INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1274/201x/00–0–000–000
DOI: 10.17957/IJAB/15.0835
http://www.fspublishers.org

Full Length Article

Vegetative Propagation Method for *Ex situ* Conservation of *Sida ramoniana* Krapov. (Malvaceae): an Endemic Species with Medicinal Potential in Danger of Extinction

Evelyn Duarte[1,2,3*], Belén González Rondán[3], Rosana Rubenich[3] and Patricia Rocha[3]
[1]*National Council of Science and Technology Research (CONICET), Corrientes 3400, Argentina*
[2]*Executive Committee on Development and Technological Innovation (CEDIT), Posadas 3300, Argentina*
[3]*School of Forestry Sciences (FCF)-National University of Misiones (UNaM), Eldorado 3380, Argentina*
[*]For correspondence: evelynduarte1982@gmail.com

Abstract

Sida ramoniana is an endemic shrub of the Upper Parana Atlantic Forest with potential medicinal properties. Moreover, it is endangered due to the low frequency of individuals found in natural populations and its low seed viability. The aim of the present work was to evaluate the factors influencing the rooting ability in cuttings with and without apex, using auxins such as 3-indolebutyric acid (IBA) and 1-naphthaleneacetic acid (NAA) in the concentrations of 0, 100 and 1000 mg kg^{-1}. The time of immersion in water and in a fungicide solution at a dose of 20 g L^{-1} and the size of the cuttings: 5, 10 and 15 cm in length, were also evaluated. The best results were obtained in the absence of auxins, 4 h of immersion in fungicide solution and cuttings of 15 cm in length. In these treatments, the rooting capacity was between 90 to 100%, the average number of roots was higher than 10 with root length of 3.93 to 6.76 cm. It is concluded that *S. ramoniana* can be propagated asexually by apical and sub-apical cuttings without the need auxins induction. This methodology can contribute to the long-term conservation of this species, for future ecological or medicinal studies and to comply with the 15.5 target of the Sustainable Development Goals (SDGs), and the target 8 of the Convention on Biological Diversity (CBD). © 2018 Friends Science Publishers

Keywords: Vegetative propagation; Auxins; Immersion time; Cuttings; Conservation

Introduction

Misiones is one of the provinces with the highest floristic diversity in Argentina, with 3,166 vascular plants (Zuloaga and Morrone, 1999; Zuloaga *et al*., 1999; Biganzoli and Múlgura de Romero, 2004; Zuloaga *et al*., 2008), of which, 34 species are endemic, according to the PlanEAr (Endemic Plants of Argentina) database. Endemic species is any native plant exclusive to a particular area (Font Quer, 2001), therefore, restricted to a very specific geographic location, and there is no evidence that they can grow anywhere else on the planet.

Sida ramoniana Krapov. is an endemic shrub species of Misiones province-Argentina, and belongs to the Malvaceae family, which presents several genera with medicinal species, including *Abutilon, Gossypium, Hibiscus, Thespesia* and *Sida* (Krapovickas *et al*., 2008; Rahman and Gondha, 2014). In the genus *Sida*, three species widely used in popular medicine to treat various conditions such as inflammation of the oral mucosa, blenorrhea, asthmatic bronchitis and nasal congestion, stomatitis, asthma and nasal congestion have been recognized so far, using *Sida cordifolia* (Nawaz *et al*., 2009; Rahmatullah *et al*., 2011), for the uterine prolapse, *Sida acuta* (Biswas *et al*., 2011) and for leucorrhoea, *Sida rhombifolia* (Rahmatullah *et al*., 2009). These antecedents raise the hypothesis that *S. ramoniana* could also have some medicinal property.

Considering that endemic species with small populations have little dispersal capacity (Beissinger, 2000), analyzes genetic variation, hereditary structure and gene flow indicate that these populations present poor genetic diversity and are susceptible to extinction due to changes in the use of soil, habitat destruction and removal of individuals (Matthies *et al*., 2004; González-Astorga *et al*., 2005).

Conservation of highly diverse ecosystems is a recommended strategy for preserving biological wealth. It is therefore important to account with more studies on the different species that make up the ecosystem to understand their role in the ecological system and the processes that favor its persistence in the ecosystem (Margules and Nichols, 1988; Golubov *et al*., 2007).

