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

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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, 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
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 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).

Cuttings Sizes Experiment

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

Cuttings Growth Conditions

After each treatment, the cuttings were placed in 60 cm³ 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.

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.

Fungicide Preventive Experiment

The basal ends of cuttings with and without apex 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.
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-sinensis 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-Garcia 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.
Duarte et al. / Int. J. Agric. Biol., Vol. 00, No. 0, 201x

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

<table>
<thead>
<tr>
<th>Apex</th>
<th>Basal Immersión Solution</th>
<th>Rooting (%)</th>
<th>Root Number</th>
<th>Root Length (cm)</th>
<th>Shoot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Water</td>
<td>70 ± 42.43 ab</td>
<td>8.91 ± 3.65 ab</td>
<td>2.73 ± 1.35 ab</td>
<td>0 ± 0 b</td>
<td></td>
</tr>
<tr>
<td>- Water</td>
<td>90 ± 14.42 a</td>
<td>10.20 ± 4.19 a</td>
<td>3.93 ± 2.09 a</td>
<td>1.00 ± 0.66 a</td>
<td></td>
</tr>
<tr>
<td>+ NAA (100 mg kg⁻¹)</td>
<td>70 ± 42.43 ab</td>
<td>7.63 ± 2.83 ab</td>
<td>2.24 ± 1.91 ab</td>
<td>0 ± 0 b</td>
<td></td>
</tr>
<tr>
<td>- NAA (100 mg kg⁻¹)</td>
<td>90 ± 14.42 a</td>
<td>11.12 ± 4.34 a</td>
<td>3.51 ± 1.92 ab</td>
<td>1.31 ± 0.82 a</td>
<td></td>
</tr>
<tr>
<td>+ NAA (1000 mg kg⁻¹)</td>
<td>0 ± 0 b</td>
<td>0 ± 0 c</td>
<td>0 ± 0 c</td>
<td>0 ± 0 b</td>
<td></td>
</tr>
<tr>
<td>- NAA (1000 mg kg⁻¹)</td>
<td>0 ± 0 b</td>
<td>0 ± 0 c</td>
<td>0 ± 0 c</td>
<td>0 ± 0 b</td>
<td></td>
</tr>
<tr>
<td>+ IBA (100 mg kg⁻¹)</td>
<td>10 ± 4.16 b</td>
<td>1.20 ± 0.79 c</td>
<td>0.45 ± 0.22 c</td>
<td>0 ± 0 b</td>
<td></td>
</tr>
<tr>
<td>- IBA (100 mg kg⁻¹)</td>
<td>30 ± 14.14 a</td>
<td>3.21 ± 1.49 bc</td>
<td>0.93 ± 0.76 bc</td>
<td>0.13 ± 0.06 b</td>
<td></td>
</tr>
<tr>
<td>+ IBA (1000 mg kg⁻¹)</td>
<td>30 ± 14.14 a</td>
<td>4.00 ± 2.48 bc</td>
<td>1.90 ± 0.60 ab</td>
<td>0 ± 0 b</td>
<td></td>
</tr>
<tr>
<td>- IBA (1000 mg kg⁻¹)</td>
<td>30 ± 14.14 a</td>
<td>3.73 ± 1.84 bc</td>
<td>0.91 ± 1.84 bc</td>
<td>0.36 ± 0.23 b</td>
<td></td>
</tr>
</tbody>
</table>

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 fungicide solution of 20 g L⁻¹ on rooting capacity (%), shoots number, root length and number

