Research Article

In Vitro Propagation of Muña-Muña (Clinopodium odorum (Griseb.) Harley)

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A micropropagation protocol was developed which may assist in the safeguarding and augmentation of dwindling natural populations of Clinopodium odorum (Griseb.) Harley, a critically and endangered medicinal plant. Factors affecting culture initiation bud sprouting and growth, rooting, and acclimatization were studied, using nodal segments of in vitro germinated seedling as primary explants on six media supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP) (0.5–1.5) and 2-Naphthalene acetic acid (NAA) (0.5–1.5). Best results for culture initiation with sustainable multiplication (100%) were obtained on WP medium without any growth regulator. WP with the addition of 0.5 : 1 or 0.5 : 1.5) of BAP and NAA promoted a higher elongation; however, the optimum number of nodes were obtained in plantlets grown on 1/2 MS with the addition of 1 : 1.5 of BAP and NAA. Culture of sectioned individual nodes transferred to the media with different rates of BAP and NAA 1/2 MS-9 (1.5 : 1.5), SH-8 (1.5 : 1.0), and 1/2 B5-4 (1.0 : 0.5) media resulted in no proliferated shoots. The in vitro plants were successfully acclimatized garden soil and sand (2 : 1) in the greenhouse, with over 90% survival rate. The in vitro-grown plants could be transferred to ex vitro conditions and the efficacy in supporting ex vitro growth was assessed, with a view to develop longer-term strategies for the transfer and reintroduction into natural habitats.

1. Introduction

Clinopodium odorum (Griseb.) Harley is a small deciduous shrub of the family Lamiaceae commonly known as muña-muña. In Argentina, the species is restricted to a very specific niche; plant exploration studies in the region has revealed the occurrence of only small populations that is especially characteristic of Pampa de Achala (Córdoba) distributed at an elevation of 1200 m [1].

The fresh herb is used as a flavoring agent for aliments and an infusion of the aerial parts is utilized as an anti-catarhral, antispasmodic, stringent, carminative, digestive, diuretic, laxative, stomachic, soporific, vermifuge, menstrual suppression, flatulent, colic, and tonic digestive and antispasmodic and to help in parturition [2, 3].

This plant species have been excessively collected from its habitats and become endangered due to different contributory factors: extensive denudation of the forest floor, caused by cattle grazing and collection of leaf litter, and removal from the wilderness which is highly used in the preparation of liquor companies “Amargos serranos” [4, 5].

Seed is only the means of propagating C. odorum, but seeds have reduced probability of germination when adult plants are already growing in the area, thus reducing species dissemination [6].

The use of in vitro techniques for rapid and mass propagation offers possibilities for recovery of endangered species thus reducing the risk of extinction. This technique could enable production of large numbers of clonal plants in relatively short-time periods using very little starting material [7].

The establishment of in vitro germplasm banks in developing countries has great importance, but these techniques must be associated with other plant genetic resources conservation practices [8, 9]. The in vitro conservation techniques allow material exchanges among germplasm banks, and the germplasm keeps its sanitary conditions and viability during the transport [10, 11].
To our knowledge, there are no reports for in vitro propagation of C. odorum; it is known that developing efficient micropropagation procedures for particular species generally requires detailed studies to define specific composition of mineral salts, plant growth regulators, and organic compounds in the culture medium.

In view of the importance of this species, a holistic approach for the propagation using in vitro methods for its conservation have been described in this paper.

2. Materials and Methods

2.1. Plant Material. Seeds of C. odorum were collected in 2010 in their natural habitat during the months of February and March when the fruits are ripened and kept at room temperature until the initiation of the experiments. A voucher specimen was deposited in the International Herbarium of the National University of Río Cuarto, Argentine.