Conservation, diversity and ecological restoration are terms that have had relevance in recent times. But ecosystems can only be maintained in time and space if they have high levels of biodiversity and ecological restoration is feasible when the full potential of local and regional species is

To cite this paper: Duarte, E., B.G. Rondán, R. Rubenich and P. Rocha, 201x. Vegetative propagation method for *ex situ* conservation of *sida ramoniana* krapov. (malvaceae): an endemic species with medicinal potential in danger of extinction. *Int. J. Agric. Biol*. 00: 000–000

conserved. If the ecosystem presents a high degree of disturbance and has lost its mechanisms of regeneration, it is necessary to apply a restoration with human intervention, to overcome the barriers that prevent the regeneration and recovery of the biological system (Vargas, 2011).

The 15.5 target of the Sustainable Development Goals (SDGs) aim to succeed the Millennium Development Goals adopted in 2015 by the international community through the United Nations, proposed to take urgent and significant action to reduce the degradation of natural habitats, halt the loss of biodiversity, and, by 2020, protect and prevent the extinction of threatened species. Meanwhile de Convention on Biological Diversity (CBD), updated and revised in 2010, with targets set to be achieved by 2020, in its 8 target, propose that at least 75% of threatened plant species should be conserved in *ex situ* collections, preferably in the country of origin, and at least 20% available for recovery and restoration programs (Sharrock and Jackson, 2017). Therefore, to assure long-term *ex situ* conservation, for species with small populations and restrictions on seed production, such as *S. ramoniana*, efforts should be taken, requiring vegetative propagation methods in order to obtain more individuals to carry out controlled crossings to increase seed availability and thus maintain the structure population genetics (León-Lobos *et al*., 2008; Feria-Arroyo *et al*., 2010). An *ex vitro* vegetative propagation method, such as rooting cuttings, become a viable technique, where its success is related to the size, type of cuttings and the use of growth regulators (San Miguel *et al*., 1999; Castrillón *et al*., 2008; Uribe *et al*., 2011). Therefore, the objective of this work is to generate a vegetative propagation protocol, using cuttings of different characteristics, to ensure the multiplication and conservation of *S. ramoniana* for future studies.

Material and Methods

The study was carried out at the Laboratory of Vegetative Propagation of the School of Forestry Sciences-National University of Misiones. The plant material used was *Sida ramoniana* Krapov. plants, harvested in the department of Oberá (province of Misiones, 27º 27' 55.6 "S - 55º 02' 07.3" W), in a juvenile state and transplanted in pots with composted pine bark as substrate and were kept under greenhouse conditions with manual irrigation in the pot until their sprout, in order to form stock plants. Once the plants reached maturity (Fig. 1) and after the first flowering, the plants were pre-treated with Zineb® fungicide (5 g L^{-1}) every three days, for two weeks to prevent fungal contamination and assure the health of the sprouts. After that period, cuttings with and without apex were harvested. Three types of trials were performed, considering the use of different concentrations of growth regulators, the time of immersion in water, fungicide and the size of the cutting.

Growth Regulator Experiment

Cuttings with and without apical meristem, immediately after harvesting, were immersed in solutions containing naphthaleneacetic acid (NAA) and indolebutyric acid (IBA) in the concentrations of 0, 100 and 1000 mg kg^{-1}; for 4 h and then in fungicide solution (20 g L^{-1}) for 20 min.

Fungicide Preventive Experiment

The basal ends of cuttings with and without apex were immersed in water and a solution with fungicide (20 g L^{-1}) for 20 min, 4 and 24 h.

Cuttings Sizes Experiment

The basal ends of cuttings with and without apex, 5, 10 and 15 cm in length were immersed in a solution with fungicide (20 g L^{-1}) for 20 min.

Cuttings Growth Conditions

After each treatment, the cuttings were placed in 60 cm^3 capacity containers, with composted pine bark as substrate, and a dose of 30 g m^{-3} of slow release fertilizer (Plantacote Plus ®). The trays with the cuttings were kept in a greenhouse with automated irrigation, where provided with 40.89 $mm.m^{-2}$ of water for 24 h with irrigations by microsprinkling.

Experiments 1 and 2 were established in completely randomized experimental design, with a 2 × 2 × 3 factorial arrangement of the treatments and the third in 2 × 3 factorial arrangements respectively. The experimental units for each experiment were 5 cuttings, with 6 replicates for a total of 30 cuttings for each treatment.

