Techniques for the In Vitro Propagation of *Rhodophiala* and *Leucocoryne* spp.

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**Keywords:** geophyte, floriculture, plant tissue culture, propagation

**Abstract**

*Leucocoryne coquimbensis* and *Rhodophiala bagnoldii* are endogenous geophytes from Chile that have the potential to be used as cut flower crops, potted plants, and garden plants. However, traditional propagation techniques with these species can be difficult. Different mechanical, environmental, and chemical treatments were used in vitro to determine the optimum method to increase bulb production rates. Both bulbs and seeds of each species were used. Traditional propagation techniques that are used to increase the number of bulbs were attempted under in vitro conditions with the *Leucocoryne* and *Rhodophiala*. These include scooping, scoring, and sectioning of the basal plate. Scoring and sectioning techniques increased the number of bulblets produced in vitro, but scooping had no advantage. With *Rhodophiala*, the effects of the cytokinins BA (N$_6$-benzyladenine) and 2iP (2-isopentenyl-adenine) each at 2.5, 5.0, and 10.0 µM were tested. BA was shown to be advantageous in vitro, but there was no advantage to incorporating 2iP in the propagation medium. The effect of sucrose level in the medium on the growth of *Rhodophiala* bulbs was tested in vitro. The concentrations 30, 60, and 90 grams per liter were evaluated as well as a control with no sucrose. There is increased bulb growth of *Rhodophiala* in vitro when increased levels of sucrose are added to the medium. However, the addition of higher levels of sucrose does not affect the number of bulbs that are produced. This research determined that it is possible to propagate *Leucocoryne* and *Rhodophiala* bulbs in vitro.

**INTRODUCTION**

The constant search for new forms and colors of plants for the horticultural industry is making the demand for new cultivars or species a permanent endeavor. Traditional breeding is still the most important source for releasing new cultivars to the market. However, there is an enormous potential to introduce non-cultivated species from nature and to breed new ornamentals from these native species (Brickell, 2001).

Horticulturists have long recognized the ornamental importance of unique geophytes. As a result, bulbs, corms, tubers, and other geophytes have become one of the most important groups of plants for the floriculture industry in the last two decades. Chile’s vast selection of unique geophytes has attracted the attention of breeders and horticulturists in recent years. The Chilean territory is an “ecological island” with geographical barriers that isolate the biological communities from the rest of the continent and produce a high percentage of endemism. Continental Chile is home to some 5,100 species, of which about 2,630 are endemic (Marticorena, 1990; Marticorena and Rodriguez, 1995). Such a proportion of endemism rivals that of many islands, and is one of the highest found in any region on Earth. The temperate ecosystems of southern South America can be appreciated best in comparison with similar plant communities that exist today in South Africa, Australia and New Zealand. As with those three examples, the Mediterranean climate and desert ecosystems of Chile have evolved in isolation.

Extensive studies have been published on the breeding and development of the Chilean genus, *Alstroemeria*, as an ornamental crop (Bridgen, 1993, 2001; Chiari and Bridgen, 2000). However, there is still much research that can be completed on the other
geophyte species from Chile. The research that is being presented will assist with the breeding, development and introduction of two of Chile’s wonderful and unique plant species, *Leucocoryne* and *Rhodophiala*. They will be able to be used in the ornamental industry as cut flowers, flowered pot plants or garden plants.

*Leucocoryne* of the Alliaceae, is an endemic genus of Chile with more than a dozen species (Muñoz and Moreira, 2000; Zöllner and Arriagada, 1998). Most of these species have some ornamental interest, but *Leucocoryne ixioides*, *L. coquimbensis*, *L. vittata* and *L. purpurea* have very showy and attractive flowers. Species of *Leucocoryne* are widely distributed in Central Chile mainly at latitudes 30 to 35°S, in dry habitats from sea level up to an altitude of 1,000 meters above sea level. Nevertheless, the largest populations of these plants are found in the coastal areas north from Santiago (latitude 30 to 33°S). This area is quite dry, with scattered rain between May and August with an average rainfall of 70 mm. From October to April it seldom rains. The average temperature is constantly fluctuating from 12°C (June) to 17°C (January).

