute a source of natural antioxidants for human consumption, as well as for agro-food, cosmetic and pharmaceutical industries (Trabelsi et al., 2010, 2012, 2013).

The ability of L. monopetalum to grow in harsh environments along with its ornamental characteristics, led to the investigation of its asexual propagation aiming to introduce it as an ornamental plant, in urban and suburban areas and historical Mediterranean landscapes. There is no relevant information in the literature till now. The asexual propagation by stem cuttings is a simple and easily applied method of plant propagation. However, experimentation for each specific plant is necessary in order to determine the appropriate rooting hormone treatment, as well as cutting collection period. It is well established that exogenous application of auxin accelerates the rates of rooting, increases final rooting percentage and the number of produced roots in leafy cuttings (Leakey, 1990; Larson, 1992; De Klerk et al., 1999), which could be attributed to the translocation of carbohydrates and other nutrients to the rooting zone (Middleton et al., 1980; Leakey et al., 1982). However, exogenous application of auxin may be promotive, ineffective or even inhibitory for the rooting of cuttings, depending on the endogenous level of growth-regulating substances (Haissig, 1979) or the tissue sensitivity (Visser et al., 1996). Relatively high concentrations of auxins have been reported to be inhibitory to rooting, indicating that in many species, optimal concentrations for rooting have to be defined (Leakey et al., 1982). The time of collecting cuttings plays an important role in rooting success and the development of cuttings (Klein et al., 2000). This may be related to changes in the endogenous plant growth regulators or carbohydrate conditions of cuttings and the environmental conditions in nursery (Abdou et al., 2004; Elgimabi, 2008).

The aim of this study was (a) to define the appropriate season for cutting collection, (b) to determine the appropriate rooting-hormone concentration and the duration of hormone treatment (dipping time), in order to improve rooting of cuttings and (c) to test various growth mixtures and fertilizations, in order to accelerate growth of rooted cuttings, so that a complete production protocol will be presented.

#### MATERIALS AND METHODS

#### Rooting of cuttings

Stem-tip cuttings, 12 to 14 cm long, were excised from native L. monopetalum adult plants (about eight years old), grown wild in Piraeus (37°56'56.1"N, 23°38'6.5"E), in January (Figure 1a), April (Figure 1b), August (Figure 1c), and October (Figure 1d), indicative of four seasons, that is, winter, spring, summer and autumn. The experiments were carried out in two years, 2013 and 2014, but due to the similarity of the results, only data of one year are presented. In winter, cuttings were excised from the new growth, which had just sprouted. During spring, new shoots were elongated and immature inflorescences were formed at the top of some shoots, while during summer, shoot elongation was retarded and plants were in blossom. In spring and summer, cuttings were collected from non-flowering shoots. Shoot growth stopped during autumn and collected cuttings were more lignified. Generally, cuttings bear short (1.0 to 4.0 cm) axillary shoots; all leaves and axillary shoots were removed from the basal half of the cuttings (Figure 1a to d). They were treated with IBA in the form of rooting powder for herbaceous/softwood cuttings Routon DP (0.066% w/w IBA in talcum, Coordination Company of Agricultural Enterprises SA, Greece), as well as with IBA ethanol-water (1 : 1, v/v) solutions, at concentration 0 (control), 1000, 2000 or 3000 mg L<sup>-1</sup>, for two dipping times, 1 or 5 min. The bases of the cuttings were immersed (around 1.5 cm of the bottom) in the IBA solution and then placed for rooting in plastic square plug trays (cell dimensions: 5.0 × 5.0 × 5.0 cm), containing a peat (High-more with adjusted pH up to 5.5 to 6.5, Klasmann-Delimann Gmbh, Geeste, Germany) and perlite (particles diameter 1 to 5 mm, Perloflor, ISOCON S.A., Athens, Greece) mixture 1 : 1 (v/v), in a mist system (spraying 15 s per 15 min from May to September or per 30 min from October to April; substrate temperature 22°C maintained by thermostatically controlled electric heating cable) for two weeks and then on a heated-glasshouse bench (37°58'53.94"N, 23°42'25.01"E) (Figure 2). Three replications with seven cuttings each were used per treatment. Rooting percentages were evaluated every two weeks for eight weeks, checking cutting resistance in pulling and root emergence through the hole at the bottom of each planting cell.