<table>
<thead>
<tr>
<th>Apex</th>
<th>Basal Immersión Solution</th>
<th>Rooting (%)</th>
<th>Root Number</th>
<th>Root Length (cm)</th>
<th>Shoot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Water (20 min)</td>
<td>80 ± 0 ab</td>
<td>11.00 ± 1.89 cd</td>
<td>2.33 ± 1.81 bc</td>
<td>0 ± 0 d</td>
</tr>
<tr>
<td>-</td>
<td>Water (20 min)</td>
<td>80 ± 28.28 ab</td>
<td>13.90 ± 2.22 bcd</td>
<td>2.49 ± 1.71 bc</td>
<td>0.85 ± 0.42 abc</td>
</tr>
<tr>
<td>+</td>
<td>Water (4 h)</td>
<td>10 ± 14.14 ab</td>
<td>19.51 ± 14.76 abc</td>
<td>0.93 ± 0.61 ab</td>
<td>1.00 ± 0.62 ab</td>
</tr>
<tr>
<td>-</td>
<td>Water (4 h)</td>
<td>10 ± 14.14 ab</td>
<td>19.86 ± 12.58 abc</td>
<td>0.53 ± 0.52 abc</td>
<td>0.73 ± 0.24 abc</td>
</tr>
<tr>
<td>+</td>
<td>Water (24 h)</td>
<td>100 ± 0 a</td>
<td>31.52 ± 8.99 a</td>
<td>5.45 ± 0.68 ab</td>
<td>0 ± 0 d</td>
</tr>
<tr>
<td>-</td>
<td>Water (24 h)</td>
<td>100 ± 0 a</td>
<td>18.00 ± 8.68 abcd</td>
<td>5.59 ± 1.89 ab</td>
<td>1.16 ± 0.31 bc</td>
</tr>
<tr>
<td>+</td>
<td>Fungicide (20 min)</td>
<td>80 ± 0 ab</td>
<td>19.40 ± 12.88 abc</td>
<td>4.89 ± 3.32 abc</td>
<td>0.54 ± 0.97 bcd</td>
</tr>
<tr>
<td>-</td>
<td>Fungicide (20 min)</td>
<td>90 ± 14.14 ab</td>
<td>24.81 ± 11.33 abc</td>
<td>5.42 ± 2.38 ab</td>
<td>1.00 ± 0 ab</td>
</tr>
<tr>
<td>+</td>
<td>Fungicide (4 h)</td>
<td>80 ± 28.28 ab</td>
<td>22.20 ± 15.32 ab</td>
<td>3.69 ± 2.07 abc</td>
<td>0 ± 0 d</td>
</tr>
<tr>
<td>-</td>
<td>Fungicide (4 h)</td>
<td>100 ± 0 a</td>
<td>30.12 ± 7.14 ab</td>
<td>6.76 ± 2.44 a</td>
<td>1.42 ± 0.51 a</td>
</tr>
<tr>
<td>+</td>
<td>Fungicide (24 h)</td>
<td>60 ± 28.28 ab</td>
<td>14.13 ± 4.78 abc</td>
<td>3.45 ± 1.20 ab</td>
<td>0 ± 0 d</td>
</tr>
<tr>
<td>-</td>
<td>Fungicide (24 h)</td>
<td>30 ± 14.14 ab</td>
<td>4.10 ± 2.87 d</td>
<td>1.38 ± 0.28 c</td>
<td>0.35 ± 0.13 cd</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Apex</th>
<th>Cuttings length</th>
<th>Rooting (%)</th>
<th>Roots number</th>
<th>Roots Length</th>
<th>Shoots Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>5 cm</td>
<td>90 ± 14.14 a</td>
<td>24.33 ± 15.38 a</td>
<td>4.34 ± 2.30 a</td>
<td>0.67 ± 0.33 bc</td>
</tr>
<tr>
<td>+</td>
<td>10 cm</td>
<td>100 ± 0 a</td>
<td>20.00 ± 10.45 a</td>
<td>4.84 ± 2.20 a</td>
<td>0.67 ± 0.16 bc</td>
</tr>
<tr>
<td>+</td>
<td>15 cm</td>
<td>100 ± 0 a</td>
<td>26.66 ± 8.14 a</td>
<td>5.11 ± 1.74 a</td>
<td>0 ± 0 c</td>
</tr>
<tr>
<td>-</td>
<td>5 cm</td>
<td>90 ± 14.14 a</td>
<td>30.00 ± 18.17 a</td>
<td>4.59 ± 2.44 a</td>
<td>1.17 ± 0.40 abc</td>
</tr>
<tr>
<td>-</td>
<td>10 cm</td>
<td>100 ± 0 a</td>
<td>21.66 ± 11.09 a</td>
<td>4.65 ± 0.86 a</td>
<td>2.00 ± 1.09 ab</td>
</tr>
<tr>
<td>-</td>
<td>15 cm</td>
<td>100 ± 0 a</td>
<td>22.33 ± 12.42 a</td>
<td>4.42 ± 0.4 a</td>
<td>2.33 ± 0.81 a</td>
</tr>
</tbody>
</table>

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 Paraná 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 fungicidal 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 fungicidal 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*.

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