2.2. Seeds Germination. For in vitro germination different conditions were assessed: (A) control, (B) washing overnight in running tap water, (C) soaking at 1 mg L\(^{-1}\) of Gibberellic acid (GA\(_3\)) during 12 h, (D) soaking at 10 mg L\(^{-1}\) GA\(_3\) during 12 h, (E) soaking at 100 mg L\(^{-1}\) GA\(_3\) during 12 h, (F) washing overnight in running tap water plus soaking at 1 mg L\(^{-1}\) GA\(_3\) during 12 h, (G) washing overnight in running tap water plus soaking at 10 mg L\(^{-1}\) GA\(_3\) during 12 h and (H) washing overnight in running tap water plus soaking at 100 mg L\(^{-1}\) of GA\(_3\) during 12 h.

Seeds were surface sterilized with a solution of 70% (v/v) ethanol for 2 min and rinsed 3 times with sterile distilled water, followed by 15 min in a solution of sodium hypochlorite (NaOCl) 1.5% (v/v) for 15 min and finally rinsed 3 times with sterile distilled water. These surface sterilized seeds were explanted onto Murashige and Skoog, 1962 (MS) [12], germination culture medium, supplemented with 6-benzylaminopurine (BAP) and 2-naphthalene acetic acid (NAA) at different concentrations (0.5–1.5 mg L\(^{-1}\)) and combinations. The pH media was adjusted to 5.6 with NaOH or HCl, prior to gelling with 0.7% (w/v) agar-agar, dispensed (10 mL) into culture tubes and sterilized by autoclaving (121°C for 15 min.). In all the experiments, the chemicals used were of analytical grade (Sigma-Aldrich, St. Louis, MO, USA and E. Merck, Darmstadt, Germany).

All cultures were maintained under culture conditions: 25 ± 2°C, 45–55% relative humidity, and 16 h photoperiod under cool white fluorescent light (30 \(\mu\)E m\(^{-2}\) S\(^{-1}\)). Explants were observed daily for the first week for signs of contamination and thereafter weekly for signs of growth and development.

2.3. Culture Initiation. Clonal plants (plants originally derived from seed) were initially propagated on media for 4-5 months to increase stock populations for multiplication rate assessment. Multiplication experiments were carried out using nodal segments with axillaries’ buds of in vitro germinated seedling aged on half-strength MS major and minor salts medium with 1 mg L\(^{-1}\) of indole-3-butyric acid (IBA) (initiation medium).

The regeneration potential of nodes with axillaries’ buds (0.5–1 cm) obtained from plants grown in the above in vitro culture condition was evaluated in terms of frequency (survival percentage), principal shoot length, nodes number, shoot formation, and rooting by culturing on six media: MS, Schenk and Hildebrandt, 1972 (SH) [13], Lloyd and McCown, 1980 (WP) [14], Gamborg et al., 1968 (B5) [15], and MS and B5 at half-strength salt medium (12 MS and 12 B5), containing 3% (w/v) of sucrose and supplemented with 6-benzylaminopurine (BAP) and 2-naphthalene acetic acid (NAA) at different concentrations (0.5–1.5 mg L\(^{-1}\)) and combinations. The pH media was adjusted to 5.6 with NaOH or HCl, prior to gelling with 0.7% (w/v) agar-agar, dispensed (10 mL) into culture tubes and sterilized by autoclaving (121°C for 15 min.). In all the experiments, the chemicals used were of analytical grade (Sigma-Aldrich, St. Louis, MO, USA and E. Merck, Darmstadt, Germany).

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2.4. Acclimatization. Young plants with well-developed roots were carefully removed from the glass culture vessels; the roots washed with sterile water to eliminate the excess of agar and transferred to plastic cups containing autoclaved garden soil and sand (2 : 1). Each pot was covered with plastic bag for plantlets acclimatization during 15 days, which was punctured the last 5 days to allow air exchange.

Each plantlet was irrigated with distilled water every 2 days for 2 weeks followed by tap water for two other weeks. The potted plantlets were initially maintained under culture room conditions (4 weeks) and later transferred to normal greenhouse conditions.