Based on initial results, where an inhibitory effect of ethanol on rooting was observed, an additional experiment was held in order to test the effect of ethanol on rooting of cuttings. Thus, the base of cuttings collected in the second half of February (end of winter) was dipped in an ethanol-water (1 : 1, v/v) solution for 1, 2.5, 5 or 10 min, as well as in plain water for 1 or 5 min (controls). Three replications with ten cuttings each were used per treatment, and rooting percentages were evaluated after eight weeks.

#### Establishment of rooted cuttings

Rooted cuttings (Figure 1e) were transplanted to various mixtures in plastic pots (1.3 L), and received various fertilizations and were maintained in the glasshouse. Their growth was evaluated on a monthly basis for three months, recording the length increase of the main shoots and the number of the axillary shoots. In all experiments, three replications with seven plants each were used per treatment.

Plants produced by spring cuttings were cultured either on a peat-perlite 2 : 1 (v/v) mixture and were fertilized once a month with 2 or 4 g L<sup>-1</sup> water soluble fertilizer (Nutrileaf 60, 20-20-20, Miller Chemical and Fertilizer Corp., Hanover, PA, USA), 100 ml of solution per plant, or on a peat-perlite-grape marc compost 1 : 1 : 1



**Figure 1.** Typical stem-tip cuttings of *Limoniastrum monopetalum* collected during winter (a), spring (b), summer (c) and autumn (d), as well as 8-weeks old rooted cutting (e) (Arrow points out an axillary shoot). Typical growth of rooted cuttings collected in spring (f) and summer (g), three months after transplantation, as well as of winter cuttings, three months after pinching (h). Marked transplantation mixtures (v/v): (A) peat-perlite 2 : 1, fertilization per 30 days, 4 g L<sup>-1</sup>, (B) peat-perlite 2 : 1, fertilization per 30 days, 2 g L<sup>-1</sup>, (C) peat-perlite-grape marc compost 1 : 1 : 1, without fertilization, (D) peat-perlite-grape marc compost 3 : 2 : 1, without fertilization, and (E) enriched peat-perlite 2:1, without fertilization. Size bars = 10 cm.

(v/v) mixture, in which fertilization was not applied. Grape marc compost was produced locally, as described in Papafotiou et al. (2013), and had pH 7.8, EC 1287 µmhos/cm, N 2.01% (by volume),

P 1.464 mg kg<sup>-1</sup>, K 15.190 mg kg<sup>-1</sup>, Mg 2.013 mg kg<sup>-1</sup> and Ca 3.667 mg kg<sup>-1</sup>. The experiment lasted from July to the end of September 2013.



Figure 2. Monthly average temperatures (T) in the glasshouse.

Plants produced by summer cuttings were cultured either on a peat-perlite 2 : 1 (v/v) mixture and were fertilized monthly with 2 or 4 g L<sup>-1</sup> Nutrileaf 60, or on a peat-perlite-grape marc compost 3 : 2 : 1 (v/v), without fertilization. The experiment lasted from October 2013 to the end of December 2014.

Plants produced by winter cuttings were transplanted either on a peat-perlite 2 : 1 (v/v) mixture and were fertilized monthly with 2 or 4 g L<sup>-1</sup> Nutrileaf 60, or on an enriched peat (with adjusted pH up to 5.5 to 6.5, N-P-K 14-10-18 of 1.0/1.5 kg m<sup>-2</sup>, Klasmann-Delimann Gmbh, Geeste, Germany) and perlite 2 : 1 (v/v) mixture, without fertilization. One month after transplantation, the main shoot of the plants was pinched (final height 10 cm) and the first fertilization was applied; the axillary shoots were not elongated during the rooting period and thus only the main shoot was pinched. One month later, data recordings started. The experiment lasted from March to the end of June 2014.

#### Statistical analysis

The completely randomized design was used in all experiments. The significance of the results was tested by either one-, two- or three- way analysis of variance (ANOVA) and the means of the treatments were compared by Student's *t* test at p < 0.05 (JMP software, SAS Institute, Cary, NC, USA). The data on percentage were statistically analyzed after arcsine transformation. The standard error (SE) of the mean of each treatment was calculated.