The evaluation of the experiments was performed at 45 days after installation. The variables studied were rooting percentage, number and length of roots (cm) and number of shoots. The data were analyzed with an analysis of variance (ANOVA), the normality assumption was verified using the Shapiro-Wilk test and the variables that did not show normality were transformed with $\sqrt{(x+0.5)}$, and subsequently subjected to analysis of variance and variables with significant differences in the F test had their means compared with the Tukey test at 5% probability of significance (P<0.05), using the software INFOSTAT (Di Rienzo *et al*., 2016).

Results

The different treatments tested with NAA or IBA addition showed significant differences (P<0.0085) with respect to the control (water) in all variables evaluated. The application of IBA as well as the high concentrations of NAA had a negative influence on the percentage of rooting. Nevertheless cuttings with or without apex treated with 100 mg kg^{-1} NAA, showed the highest number of shoots per cuttings, high rooting capacity, root number and root length, but no significant difference with the control treatment (Table 1 and Fig. 1).

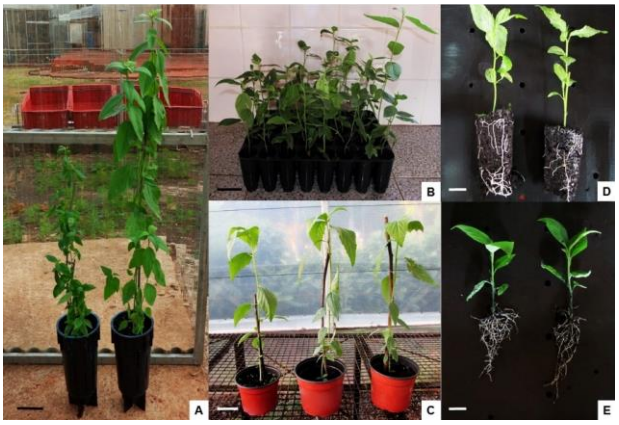

Fig. 1: Rooting of *S. ramoniana*. A) Adult plants can reach 2 m. B) Tray with cuttings at the end of the experiment. C) Root cuttings after 4 months. D) Cuttings without shoot tips in water (left) and NAA 100 mg kg^{-1} (right). E) Shoot tips cuttings in water (left) and NAA 100 mg kg^{-1} (right). The bar indicates 10 cm in A, 5 cm in B-C and 2 cm in D-E

Regarding time and immersion solution treatment, statistically significant differences (P<0.0036) were observed between them for all variables evaluated, without significant interaction between type of cutting and immersion time. The cuttings without apex induced in fungicide for 4 h, showed the highest value for all variables, percentage of rooting (100%), number of roots (30.12 ± 7.14), root length (6.76 ± 2.44) and number of shoots (1.42 ± 0.51) (Table 2).

The least desirable treatment to root *S. ramoniana* cuttings was the one cuttings with no apex subjected to fungicide solution for 24 h, where rooting percentage was only 30%, number of roots 4.10 and the average length of roots of was 1.38 cm. Long times exposure to fungicide solution of 20 g L^{-1}, induced a high level of mortality.

Cuttings size, showed no significant differences for the roots number and length variable, meanwhile significant difference were observed for shoot number (P<0.0001), where the cuttings with no apex had the highest number of shoots and as the size of the cutting increased, the number of shoots increased. The rooting percentage was higher than 90% in all treatments, the number of roots varied from 20 to 30 and the average length of roots between 4 and 5 cm (Table 3). However, the lowest percentage of rooting occurred in cuttings of 5 cm in length, while the highest percentages were observed in cuttings of larger size (10 - 15 cm).

In all treatments tested either with auxins, time and immersion solution, and size of cuttings, the percentage of survival showed equal to the percentage of rooting, *i.e.*, all cuttings that survived succeeded in rooting.

Discussion

The genus *Sida* belongs to the Malvaceae family, comprises of grasses, shrubs and rarely trees (Krapovickas *et al*., 2008) and can be propagated by both seed and cuttings (Patel, 2014). The species may present different endogenous auxin contents, some may require the exogenous addition of rooting hormones to induce rhizogenesis, such as *Ceiba pentandra*, which requires high IBA contents to be able to root a stem cutting. Meanwhile, species such as, *Hibiscus rosa-sinesis* and *Commersonia adenothalia* do not need the incorporation of auxins to achieve the morphogenic process (Moreno *et al*., 2009; Inga *et al*., 2014; Patel, 2014).