2.5. Statistical Analysis. All experiments were conducted at least three times. For each treatment, a minimum of 14 and a maximum of 25 explants were used. For statistical analysis, all quantitative data expressed as percentages were first submitted to arcsine transformation and the means corrected for bias before a new conversion of the means and standard error back into percentages [16]. Statistical analysis was performed by ANOVA, and significantly different means were identified using Duncan’s test \(P \leq 0.05\).

3. Results and Discussion

3.1. Effect of Different Strategies on Seed Germination. Many studies evaluating adequate in vitro conditions for propagation of several species have been conducted using in vitro-germinated plants, to avoid disinfection of explants, because of extreme sensitivity to common disinfection procedures and subsequent low survival rates [17, 18]. A seed was scored as “germinated” when the root emerged and grew to 1 cm. C. odorum seeds were capable of germinating in all the treatments used, although several rates of germination were obtained.

As shown in Table 1, only at 28.1% germination was observed in the control. It is known that in some plant species, seed dormancy is a condition which prevents the
Figure 1: *In vitro* plant regeneration from single node of *C. odorum*. (a) General aspect of the wild plant in its habitat, (b) *in vitro* germinated plant after 10 weeks on MS medium, (c) *in vitro* grown plant after 2 months of culture on WP-2 medium, and (d) acclimatized plant, 1 month after potting into a mixture of garden soil and sand (2:1) substrates.

Table 1: Effect of pregermination conditions on *in vitro* *C. odorum* culture, (A) control, (B) washing overnight in running tap water, (C) soaking at 1 mg L\(^{-1}\) GA\(_3\) during 12 h, (D) soaking at 1 mg L\(^{-1}\) of GA\(_3\) during 12 h, (E) soaking at 100 mg L\(^{-1}\) of GA\(_3\) during 12 h, (F) washing overnight in running tap water + soaking at 1 mg L\(^{-1}\) GA\(_3\) during 12 h, (G) washing overnight in running tap water + soaking at 10 mg L\(^{-1}\) GA\(_3\) during 12 h, and (H) washing overnight in running tap water + soaking at 100 mg L\(^{-1}\) GA\(_3\) during 12 h.

| Pregermation condition | Percentage of germination |
|------------------------|---------------------------|
| A                      | 28.1 %                    |
| B                      | 41.8 %                    |
| C                      | 60.2 %                    |
| D                      | 37.9 %                    |
| E                      | 55.8 %                    |
| F                      | 62.2 %                    |
| G                      | 42.9 %                    |
| H                      | 54.8 %                    |

Seed from germinating, even when it is perfectly healthy and all conditions for germination are at an optimum.

Seed dormancy is also a prevalent cause of very slow and erratic germination in the majority of wild plants [19–22].

Therefore, strategies to induce physiological and mechanical seed dormancy rupture were carried out, which caused a positive reaction on the *C. odorum* seed germination response. The washing overnight in running tap water condition was not enough mechanism to prevent total seed dormancy, with the resulting germination rate being only 41.8%. Germination rate is the “speed or velocity” of germination and can be expressed as the time it takes for a defined percentage of seed to germinate in our case at 15 days.

More than 2-fold improvement in germination was recorded in seeds washing overnight in running tap water plus soaking at 1 mg L\(^{-1}\) GA\(_3\) during 12 h, in which a physiological dormancy release increasing germination rates at 62.2% was obtained (Table 1).