# **RESULTS AND DISCUSSION**

#### **Rooting of cuttings**

Three-way ANOVA of cuttings' rooting percentages showed significant interactions between season of cutting collection, IBA solution concentration and dipping time (3way ANOVA results not presented), so rooting data were analyzed separately for each season using two-way ANOVA.

Cuttings collected in winter rooted at 100% in all treatments regardless of IBA application (Table 1). In

spring, there was significant interaction between the main factors of the experiment. As in winter, high rooting percentages were observed that reached 100%, with the exception of cuttings dipped for 5 min in the control or in the 3000 mg L<sup>-1</sup> IBA solution (Table 1). In summer, there was also significant interaction between the main experimental factors. Cuttings rooted at a quite high percentage after dipping their base in a 1000 or 2000 mg L<sup>1</sup> IBA solution for 1 min, while increasing IBA concentration or dipping time reduced the response; particularly, the latter inhibited rooting, as can be seen by the comparison with the 1-min control (Table 1). In autumn, both IBA concentration and dipping time affected response; IBA application increased rooting the significantly, while longer dipping time reduced rooting. Similarly to summer, cuttings rooted at higher percentage after dipping their base in an IBA solution for 1 min compared to a 5-min dipping; maximum rooting was induced by the lower IBA concentration, but rooting was high in all three concentrations tested (Table 1). Comparison of the two controls indicates that the negative effect of 5-min dipping on rooting could be attributed to the ethanol content of the solution and not to a prolonged exposure to IBA. This indication was confirmed in the additional experiment, carried out to test a possible ethanol effect on rooting. Thus, cuttings treated with an ethanol-water solution for various dipping times rooted at higher percentage after dipping in ethanol-water solution for 1 min or the water controls as compared to those that were dipped in ethanol-water solution for longer time, 2.5, 5 or 10 min (Figure 3). Inhibition of rooting in stem cuttings by ethanol has been indicated in some previous works, as well (Middleton et al., 1978; Chong et al., 1992; De Klerk et al., 1997).

There are no reports found in the literature on propagation of the two species of *Limoniastrum* genus, *L. monopetalum* and *Limoniastrum guyonianum*; a work on

IPA concentration/dimping time	Season of cutting collection			
IBA concentration/dipping time	Winter	Spring	Summer	Autumn
0 / 1	$100.0 \pm 0.0^{a}$	$93.4 \pm 6.6^{ab}$	$35.0 \pm 6.1^{bc}$	45.0 ± 12.2 <sup>bc</sup>
1000/1	$100.0 \pm 0.0^{a}$	$100.0 \pm 0.0^{a}$	$70.0 \pm 5.0^{a}$	$100.0 \pm 0.0^{a}$
2000/1	$100.0 \pm 0.0^{a}$	$100.0 \pm 0.0^{a}$	$70.0 \pm 5.0^{a}$	$85.0 \pm 6.1^{ab}$
3000/1	$100.0 \pm 0.0^{a}$	$100.0 \pm 0.0^{a}$	40.0 ± 6.1 <sup>b</sup>	90.0 ± 10.0 <sup>a</sup>
0/5	$100.0 \pm 0.0^{a}$	75.2 ± 6.4 <sup>b</sup>	$10.0 \pm 6.1^{\circ}$	15.0 ± 6.1 <sup>°</sup>
1000/5	$100.0 \pm 0.0^{a}$	$93.4 \pm 6.6^{ab}$	$10.0 \pm 6.1^{\circ}$	75.0 ± 7.9 <sup>ab</sup>
2000/5	$100.0 \pm 0.0^{a}$	$100.0 \pm 0.0^{a}$	15.0 ± 6.1 <sup>bc</sup>	$50.0 \pm 7.9^{bc}$
3000/5	$100.0 \pm 0.0^{a}$	75.2 ± 6.4 <sup>b</sup>	$10.0 \pm 6.1^{\circ}$	75.0 ± 11.2 <sup>ab</sup>
F dipping time	NS	-	-	**
F IBA concentration	NS	-	-	**
F interaction	NS	*	*	NS

**Table 1.** Effect of collection season, IBA solution concentration (mg L<sup>-1</sup>) and dipping time (min) on rooting percentage of *Limoniastrum monopetalum* cuttings.