In the case of *S. ramoniana*, the results showed that the external addition of auxins is not necessary, and the presence of IBA or high concentrations of NAA makes it difficult to survive and root cuttings. It can be deduced that it is a species with high endogenous auxin contents, which leads to a spontaneous rooting of a stem cutting (Hartmann *et al*., 2002). The auxins are the most appropriate to promote the adventitious rhizogenesis, and these phytohormones have the capacity to increase the transport of carbohydrates at the base of the cut, in this sense the most suitable to root are the NAA and IBA (Hartmann *et al*., 2002; Heberle, 2010). However, several studies have already shown that synthetic auxins and their different concentrations have not had a significant effect on the variables analyzed in present study (Ruiz-García *et al*., 2005; Latsague *et al*., 2009; Uribe *et al*., 2011).

Another important aspect to consider in this phase, is the quality of the shoot and the length, as well as if they come from woody or herbaceous species or are apical or sub-apical (Grattapaglia and Machado, 1998; Ramírez-Villalobos *et al*., 2004). The apical cuttings may be the best option to induce rhizogenesis, because in these portions of the plant, there is a higher concentration of endogenous auxins. However, sub-apical cuttings in some shrub species have shown higher rooting capacity than apical cuttings (Moratinos *et al*., 2008).

The results of the present work have demonstrated that the apical or sub-apical cuttings of *S. ramoniana* showed no interaction or significant difference between the treatments, namely different types of cuttings had a similar response for the same treatment in the evaluated variables. Similar results were reported by Niella *et al*. (2016), on cuttings of *Peltophorum dubium*. Also the cuttings size, showed no interaction or significant differences for any of the rooting variables, however, shoots with no apex, 10 and 15 cm in length, showed a greater number of shoots, therefore if large plants size in a short period of time is required, it is important to consider cuttings between 10 and 15 cm. Both the type of cutting and its size may be of vital importance to induce roots, because the highest concentration of auxins is found in the apical parts (Ramírez-Villalobos *et al*., 2004) and if the cuttings have an appropriate size to supplement the need for carbohydrates at the base of the cutting to allow for the dedifferentiation of tissue, will give rise to the formation of new roots (Moe and Andersen, 1988; Barbat, 2006).

The time of immersion in water or in a fungicide solution is relevant, especially when it is necessary to transport plant material for several h and to reach the destination without suffering dehydration or hyper-hydration.

Table 1: Effect of NAA and IBA addition on rooting capacity (%), shoots number, root length and number

Apex	Basal Inmersión Solution	Rooting (%)	Root Number	Root Length (cm)	Shoot Number
+	Water	70 ± 42.43 ab	8.91 ± 3.65 ab	2.73 ± 1.35 abc	0 ± 0 b
-	Water	90 ± 14.142 a	10.20 ± 4.19 a	3.93 ± 2.09 a	1.00 ± 0.66 a
+	NAA (100 mg kg^{-1})	70 ±42.43 ab	7.63 ± 2.83 ab	2.24 ± 1.91 abc	0 ± 0 b
-	NAA(100 mg kg^{-1})	90 ± 0 a	11.12 ± 4.34 a	3.51 ± 1.92 ab	1.31 ± 0.82 a
+	NAA (1000 mg kg^{-1})	0 ± 0 b	0 ± 0 c	0 ± 0 c	0 ± 0 b
-	NAA (1000 mg kg^{-1})	0 ± 0 b	0 ± 0 c	0 ± 0 c	0 ± 0 b
+	IBA (100 mg kg^{-1})	10 ± 4.16 b	1.20 ± 0.79 c	0.45 ±0.22 c	0 ± 0 b
-	IBA (100 mg kg^{-1})	30 ± 14.14 ab	3.21 ± 1.49 bc	0.93 ± 0.76 bc	0.13 ± 0.06 b
+	IBA (1000 mg kg^{-1})	30 ± 14.14 ab	4.00 ± 2.48 bc	1.90 ± 0.60 abc	0 ± 0 b
-	IBA (1000 mg kg^{-1})	30 ± 14.14 ab	3.73 ± 1.84 bc	0.91 ± 1.84 bc	0.36 ± 0.23 b