This germination value was similar to those obtained with soaking 1 mg L\(^{-1}\) GA\(_3\); it suggests that *C. odorum* seeds had among others a requirement to GAs which is known that induce the production of enzymes to digest the endosperm that would otherwise from a mechanical barrier to radicle emergence reported that the use of this plant growth regulator (GA\(_3\)) is an improved alternative to release

Table 2: Percentage of survival (%) of *C. odorum* explants on different culture media (1/2 MS, SH, WP, 1/2 B5) with the addition of different concentrations and combination of PGRs.

| PGRs (mg L\(^{-1}\)) | Culture media |
|----------------------|---------------|
| BAP/NAA              | 1/2 MS  | SH  | WP  | 1/2 B5    |
| 0.00                 | 72.22   | 62.50 | 100.00 | 15.78    |
| 0.5/0.5              | 87.50   | 70.00 | 84.61   | 45.00    |
| 0.5/1.00             | 77.77   | 50.00 | 93.75   | 40.00    |
| 0.5/1.50             | 62.50   | 28.57 | 57.14   | 45.00    |
| 1.00/0.5             | 53.84   | 62.50 | 38.88   | 21.05    |
| 1.00/1.00            | 40.00   | 63.63 | 53.33   | 0.00     |
| 1.00/1.50            | 88.88   | 50.00 | 83.33   | 31.57    |
| 1.50/0.50            | 37.50   | 40.00 | 61.90   | 33.33    |
| 1.50/1.00            | 52.63   | 0.00  | 15.78   | 5.26     |
| 1.50/1.50            | 0.00    | 33.33 | 33.33   | 31.57    |
Table 3: Effect of nutrient media and PGRs (mg -1) concentrations on in vitro C. odorum. Values followed by the same letter within a column are not significantly different at the P ≤ 0.05, according ANOVA analysis and by Duncan’s test.