*Leucocoryne* plants have a bulb approximately 1.5-2.5 cm wide, covered with a brown dry tunic. In some cases, the bulbs form droppers, which draw the new bulb down to 40 cm below the surface to prevent drying. Plants have 2 to 7 filiform leaves, which are 20-30 cm long and 0.2-0.3 cm wide, which can be present or not during anthesis depending on the species. The solitary inflorescence stem can reach 30-80 cm tall with an umbel of 2-9 flowers depending on the species. Flowers are actinomorphic with six tepals and three prominent white, yellow or bicolor staminodes (sterile stamens). True fertile stamens are sessile and not visible on the inside of the flower tube. Tepal colors range from solid white to light or deep purple, with some bicolor forms depending on the species (Schiappacasse et al., 2002; Zöllner, 2002). It is very likely that natural hybrid plants have developed. *L. coquimbensis* inflorescences have a vase life of 8-9 days depending on the stage of harvest (Elgar et al., 2003).

The genus *Rhodophiala* belongs to the Amaryllidaceae and is endemic to South America. There are a total of 26 species described: 23 species endemic to Chile and three species native to Chile and Argentina. For several years it was considered part of *Hippeastrum*, but after Meerow et al. (2000) *Rhodophiala* is now generally accepted as a different genus. In Chile the distribution of *Rhodophiala* spp. ranges from latitude 24 to 42°S, but 60% of those species are concentrated between latitude 30 to 38°S (Ravena et al., 1998). There are several species with ornamental interest like *R. advena* and *R. phycelloides*, both with red flowers, *R. bagnoldii* with a unique solid yellow color, and *R. rhodolirion* with large showy pink-white flowers.

Plants of *Rhodophiala* have a tunicate bulb of 4-6 cm diameter, with filiform leaves that dry out during anthesis (Muñoz, 1985). The bulbs are set 20-30 cm underground. The species has a one-flowered umbel, openly funnel-shaped flowers, styles overtopping the stamens, and capitate stigma (Arroyo-Leuenberger and Leuenberger, 1991). The umbel can hold up to six flowers, and each flower is 4-6 cm wide. The length of the flower stem ranges between 35 and 50 cm (Riedemann and Aldunate, 2001). Plants bloom during the spring depending on the species and the location.

The objective of this project was to evaluate traditional bulb propagation techniques under in vitro conditions by using different mechanical, environmental, and chemical treatments. Because conventional vegetative and seed propagation techniques of bulbs are slow or genetically variable, the in vitro procedures will be able to increase bulb regeneration rates. In addition, by developing micropropagation techniques, new cultivars can be rapidly, asexually propagated and breeding, germplasm preservation, and the production of pathogen-free plants will be expedited. The authors would like to thank the Gloeckner Foundation for its funding of part of the *Leucocoryne* research.

**MATERIALS AND METHODS**

In all experiments, unless otherwise indicated, the culture medium consisted of plain MS salts (Murashige and Skoog, 1962) plus 100 mg L⁻¹ myo-inositol, 2 mg L⁻¹ glycine, 0.5 mg L⁻¹ nicotinic acid, 0.5 mg L⁻¹ pyridoxine-HCl and 0.1 mg L⁻¹ thiamine-HCl.
All the explants were cultured in test tubes with 15 ml medium, 30 g L⁻¹ of sucrose, 7 g L⁻¹ of agar and pH adjusted to 5.7 before autoclaving. The cultures were maintained at 23°C under 24 hours/day cool white fluorescent light (24.2 µmol s⁻¹ m⁻² PAR) and transferred to the same medium every four weeks (six weeks for Leucocoryne coquimbensis). At the initiation stage and at each transfer, data on the number of bulblets produced, shoot number, root number, total fresh weight and fresh weight of the bulbs, presence or absence of roots and dormancy were evaluated. There were a minimum of 16 replications per treatment and the statistical design for all experiments was a complete randomized design. Data were analyzed using SAS ANOVA procedures (SAS, 1999).