Mean ± Standard Deviation (SD). N=30. Different letters indicate significant differences between treatments (*P≤0.05*, Tukey Multiple Comparison Test). Signs +/- indicate presence or absence of apex

Table 2: Effect of immersion time on water and on a fungucide solution of 20 g L^{-1} on rooting capacity (%), shoots number, root length and number

Apex	Basal Inmersión Solution	Rooting (%)	Roots number	Root Length (cm)	Shoot Number
+	Water (20 min)	80 ± 0 ab	11.00 ± 1.89 cd	2.33 ± 1.81 bc	0 ± 0 d
-	Water (20 min)	80 ± 28.284 ab	13.90 ± 2.22 bcd	2.49 ± 1.71 bc	0.83 ± 0.42 abc
+	Water (4 h)	90 ± 14.142 ab	22.51 ± 14.78 abc	6.55 ± 3.15 a	1.00 ± 0.47 ab
-	Water (4 h)	90 ± 14.142 ab	19.80 ± 12.51 abcd	4.83 ± 2.66 abc	0.92 ± 0.32 abc
+	Water (24 h)	100 ± 0 a	31.52 ± 9.89 a	5.45 ± 0.68 ab	0 ± 0 d
-	Water (24 h)	100 ± 0 a	18.00 ± 8.68 abcd	5.59 ± 1.89 ab	1.16 ± 0.31 ab
+	Fungicide (20 min)	80 ± 0 ab	19.40 ± 12.88 abcd	4.89 ± 3.32 abc	0.54 ± 0.97 bcd
-	Fungicide (20 min)	90 ± 14.142 ab	24.21 ± 11.35 abc	5.42 ± 2.88 ab	1.00 ± 0 ab
+	Fungicide (4 h)	80 ± 28.284 ab	22.20 ± 15.32 abc	3.69 ± 2.67 abc	0 ± 0 d
-	Fungicide (4 h)	100 ± 0 a	30.12 ± 7.14 ab	6.76 ± 2.44 a	1.42 ± 0.51 a
+	Fungicide (24 h)	60 ± 28.28 ab	14.13 ± 4.78 abcd	3.45 ± 1.20 abc	0 ± 0 d
-	Fungicide (24 h)	30 ± 14.14 b	4.10 ± 2.87 d	1.38 ± 0.28 c	0.35 ± 0.13 cd

Mean ± Standard Deviation (SD). N=30. Different letters indicate significant differences between treatments (*P≤0.05*, Tukey Multiple Comparison Test). Signs +/- indicate presence or absence of apex

Table 3: Effect of cuttings size on evaluated variables on rooting capacity (%), shoots number, roots length and number

Apex	Cuttings length	Rooting (%)	Roots number	Roots length	Shoots Number
+	5 cm	90 ± 14.14 a	24.33 ± 15.38 a	4.34± 2.30 a	0.67 ±0.33 bc
+	10 cm	100 ± 0 a	20.00 ± 10.45 a	4.84 ± 2.20 a	0.67 ± 0.16 bc
+	15 cm	100 ± 0 a	26.66 ± 8.14 a	5.11 ± 1.74 a	0 ± 0 c
-	5 cm	90 ± 14.14 a	30.00 ± 18.17 a	4.59 ± 2.44 a	1.17 ± 0.40 abc
-	10 cm	100 ± 0 a	21.66 ± 11.09 a	4.65 ± 0.86 a	2.00 ± 1.09 ab
-	15 cm	100 ± 0 a	22.33 ± 12.42 a	4.42 ±1.04 a	2.33 ± 0.81 a

Mean ± Standard Deviation (SD). N=30. Different letters indicate significant differences between treatments (*P≤0.05*, Tukey Multiple Comparison Test). Signs +/- indicate presence or absence of apex

However, some species only need to be kept in water, to be able to root a cutting, due to which water is known as the universal rooting (Ramírez-Franco *et al*., 2011). Both the rooting type and the time of exposure of the cuttings in the rooting solution significantly influenced the rhizogenic process of *S. ramoniana* cuttings, an aspect also observed by Castrillón *et al*. (2008) in *Vaccinium meridionale*.