| Culture media | BAP  | NAA  | Principal shoot length (cm) | Number of nodes (principal shoot) | Number of axillaries shoots |
|---------------|------|------|-----------------------------|-----------------------------------|-----------------------------|
| 1/2 MS-0      | 0.00 | 0.00 | 6.06 ± 0.71a               | 9.46 ± 0.91a                      | 1.00 ± 0.35a               |
| 1/2 MS-1      | 0.50 | 0.50 | 8.91 ± 0.68a               | 11.14 ± 0.88a                     | 0.79 ± 0.34a               |
| 1/2 MS-2      | 0.50 | 1.00 | 9.81 ± 0.68a               | 11.93 ± 0.88a                     | 0.79 ± 0.34a               |
| 1/2 MS-3      | 0.50 | 1.50 | 7.13 ± 0.81a               | 7.89 ± 1.09a                      | 0.44 ± 0.43a               |
| 1/2 MS-4      | 1.00 | 0.50 | 3.91 ± 0.96a               | 5.14 ± 1.24a                      | 1.00 ± 0.48a               |
| 1/2 MS-5      | 1.00 | 1.00 | 4.69 ± 0.90a               | 7.89 ± 1.09a                      | 1.22 ± 0.43a               |
| 1/2 MS-6      | 1.00 | 1.50 | 12.14 ± 0.64b              | 16.38 ± 0.82b                     | 1.75 ± 0.32a               |
| 1/2 MS-7      | 1.50 | 0.50 | 2.70 ± 1.04a               | 4.17 ± 1.34a                      | 1.50 ± 0.32a               |
| 1/2 MS-8      | 1.50 | 1.00 | 5.22 ± 0.81a               | 7.50 ± 1.04a                      | 3.60 ± 0.40a               |
| 1/2 MS-9      | 1.50 | 1.50 | —                           | —                                 | —                           |
| SH-0          | 0.00 | 0.00 | 7.46 ± 1.14a               | 6.60 ± 1.47a                      | 1.80 ± 0.57a               |
| SH-1          | 0.50 | 0.50 | 8.89 ± 0.96a               | 10.14 ± 1.24b                     | 1.29 ± 0.48a               |
| SH-2          | 0.50 | 1.00 | 8.60 ± 1.47a               | 10.00 ± 1.89b                     | 1.67 ± 0.74a               |
| SH-3          | 0.50 | 1.50 | 4.15 ± 1.80a               | 4.50 ± 1.32a                      | 1.00 ± 0.90a               |
| SH-4          | 1.00 | 0.50 | 3.56 ± 1.14a               | 4.40 ± 1.47a                      | 0.80 ± 0.57a               |
| SH-5          | 1.00 | 1.00 | 6.84 ± 0.96a               | 7.71 ± 1.24a                      | 0.71 ± 0.48a               |
| SH-6          | 1.00 | 1.50 | 5.85 ± 1.04a               | 8.33 ± 1.34a                      | 1.17 ± 0.52a               |
| SH-7          | 1.50 | 0.50 | 4.38 ± 1.27a               | 6.25 ± 1.64a                      | 1.03 ± 0.48a               |
| SH-8          | 1.50 | 1.00 | —                           | —                                 | —                           |
| SH-9          | 1.50 | 1.50 | 6.20 ± 1.47a               | 8.33 ± 1.89a                      | 4.67 ± 0.74a               |
| WP-0          | 0.00 | 0.00 | 7.74 ± 0.68a               | 7.71 ± 0.88a                      | 0.93 ± 0.34a               |
| WP-1          | 0.50 | 0.50 | 11.24 ± 0.77a              | 12.91 ± 0.99a                     | 1.27 ± 0.39a               |
| WP-2          | 0.50 | 1.00 | 12.67 ± 0.66b              | 13.07 ± 0.85a                     | 1.53 ± 0.33b               |
| WP-3          | 0.50 | 1.50 | 12.61 ± 0.74b              | 14.42 ± 0.95a                     | 1.17 ± 0.37b               |
| WP-4          | 1.00 | 0.50 | 9.39 ± 0.96a               | 9.71 ± 1.24a                      | 0.29 ± 0.42a               |
| WP-5          | 1.00 | 1.00 | 10.19 ± 0.90a              | 10.38 ± 1.16a                     | 1.25 ± 0.45a               |
| WP-6          | 1.00 | 1.50 | 9.14 ± 0.81a               | 10.50 ± 1.04a                     | 0.80 ± 0.40a               |
| WP-7          | 1.50 | 0.50 | 5.74 ± 0.71a               | 8.85 ± 0.91a                      | 0.69 ± 0.35a               |
| WP-8          | 1.50 | 1.00 | 2.57 ± 1.47a               | 6.33 ± 1.89a                      | 0.33 ± 0.74a               |
| WP-9          | 1.50 | 1.50 | 2.55 ± 1.27a               | 6.25 ± 1.64a                      | 0.75 ± 0.64a               |
| 1/2 B5-0      | 0.00 | 0.00 | 2.37 ± 1.44a               | 4.33 ± 1.77a                      | 0.67 ± 1.00a               |
| 1/2 B5-1      | 0.50 | 0.50 | 3.70 ± 0.83a               | 4.33 ± 1.02a                      | 5.33 ± 0.58b               |
| 1/2 B5-2      | 0.50 | 1.00 | 6.21 ± 0.88a               | 6.63 ± 1.08a                      | 6.63 ± 0.61a               |
| 1/2 B5-3      | 0.50 | 1.50 | 4.93 ± 0.83a               | 6.78 ± 1.02a                      | 7.00 ± 0.58b               |
| 1/2 B5-4      | 1.00 | 0.50 | 4.72 ± 1.25a               | 7.00 ± 1.53a                      | 6.75 ± 0.87a               |
| 1/2 B5-5      | 1.00 | 1.00 | —                           | —                                 | —                           |
| 1/2 B5-6      | 1.00 | 1.50 | 6.12 ± 1.02a               | 6.83 ± 1.25a                      | 3.00 ± 0.71a               |
| 1/2 B5-7      | 1.50 | 0.50 | 2.39 ± 0.94a               | 3.71 ± 1.16a                      | 4.14 ± 0.65a               |
| 1/2 B5-8      | 1.50 | 1.00 | 1.90 ± 2.49b               | 5.00 ± 3.06b                      | —                           |
| 1/2 B5-9      | 1.50 | 1.50 | 4.03 ± 1.02a               | 4.33 ± 1.25a                      | 3.00 ± 0.71a               |