Cuttage Experiments

The species used in this set of experiments were Rhodophiala bagnoldii and Leucocoryne coquimbensis. The treatments tested were the following:

i Scooping. Total removal of the basal plate to obtain adventitious bulb formation from the base of the remaining scales.

ii Scoring. Partial vertical sectioning of the basal plate in order to damage the apical meristem and break the apical dominance. Two treatments were tested: one incision and two incisions.

iii Sectioning. Complete transversal sectioning of the bulb or corm. In this case, two treatments were tested: two piece sections and four piece sections.

iv Control with no mechanical intervention.

Cytokinin Experiments

Plant material for Rhodophiala bagnoldii was collected in Chile during October 1998. Seeds were initiated in vitro. Factorial treatments were designed using two different sources of cytokinin, N₆-benzyladenine (BA) and 2-isopentenyl-adenine (2iP), each at the rates of 2.5, 5.0 and 10.0 µM plus a control with no plant regulators added to the medium.

Sucrose Experiments

Seeds and fruits of Rhodophiala bagnoldii were collected during October 2000 and 2001 in central Chile. Seeds were initiated in vitro and cultured for 8 weeks. The experiment was designed with different levels of sucrose in the growing medium: 30, 60 and 90 g L⁻¹, plus a control with no sucrose added to the medium.

RESULTS

Cuttage Experiments

1. Rhodophiala bagnoldii. After 12 weeks of culture it was possible to observe differences among treatments for the total fresh weight of plants. The largest growth was produced by sectioning in four pieces and scoring with only one cut (Fig. 1). Looking at the final number of bulbs produced (Fig. 2), it was observed that by scoring R. bagnoldii bulbs with one incision or sectioning them into four pieces, they produced 10 times more bulblets than bulbs that have had no cuttage. Although greater in number, bulbs that were produced by those treatments had 30% less average weight than those bulbs that were produced by natural propagation (Fig. 2). Considering both number and average weight of the bulbs formed, scoring and sectioning of R. bagnoldii bulbs produced the largest total fresh weight of plants. Scooping treatment did not produce any adventitious growth or bulbs and the plants eventually died. Control plants maintained a normal growth during the culture period, but they did not produce any adventitious bulblets.

2. Leucocoryne coquimbensis. Bulbs of L. coquimbensis that were treated with either scoring technique or sectioned into four pieces produced four or eight times more bulblets than the control plants (Fig. 3) and the greatest total fresh weight (Fig. 4). However, the gain of individual fresh weight of the bulbs sectioned into four pieces was similar to the increase obtained by the control plants (Fig. 3). Bulbs that had their basal plates scored with two incisions increased in fresh weight of individual bulbs the most. The scooping

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treatment produced a few of adventitious bulbs and the plants eventually died, as it happened with bulbs of *R. bagnoldii*. This effect of the scooping treatment demonstrates the importance of keeping at least part of the basal plate in any propagation technique to obtain adventitious growth. Only a few control bulbs produce some adventitious bulblets. Most of the control bulbs remained inactive with no growth of shoots or roots during the culture period. This research demonstrated the importance of basal plate cuttage on *L. coquimbensis* bulbs for increased in vitro bulb production.

**Cytokinin Experiments**

After 12 weeks of culture, the increase of total plant weight was the only factor that showed significant differences among treatments (Fig. 5). Treatments with 2.5 and 5.0 µM BA demonstrated the greatest fresh weights, showing values over four times greater than those from the control plants. The smallest increase of total fresh weight was with the 2.5 µM 2iP treatment. Similar results were possible to observe for the final number of bulbs that were produced (Fig. 6). Treatments with BA showed the greatest number of bulbs produced but there were no differences among the BA levels. Treatments with different doses of 2iP produced similar number of bulbs to those produced by the control plants.

**Sucrose Experiments**

After 12 weeks of culture it was possible to observe a significant effect of sucrose on total plant weight of *Rhodophiala bagnoldii* plantlets (Fig. 7), and weight and number of bulbs produced (Fig. 8). For all of these parameters, the highest level of sucrose (90 g L⁻¹) produced the highest values and no growth suppression was observed, meaning that a higher dose of sucrose can increase the growth of plants and/or bulbs of *R. bagnoldii* in vitro.