Vegetative propagation may be an advantageous method to obtain plantlets in small populations where sexual reproduction is diminished by incompatibility among individuals of the population because of a high degree of kinship (Nuortila *et al*., 2002; Honnay and Bossuyt, 2005). Vegetative propagation is a method that has enabled the reproduction of endemic species in danger of extinction (Uribe *et al*., 2011). Considering this technique as an alternative to propagate endemic species of the Upper Parana Atlantic Forest is vital because it could contribute to the conservation of these species. Since preserving the biological diversity of ecosystems is of paramount importance to society and especially to endangered species as a genetic reserve (Wilken, 1998; Albarrán *et al*., 2011).

Conclusion

Based on the results obtained, it is concluded that it is feasible to achieve the rooting of cuttings with or without apex of *S. ramoniana* without the addition of auxins. The time of exposure in water or fungicide can affect positively or negatively; because if the cuttings remain in water for 24 h, survival and rooting are favored, while if the cuttings remain

for 24 h in a fungicide solution of 20 g L^{-1} increases the mortality of cuttings. Therefore, it is more advisable to leave the cuttings for several hours in water rather than in a fungicide solution. A water immersion is sufficient to promote the development of roots and shoots in *S. ramoniana*. On the other hand, the size of the cuttings did not become relevant in the rhizogenic process of this species, since cuttings of 5, 10 or 15 cm in length promote good induction and development of roots. The research described in the present study, provides a simple but valuable technology at the moment of rescuing, propagating and restoring endemic species in a critical state of extinction, as is the case of *S. ramoniana*.

Acknowledgments

To the Faculty of Forestry Sciences for their collaboration on the execution of this research and CONICET and CEDIT for the grant awarded. To Dr. Keller Hector, for providing the plant material.

References

Albarrán, J.G., F. Fuenmayor, M. Fuchs, G. Martínez, A. Rodríguez, E. Manzanilla, E. Díaz, R. León and M. Torrealba, 2011. Estrategias biotecnológicas para la conservación de germoplasma en el INIA-CENIAP Venezuela. Caso yuca y musáceas. *Agron. Trop.*, 1: 85–94

Barbat, T., 2006. La multiplicación de las plantas. *Hortic. Int.*, 1: 32–43

Beissinger, S.R., 2000. Ecological mechanisms of extinction. *Proc. Nat. Acad. Sci*., 97: 11688–11689

Biganzoli, F. and M.E. Múlgura de Romero, 2004. Inventario florístico del Parque Provincial Teyú Cuaré y alrededores (Misiones, Argentina). *Darwiniana*, 42: 1–24

Biswas, K.R., T. Khan, M.N. Monalisa, A. Swarna, T. Ishika, M. Rahman and M. Rahmatullah, 2011. Medicinal plants used by folk medicinal practitioners of four adjoining villages of Narail and Jessore districts, Bangladesh. *Amer.-Eur. J. Sustain. Agric*., 5: 23–33

Castrillón, J.C., E. Carvajal, G. Ligarreto and S. Magnitskiy, 2008. El efecto de auxinas sobre el enraizamiento de las estacas de agraz (*Vaccinium meridionale* Swartz) en diferentes sustratos. *Agron. Colomb.*, 1: 16–22

Di Rienzo, J.A., F. Casanoves, M.G. Balzarini, L. González, M. Tablada and C.W. Robledo, 2016. InfoStat, versión 2016, Grupo InfoStat, FCA, Argentina, Universidad Nacional de Córdoba

Feria-Arroyo, T.P., E. Solano and A. García-Mendoza, 2010. Reevaluación del riesgo de extinción de cinco especies del género *Polianthes* L. (Agavaceae). *Act. Bot. Mex.*, 92: 11–28

Font Quer, P., 2001. *Diccionario de Botánica*, 2ª edición. Ediciones Península, Barcelona, España

Golubov, J., M.C. Mandujano, S. Arizaga, A. Martínez-Palacios and P. Koleff, 2007. Inventarios y conservación de Agavaceae y Nolinaceae. *In*: *En lo Ancestral hay Futuro: del Tequila, los Mezcales y otros Agaves*, pp: 33–52. Colunga, P., L. Eguiartey and A. García (eds.). Centro de Investigaciones Científicas de Yucatán, Conacyt, UNAM, Mérida, México

González-Astorga, J., A.P. Vovides, A. Cruz-Angon, P. Octavio-Aguilar and C. Iglesias, 2005. Allozyme variation in the extant populations of the narrowly endemic cycad *Dioon angustifolium* Miq. (Zamiaceae) from north eastern Mexico. *Ann. Bot.*, 95: 999–1007