Means  $\pm$  SE within a column followed by the same letter are not significantly different according Student's *t* test at p  $\leq$  0.05. \* and \*\*Significant at p  $\leq$  0.05 and p  $\leq$  0.01, respectively; NS: not significant at p  $\leq$  0.05.



#### Ethanol treatment

**Figure 3.** Effect of dipping time (min) in ethanol-water solutions (1:1, v/v) on rooting percentage of *Limoniastrum monopetalum* cuttings. Means  $\pm$  standard error (SE) followed by the same letter are not significantly different according Student's t test at  $p \le 0.05$ .

vegetative propagation of *Plumbago capensis*, a relative plant of the Plumbaginaceae family, showed that IBA or NAA application at 1000 to 3000 mg L<sup>-1</sup> was necessary for rooting of subapical cuttings collected during cold period (Hernández et al., 2007), resembling the results of the present work, while hard wood cuttings of this species showed better rooting results and vegetative growth characteristics when treated with 1500 and 2000 mg L<sup>-1</sup> IBA (Abdulrahman and Faizy, 2013). Similarly, in the salt desert shrubs *Atriplex canescens* and *Atriplex cuneata*,

cuttings treated with various concentrations of IBA (0.1 to 2.0% in talc powder) also rooted at higher percentages than the untreated ones, particularly in seasons where rooting ability of cuttings was low (Richardson et al., 1979), as shown in the present work, too. Thus, as in most cases of leafy cuttings (Leakey et al., 1990; Larson, 1992; De Klerk et al., 1999), exogenous application of auxin promoted rooting, probably due to the translocation of carbohydrates and other nutrients to the rooting zone (Middleton et al., 1980).

	Transplantation mixture				
Time (days)	Peat-perlite 2 : 1 (v/v), fertilization monthly (2 g L <sup>-1</sup> )	Peat-perlite 2 : 1 (v/v), fertilization monthly (4 g L <sup>-1</sup> )	Peat-perlite-grape marc compost 1 : 1 : 1 (v/v), no fertilization	F	
Main shoot	length increase (cm)				
30	$10.0 \pm 0.5^{a}$	$9.6 \pm 0.5^{a}$	$9.3 \pm 0.4^{a}$	NS	
60	$6.0 \pm 0.7^{a}$	$5.2 \pm 0.5^{a}$	$2.2 \pm 0.3^{b}$	**	
90	$9.5 \pm 0.8^{a}$	$9.1 \pm 0.4^{ab}$	$7.6 \pm 0.6^{b}$	*	
Axillary sho	ot number				
30	$4.3 \pm 0.3^{a}$	$3.9 \pm 0.3^{a}$	$4.1 \pm 0.3^{a}$	NS	
60	$5.3 \pm 0.2^{a}$	$4.2 \pm 0.3^{b}$	$4.3 \pm 0.3^{b}$	*	
90	$6.0 \pm 0.2^{a}$	$4.7 \pm 0.3^{b}$	$4.7 \pm 0.3^{b}$	*	

**Table 2.** Effect of transplantation mixture and fertilization on growth of spring cuttings over the three months of the establishment period (July to end of September).

Means  $\pm$  standard error (SE) within a line followed by the same letter are not significantly different according Student's t test at p  $\leq$  0.05. \* and \*\*Significant at p  $\leq$  0.05 and p  $\leq$  0.01, respectively; NS: not significant at p  $\leq$  0.05.

The superiority of winter may be due to the fact that new vegetation of L. monopetalum sprouts in the middle of winter, and cuttings collected during this period, may be richer in endogenous auxins produced at the active apex of the young shoot and transported basipetally to the base of the cutting acting as a trigger for rooting (Nordström and Eliasson, 1991). Higher rooting percentages of cuttings during growing season has been reported for other salt desert shrubs, such as A. canescens and A. cuneata, too (Richardson et al., 1979), while the opposite was shown for Artemisia tridentata, plant that can grow in very alkaline and dry soils, where peak rooting percentages of cuttings were produced in late winter and root formation was much reduced after the onset of growth in spring (Alvarez-Cordero, 1979). Ambient temperature is rather unlikely to have affected rooting, as temperature in the mist was guite constant.