3.2. Plant Tissue Culture. Successful plant tissue culture depends on the choice of nutrient medium. The explants of most plant species can be grown on completely defined media and there is a small number of standard culture media that are widely used with inorganic supplements. No single medium can be used for all types of plants and organs, so the composition of the culture medium for each plant material has to be worked out [24]. Best results for culture initiation with sustainable multiplication rates (100%) were obtained on WP medium without any growth regulator.

Significant differences were observed among the media for shoot elongation (Table 2). The maximum shoot length was significantly higher on hormone-free media WP and SH media resulting 7.74 ± 0.68 and 7.46 ± 1.14 cm, respectively (Table 3).

A scarce elongation (2.37 ± 1.44 cm) was observed when the explants were placed on 12 B5. The plants produced on 12
MS showed significant differences for the number of nodes per plant among other treatments resulting with a value of $9.46 \pm 0.91$ at $P \leq 0.05$.

The media 12 MS-9, SH-8, and 12 B5-4 were not effective to induce growth and caused necrosis, thus resulting in death of nodal segments.

The steps in plant tissue culture protocols can impose a series of PGRs; in many plants, a balance between two groups of PGRs; auxins and cytokinins, constitutes the most conspicuous group of plant hormones regulating cell division and elongation and determining morphogenesis. An adequate assessment of the suitability requirement of PGRs depends upon the type of plant tissues or explants that are used for culture.

The use of culture media supplemented with BAP and NAA has been associated with in vitro morphogenetic events and it is known that cytokinins have the ability to induce the shoot formation.

Mc Cown and Sellmer, 1987 [25], reported that the effect of growth regulators can be strongly modified by the medium on which the culture is grown. When the experiments were conducted to determine how different ratios of plant growth regulators would support microplant growth, significant difference with an excellent growth response (length of principal shoot and node number) corresponded to plantlets grown on 12 MS-6 (12.14 ± 0.64 cm) and WP-2 (12.67 ± 0.66 cm) and WP-3 (12.61 ± 0.74 cm). 12 B5-3 affected significantly shoot proliferation as it recorded the highest number of new shoots/explants, 7.0 ± 0.58 after 8 weeks in culture (Table 3).

A rooting rate of 90% was attained when the plantlets were placed in inductive media or included in hormone-free, suggesting NAA incorporated to the media was not necessary.

Plantlets were acclimatized successfully when transferred to trays containing a mix substrate of plastic cups containing autoclaved garden soil and sand (2:1). Rooted plantlets were transferred into pots and the survival rate was the 85% after 1 month (Figure 1(d)).

**4. Conclusion**

In conclusion, an efficient protocol for micropropagation of an important medicinal plant *C. odorum* was developed by testing various concentrations of growth regulators and nutrition conditions. The success of plant tissue culture as a mean of plant propagation is greatly influenced by nature of the culture medium used; they are grown in vitro on artificial media, which supplies the nutrients necessary for growth.

Different nutrient media hormone-free tested to find out the suitable starting nutrient medium supplemented with 3% (w/v) sucrose, for 8 weeks for in vitro nodal cuttings establishment, showed that an efficient survival percentage was obtained on plantlets grown on free-hormone WP medium. The free-hormone medium contains the proportion of inorganic nutrients to satisfy the nutritional as well as the physiological needs of this species in vitro culture. However, the optimum growth depended on the addition of PGRs. The positive response to these additions indicated a requirement of the explants for a morphogenetic induction as was observed when the explants grown on 12 B5-3, this medium appear to be adequate for new shoots formation.

Our investigation revealed that no auxin supplementation was necessary for rooting differentiation (100%) in proliferation of regenerated shoots.