Grattapaglia, D. and M. Machado, 1998. Micropropagação. *In*: *En Cultura de tecidos e transformação genética de plantas*, pp: 183–260. Torres, A.C. and L. S. Caldas (eds.). ABCTP/EMBRAPA CNPH, Brasília, Brasil

Hartmann, H.T., D.E. Kester, F.T. Davies and R.L. Geneve, 2002. *Plant Propagation: Principles and Practices*, 7th edition, Prentice Hall, New Jersey, USA

Heberle, M., 2010. Propagação *in vitro* e *ex vitro* de louro-pardo (*Cordia trichotoma* (Vell.) Arrabida ex Steudel). *Dissertação de Mestrado em Engenharia Florestal*, p: 76. Universidade Federal de Santa Maria, Santa Maria

Honnay, O. and B. Bossuyt, 2005. Prolonged clonal growth: escape route or route to extinction? *Oikos*, 108: 427–432

Inga, H., M. Pinedo, R. Farroñay, E. Paredes and D. Del Castillo, 2014. Enraizamiento de esquejes de lupuna (*Ceiba pentandra*) mediante Ácido indol-3-butírico (AIB), en Jenaro Herrera, Loreto. *Xilema*, 1: 57–61

Krapovickas, A., J.A. Tolaba and L. Novara, 2008. Malvaceae. *In*: *Aportes Botánicos de Salta-Ser. Flora*, pp: 1–136. HERBARIO MCNS, Facultad de Ciencias Naturales Universidad Nacional de Salta, Salta, Argentina

Latsague, M., P. Sáez and J. Yáñez, 2009. Efecto del ácido indolbutírico en la capacidad rizogénica de estacas de *Eucryphia glutinosa*. *Bosque*, 2: 102–105

León-Lobos, P., M. Rosas, P.C. Guerrero, A. Sandoval and M. Way, 2008. Conservación *Ex Situ* de la Flora de la Región de Atacama: Métodos, Experiencias y Desafíos Futuros. *In*: *En Libro Rojo de la Flora Nativa y de los Sitios Prioritarios para su Conservación: Región de Atacama*, pp: 347–356. Squeo, F.A., G. Arancio and R.J. Gutiérrez (eds.). Universidad de la Serena, Serena, Chile

Margules, C.R. and A.O. Nichols, 1988. Selecting networks of reserves to maximize biological diversity. *Biol. Conserv.*, 43: 63–76

Matthies, D.I., W. Brauer, T. Maibom and T. Tscharntke, 2004. Population size and the risk of local extinction: empirical evidence from rare plants. *Oikos*, 105: 481–488

Moe, R. and A. Andersen, 1988. Stockplant environment and subsequent adventitious rooting. *In: Adventitious Root Formation in Cuttings*, pp: 214–234. Davis, T.D., B.E. Haissig and N. Sankhla, (eds.). B.E. Dioscorides Press, Portland, Oregon

Moratinos, P., E. Flores and M. Ramírez-Villalobos, 2008. Enraizamiento de estacas de semeruco (*Malpighia qlabra* L. y M. emarginata Sessé & Moc. ex DC). *Rev. Fac. Agron.*, 3: 405–420

Moreno, N.H., J.G. Álvarez-Herrera, H.E. Balaguera-López and G. Fische, 2009. Propagación asexual de uchuva (*Physalis peruviana* L.) en diferentes sustratos y a distintos niveles de auxina. *Agron. Colomb.*, 3: 341

Nawaz, A.H.M.M., M. Hossain, M. Karim, M. Khan, R. Jahan and M. Rahmatullah, 2009. An ethnobotanical survey of Rajshahi district in Rajshahi division, Bangladesh. *Am.-Eurasian J. Sustain. Agric*., 2: 143–150

Niella, N., P. Rocha, B. Eibl, C. Schoffen, M. Martinez, P. Conti, M. Franco and L. Ayala, 2016. Propagación clonal de *Peltophorum dubium* (Caña fistola), *Myrocarpus frondosus* (Incienso), y *Cordia trichotoma* (Peteribi) para su conservación y domesticación. *Yvyrareta*, 21: 43–50