Auxin concentrations exceeding a certain level have been reported to inhibit or reduce rooting ability of stem cuttings of various species (Leakey et al., 1982; Chong et al., 1992; Puri and Verma, 1996; Akwatulira et al., 2011), indicating that optimal concentrations for rooting have to be defined (Leakey et al., 1982; Chong et al., 1992).

Cuttings collected from spring to autumn and treated with powder IBA for soft-wood cuttings rooted at lower percentages (5 to 45%), compared to those that were dipped in 0 or 1000 mg L<sup>-1</sup> IBA solution (the latter contains similar quantity of rooting hormone to powder) for 1 min. Only those collected in winter, during which cuttings generally rooted easily at high percentages irrespectively of treatment, rooted at 90% (data not shown). These results are consistent with those obtained in various landscape shrubs and trees, where talc-IBA formulations were less effective than IBA in solution at comparable concentrations in rooting of stem cuttings (Chong et al., 1992). Regarding the required time for rooting, cuttings collected in winter and spring and dipped in solutions 1000 to 3000 mg  $L^{-1}$  IBA for 1 min (the best treatments throughout the year) rooted faster, reaching the maximum of their rooting percentage at only two weeks, compared to cuttings collected in summer or autumn, which reached their maximum rooting percentage after 6 to 8 weeks (data not shown).

# Establishment of rooted cuttings

Establishment of rooted cuttings was successful and all plantlets survived three months after transplantation independently of substrate and fertilization type, season and culture technique applied. Plantlets that were fertilized monthly with a water soluble fertilizer exhibited bigger elongation of the main shoot and, in general, produced more axillary shoots compared to those that were not fertigated, but instead their substrate was amended with grape marc compost or enriched peat (Tables 2 to 4 and Figure 1f to h). Grape marc compost is of high quality, and it is degraded very slowly providing good physical structure to amended mixture and releasing slowly its nutrients (Manios, 2004). Thus, plantlets cultured in the compost amended mixture probably had less nitrogen available to promote shoot elongation compared to those that were fertigated, particularly during periods with lower temperatures (November to January, Figure 2), when nitrogen release from compost was probably lower compared to periods with high temperatures (Agehara and Warncke, 2004). Similarly enriched peat provided much fewer nutrients to the plants compared to monthly fertilization (see materials and methods), resulting in the smallest shoot elongation (Table 4 and Figure 1h). During the hottest

	Transplantation mixture				
Time (days)	Peat-perlite 2 : 1 (v/v), fertilization monthly (2 g l <sup>-1</sup> )	Peat-perlite 2 : 1 (v/v), fertilization monthly (4 g l <sup>-1</sup> )	Peat-perlite-grape marc compost 3 : 2 : 1 (v/v), no fertilization	F	
Main shoot	length increase (cm)				
30	$4.6 \pm 0.2^{b}$	$7.3 \pm 0.3^{a}$	$4.0 \pm 0.2^{b}$	**	
60	$4.3 \pm 0.2^{b}$	$5.3 \pm 0.3^{a}$	$1.6 \pm 0.2^{\circ}$	**	
90	$2.7 \pm 0.1^{a}$	$3.1 \pm 0.2^{a}$	$1.3 \pm 0.1^{b}$	**	
Axillary shoot number					
30	$4.2 \pm 0.1^{a}$	$4.5 \pm 0.1^{a}$	$3.6 \pm 0.1^{b}$	**	
60	$5.9 \pm 0.1^{a}$	$6.2 \pm 0.2^{a}$	$4.3 \pm 0.3^{b}$	**	
90	$9.4 \pm 0.4^{a}$	$9.0 \pm 0.3^{a}$	$7.7 \pm 0.3^{b}$	**	

**Table 3.** Effect of transplantation mixture and fertilization on growth of summer cuttings over the three months of the establishment period (October to end of December).

Means ± standard error (SE) within a line followed by the same letter are not significantly different according Student's t test at  $p \le 0.05$ . \*\*Significant at  $p \le 0.01$ .