The in vitro-grown plants could be transferred to ex vitro conditions, with a view to develop longer-term strategies for the transfer and reintroduction of micropropagated *C. odorum* plants into natural habitat [26, 27]. The results will make the conservation and propagation of the species much easier.

**Abbreviations**

- BAP: 6-Benzylaminopurine
- B5: Gamborg medium
- GA3: Gibberellic acid
- IBA: Indole-3-butyric acid
- NAA: 2-Naphthalene acetic acid
- PGRs: Plant growth regulators
- MS: Murashige and Skoog medium
- SH: Schenk and Hildebrandt medium
- WP: Woody plant medium (Lloyd and McCown) medium
- 12MS: Half-strength Murashige and Skoog macronutrients
- 12B5: Half-strength Gamborg macronutrients

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**References**

[1] G. E. Barboza, J. J. Cantero, C. Núñez, A. Pacciaroni, and L. Ariza Espinar, *Medicinal Plants: Review and a phytochemical and ethnopharmacological Screening of the Native Argentine Flora*, vol. 34, Kurtziana, 2009.

[2] G. B. Mahady, “Medicinal plants for the prevention and treatment of bacterial infections,” *Current Pharmaceutical Design*, vol. 11, no. 19, pp. 2405–2427, 2005.

[3] R. M. Harley and A. G. Paucar, “List of species of tropical American *Clinopodium* (Labiatae), with new combinations,” *Kew Bulletin*, vol. 55, no. 4, pp. 917–927, 2000.

[4] M. E. Goleniowski, G. A. Bongiovanni, L. Palacio, C. O. Núñez, and J. J. Cantero, “Medicinal plants from the “Sierra de Comechingones”, Argentina,” *Journal of Ethnopharmacology*, vol. 107, no. 3, pp. 324–341, 2006.

[5] G. I. Martínez, A. M. Planchuelo, E. Fuentes, and M. Ojeda, “A numeric index to establish conservation priorities for medicinal plants in the Paravachasca Valley, Córdoba, Argentina,” *Biodiversity and Conservation*, vol. 15, no. 8, pp. 2457–2475, 2006.

[6] J. J. Cantero and C. A. Bianco, “Las plantas vasculares del suroeste de la provincia de Córdoba. Catálogo preliminar de
las especies," Revista Universidad Nacional De Río Cuarto, vol. 6, pp. 65–75, 1986.

[7] A. Rubluro, V. Chávez, A. P. Martínez, and O. Martínez-Vázquez, "Strategies for the recovery of endangered orchids and cacti through in-vitro culture," Biological Conservation, vol. 63, no. 2, pp. 163–169, 1993.

[8] G. M. Alves and M. P. Guerra, "Micropropagation for mass propagation and conservation of Vrieseaëfí burgensis var. paludosum from microbuds," Journal of the Bromeliad Society, vol. 515, pp. 202–212, 2001.

[9] H. K. Badola, H. K. Badola, and B. K. Pradhan, "Chemical stimulation of seed germination in ex situ produced seeds in Swertia chirayita, a critically endangered medicinal herb," Research Journal of Seed Science, vol. 3, no. 3, pp. 139–149, 2010.

[10] F. Engelmann, "In vitro conservation methods," in Biotechnology and Plant Genetic Resources, J. A. Callow, B. V. Ford-Lloyd, and H. J. Newbury, Eds., pp. 119–161, CAB International, Oxon, England, 1997.

[11] A. Rech Filho, L. L. Dal Vesco, R. O. Nodari, R. W. Lischka, C. V. Müller, and M. P. Guerra, "Tissue culture for the conservation and mass propagation of Vriesea reitzii Leme and Costa, a bromeliad threatened of extinction from the Brazilian Atlantic Forest," Biodiversity and Conservation, vol. 14, no. 8, pp. 1799–1808, 2005.