Nuortila, C.J., K. Tuomi and N. Laine, 2002. Inter-parent distance affects reproductive success in two clonal dwarf shrubs, *Vaccinium myrtillus* and *Vaccinium vitis-idaea* (Ericaceae). *Can. J. Bot.*, 80: 875–884

Patel, D.K., 2014. Some Medicinal and aromatic shrubs introduced in Herbal Garden for their Propagation and *ex situ* conservation. *J. Med. Plants*, 6: 9–12

PlanEAr (Plantas Endémicas de Argentina) http://www.lista-planear.org/ (Accessed 19 Jun 2017)

Rahman, A.H.M.M. and R. Gondha, 2014. Taxonomy and Traditional Medicine Practices on Malvaceae (Mallow Family) of Rajshahi, Bangladesh. *Open J. Bot.*, 2: 19–24

Rahmatullah, M., M.N.K. Azam, M.M. Rahman, S. Seraj, M.J. Mahal, S.M. Mou, D. Nasrin, Z. Khatun and M.H. Chowdhury, 2011. A survey of medicinal plants used by Garo and non-Garo traditional medicinal practitioners in two villages of Tangail district, Bangladesh. *Am.-Eurasian J. Sustain. Agric.*, 5: 350–357

Rahmatullah, M., A. Noman, M.S. Hossan, M.H. Rashid, T. Rahman, M.H. Chowdhury and R. Jahan, 2009. A survey of medicinal plants in two areas of Dinajpur district, Bangladesh including plants which can be used as functional foods. *Am.-Eurasian J. Sustain. Agric.*, 4: 862–876

Ramírez-Franco, L.A., Z. Amaya, C. María and J.M. Cotes-Torres, 2011. Evaluación de metodologías de enraizamiento de esquejes de tallo lateral en genotipos de *Solanum phureja*. *Rev. Fac. Cien. Bás.*, 2: 192–203

Ramírez-Villalobos, M., A. Urdaneta-Fernández and G. Vargas-Simón, 2004. Tratamientos con ácido indolbutírico y lesionado sobre el enraizamiento de estacas de icaco (*Chrysobalanus icaco* L.). *Agron. Trop.*, 2: 203–218

Ruiz-García, R., J.J. Vargas-Hernández, V.M. Cetina-Alcalá and Á. Villegas-Monter, 2005. Efecto del ácido indolbutírico (AIB) y tipo de estaca en el enraizado de *Gmelina arborea* Roxb. *Rev. Fitotec. Mex*., 4: 319–326

San Miguel, F., C. Michelangeli-De Clavijo, C. Basso and A. Trujillo, 1999. Enraizamiento de estacas de onoto. *Agron. Trop.*, 49: 60–79

Sharrock, S. and P.W. Jackson, 2017. Plant conservation and sustainable development goals: a policy paper prepared for the global partnership for plant conservation. *Ann. Missouri Bot. Gard.*, 102: 290–302

Uribe, M.E., R. Durán, G. Bravo, F. Mora, P. Cartes and C. Delaveau, 2011. Propagación vegetativa de *Berberidopsis corallina* Hook. f., una especie en peligro de extinción, endémica de Chile. *Gayana Bot.*, 2: 135–140

Vargas, J.O., 2011. Restauracion ecológica: biodiversidad y conservación. *Acta Biol. Colomb*., 2: 221–246

Wilken, P., 1998. La sustentabilidad forestal en Chile: aporte a una política integral de los bosques nativos y plantaciones exóticas. Santiago, Chile. *Serie documentos Experiencias de Cooperación*, 3: 62

Zuloaga, F. and O. Morrone, 1999. Catálogo de las Plantas Vasculares de la Argentina (Dicotyledoneae). *Monogr. Syst. Bot. Mo. Bot. Gard.*, 74: 98–353

Zuloaga, F., O. Morrone and D. Rodríguez, 1999. Análisis de la biodiversidad en plantas vasculares de la Argentina. *Kurtziana*, 27: 17–167

Zuloaga, F.O., O. Morrone and M.J. Belgrano, 2008. Catálogo de las plantas vasculares del Cono Sur (Argentina, sur de Brasil, Chile, Paraguay y Uruguay) Volúmenes I, II y III. *Monogr. Syst. Bot. Mo. Bot. Gard.*, 107: 1–983

(Received 10 November 2017; Accepted 29 June 2018)