**Table 4.** Effect of transplantation mixture and fertilization on growth of winter cuttings pinched in April, one month after transplantation, over three months (April to end of June); fertilizations started after pinching.

	Transplantation mixture				
Time (days)	Peat-perlite 2 : 1 (v/v), fertilization monthly (2 g L <sup>-1</sup> )	Peat-perlite 2 : 1 (v/v), fertilization monthly (4 g L <sup>-1</sup> )	Enriched peat-perlite 2 : 1 (v/v), no fertilization	F	
Length of ma	in shoots (cm)				
30	$6.4 \pm 0.2^{b}$	$9.0 \pm 0.3^{a}$	$5.0 \pm 0.2^{c}$	**	
60	$9.0 \pm 0.2^{b}$	$12.4 \pm 0.5^{a}$	$5.4 \pm 0.2^{c}$	**	
90	$11.4 \pm 0.3^{b}$	$16.3 \pm 0.6^{a}$	$5.7 \pm 0.2^{c}$	**	
Main shoot n	umber				
30	$6.4 \pm 0.2^{a}$	6.1 ± 0.3 <sup>ab</sup>	$5.5 \pm 0.2^{b}$	*	
60	$6.8 \pm 0.2^{a}$	$7.0 \pm 0.3^{a}$	$5.6 \pm 0.2^{b}$	**	
90	$6.9 \pm 0.2^{a}$	$7.2 \pm 0.3^{a}$	$5.7 \pm 0.2^{b}$	**	

Means ± standard error (SE) within a line followed by the same letter are not significantly different according Student's t test at  $p \le 0.05$ . \* and \*\*: significant at  $p \le 0.05$  and  $p \le 0.01$ , respectively.

months of the year, July to August, (Figure 2), the dose of fertilizer applied did not seem to affect shoot elongation (Table 2), while during cooler periods (October to December and March to June) shoot elongation was promoted by the bigger fertilizer dose (Tables 3 and 4). Plantlets growth was characterized by strong apical dominance; thus, plantlets produced by spring or summer cuttings, which were not pinched, developed only one main shoot, bearing some axillary shoots, which were not elongated (Figure 1f). Apical dominance determines the degree of branching and the form of the shoot system (Cline, 1994; Leyser, 2003). Auxin, derived from the shoot apex and moving basipetally, inhibits growth of axillary buds, while cytokinin, thought to be derived from the roots, promotes their growth (Cline, 1994; Leyser, 2003). Tanaka et al. (2006) indicated that one role of auxin is to repress local biosynthesis of cytokinins in the nodal stem and that, after decapitation, cytokinins are locally biosynthesized in the nodal stem rather than in the roots. The reduced main shoot elongation during winter (December) probably restricted apical dominance and thus more axillary buds were developed at this period (Table 3), resembling the species behaviour in the wild, where new vegetation sprouts in winter.

Plantlets produced by winter cuttings were established during spring and had their main shoot pinched one month after transplantation in order to remove apical dominance. As expected, axillary shoots were elongated and so more main shoots per plant were formed three months later; produced main shoots had no axillary shoots on (Table 4 and Figure 1h). In this way, a rounded and thus more attractive plant shape was taken, particularly when plants were fertigated with the higher fertilizer dose (Figure 1h).

In general, plantlets transplanted on a mixture of peat and perlite and received fertigation were more vigorous than those transplanted on a mixture containing grape marc compost or enriched peat and were not fertilized. The higher the dose of fertilizer used, the taller the plants became, which is not always desirable because plants may bend easily or develop less attractive shape if not pinched. During the experiments, it was observed that plants fertilized with the higher dose were also less tolerant to water deficiency.

# Conclusion

A complete production protocol of *L. monopetalum* is provided. Rooting of cuttings was affected both by season and rooting hormone treatment. Winter and spring were the most appropriate collection seasons and dipping in 1000 or 2000 mg L<sup>-1</sup> IBA for 1 min was the best treatment. As regards establishment, growth of rooted cuttings was enhanced by transplantation on a peatperlite mixture (2:1, v/v) and monthly fertigation. Pinching was necessary for the production of branched plants.

# **Conflict of interests**

The authors have not declared any conflict of interest.

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