[12] T. Murashige and F. Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," Physiology Plant, vol. 15, pp. 473–497, 1962.

[13] R. U. Schenk and A. Hildebrandt, "Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures," Canadian Journal of Botany, vol. 350, pp. 199–204, 1972.

[14] G. Lloyd and B. McCown, "Commerically-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture," Proceedings International Plant Propagators Society, vol. 30, pp. 421–427, 1980.

[15] O. L. Gamborg, R. A. Miller, and K. Ojima, "Nutrient requirements of suspension cultures of soybean root cells," Experimental Cell Research, vol. 50, no. 1, pp. 151–158, 1968.

[16] J. H. Zar, Biostatistical Analysis, Prentice-Hall, Englewood Cliffs, NJ, USA, 1996.

[17] P. E. Pérez-Molphé-Balch and C. A. Dávila-Figueroa, "In vitro propagation of Penelopephora aselliformis Ehrenberg and P. strobiliformis Werdermann (Cactaceae);" In vitro Cellular and Developmental Biology-Plant, vol. 38, no. 1, pp. 73–78, 2002.

[18] M. D. S. Santos-Díaz, R. Méndez-Onitineros, A. Arredondo-Gómez, and M. D. L. Santos-Díaz, "In vitro organogenesis of Penelopephora aselliformis Ehrenberg (Cactaceae);" In vitro Cellular and Developmental Biology-Plant, vol. 39, no. 5, pp. 480–484, 2003.

[19] P. Grappin, D. Bouinot, B. Sotta, E. Migniaccia, and M. Jullien, "Control of seed dormancy in Nicotiana plumbaginifolia: post-imbibition abscisic acid synthesis imposes dormancy maintenance," Planta, vol. 210, no. 2, pp. 279–285, 2000.

[20] G. Metzger, "Plant hormone interactions during seed dormancy release and germination," Seed Science Research, vol. 15, no. 4, pp. 281–307, 2005.

[21] W. E. Finch-Savage and G. Leubner-Metzger, "Seed dormancy and the control of germination," New Phytologist, vol. 171, no. 3, pp. 501–523, 2006.

[22] J. V. Jacobsen, D. W. Pearce, A. T. Poole, R. P. Pharis, and L. N. Mander, "Abscisic acid, phaeic acid and gibberellin contents associated with dormancy and germination in barley," Physiology Plantarum, vol. 115, no. 3, pp. 428–441, 2002.

[23] B. Kucera, M. A. Cohn, and G. Leubner-Metzger, "Plant hormone interactions during seed dormancy release and germination," Seed Science Research, vol. 15, no. 4, pp. 281–307, 2005.

[24] J. Wu, X. Zhang, Y. Nie, S. Jin, and S. Liang, "Factors affecting somatic embryogenesis and plant regeneration from a range of recalcitrant genotypes of Chinese cottons (Gossypium hirsutum L.)," In vitro Cellular and Developmental Biology - Plant, vol. 40, no. 4, pp. 371–375, 2004.

[25] B. H. Mc Gown and J. C. Sellmer, "General media and vessels suitable for woody plant culture," in Cell and Tissue Culture in Forestry, J. M. Bonga and D. J. Durzan, Eds., vol. 1 of General Principles and Biotechnology, pp. 4–16, Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 1987.

[26] M. Lambardi and A. De Carlo, "Application of tissue culture to the germplasm conservation of temperate broad-leaf trees," in Micropropagation of Woody Trees and Fruits, S. M. Jain and K. I. Ishii, Eds., pp. 241–248, Kluwer Academic, Dordrecht, The Netherlands, 2003.

[27] L. Palacio, M. C. Baæa, J. J. Cantero, R. Cusidó, and M. E. Goleniowski, "In vitro propagation of Jarilla (Larrea divaricata Cav.) and Secondary Metabolite Production," Biological and Pharmaceutical Bulletin, vol. 31, no. 12, pp. 2321–2325, 2008.