**DISCUSSION**

Bulbs of the Chilean geophyte, *Rhodophiala bagnoldii*, can be propagated in vitro. By scoring *R. bagnoldii* bulbs with one incision or by sectioning them into 4 pieces, they can produce up to 10 times more bulblets than bulbs that have had no cuttage. Although greater in number, bulbs that are produced by scoring or sectioning will have as much as 30% less total fresh weight than those bulbs that are produced by natural propagation. However, considering both the number and fresh weight of the bulbs formed, scoring and sectioning of *R. bagnoldii* produce the greatest total fresh weight. Scooping treatments do not produce any adventitious growth or bulbs and the plants eventually die. Control plants maintain normal growth during the culture period in vitro, but do not produce any adventitious bulblets.

Bulbs of *Leucocoryne coquimbensis* can also be propagated in vitro. *Leucocoryne* bulbs that have their basal plates scored with two incisions, increased in fresh weight the most of the treatments that were tested. Bulbs treated with either scoring technique or sectioned into four pieces produced 4 or 8 times more bulblets than the control plants, respectively, and obtained the greatest total fresh weight of bulbs produced. Only a few of the control bulbs produced some adventitious bulblets in vitro. Most of the control bulbs remained inactive with no growth of shoots or roots during the test period. This research demonstrates the importance of basal plate cuttage on *Leucocoryne coquimbensis* bulbs for increased bulblet production in vitro.

When propagating *Rhodophiala bagnoldii* bulbs in vitro it is advantageous to use cytokinins in the propagation medium. There will be a significant increase in total plant growth (fresh weight) and the number of bulblets produced with the addition of \(N_6\)-benzyladenine (BA) to the growing medium. In these experiments, there was no difference in the production of bulblets between any of the three levels of BA that were used, 2.5 µM, 5 µM, and 10 µM of BA. There was no benefit to adding 2-isopentenyl-adenine (2iP) at any of the three levels to the growing medium.

When growing *Rhodophiala bagnoldii* bulbs in vitro, it is advantageous to increase the amount of sucrose in the medium if larger bulbs need to be produced faster. Increases
in total plant and bulb growth were obtained by adding 60 and 90 g liter⁻¹ to the growing medium. Although the increased levels of sucrose will increase the size of the bulbs, there is not a significant increase in the number of bulbs that are produced. This procedure may be helpful to breeders who want to produce flowering bulbs from seeds in a shorter period of time.

**Literature Cited**


Figures

Fig. 1. Total fresh weight of *Rhodophiala bagnoldii* plants produced in vitro 12 weeks after initiation.

Fig. 2. Number and average fresh weight of adventitious bulbs of *Rhodophiala bagnoldii* produced in vitro 12 weeks after initiation.

Fig. 3. Individual fresh weight and number of adventitious bulbs of *Leucocoryne coquimbensis* produced in vitro 18 weeks after initiation.
Fig. 4. Total fresh weight of *Leucocoryne coquimbensis* plants produced in vitro 18 weeks after initiation.

Fig. 5. Effect of different cytokinin treatments on total plant weight increase of *Rhodophiala bagnoldii* after 12 weeks of culture in vitro. Mean separation for weight increase by Tukey’s multiple range test at $P \leq 0.05$. 


Fig. 6. Effect of different cytokinin treatments on final production of *Rhodophiala bagnoldii* bulbs after 12 weeks of culture in vitro. Mean separation for treatments by Tukey’s multiple range test at P ≤ 0.05.

![Graph showing the effect of cytokinin treatments on bulb production.](image)

Fig. 7. Effect of sucrose concentration on total plant weight of *Rhodophiala bagnoldii* after 12 weeks of culture in vitro. Mean separation for treatments by Tukey’s multiple range test at P ≤ 0.05

![Graph showing the effect of sucrose concentration on plant weight.](image)

Fig. 8. Effect of different levels of sucrose on the final number and weight of bulbs produced in vitro of *Rhodophiala bagnoldii* after 12 weeks of culture.

![Graph showing the effect of sucrose on bulb number and weight.](image)