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STUDY ON THE ASEXUAL PROPAGATION OF *BILLBERGIA NUTANS* H. WENDL. EX REGEL.

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ABSTRACT: *Billbergia nutans* H. Wendl. ex Regel (Bromeliaceae), is an ornamental species widely commercialized and produced by nurserymen. There is scarce diffusion and bibliography available in Spanish about the production and management of this species. Furthermore, the availability of improved species and hybrids is scarce in Argentina. The objective of this study was to develop a protocol for the asexual propagation of this species, useful for nurserymen. The first phase of the study was to propagate plants of *Billbergia nutans* by clumps division. Then, one group was subjected to apical bud removal of the growing apex (decapitation) by mechanical methods, while the other was a control group. Thereafter, variables such as number of offspring produced, length of offspring and leaves were measured. In the second phase, we evaluated the effect of the application of mineral nutrition with synthetic auxins on relative growth rate and root variables using RhizoVision Explorer (RVE) software (©Noble Research Institute, LLC). Apical bud removal by destruction of the apical meristem in *Billbergia nutans* plants generated a greater number of offspring and a better morphological conformation for the sale of seedlings for commercial purposes. In addition, decapitated plants had a higher relative growth rate with mineral nutrition with synthetic auxins.

Keywords: *Billbergia nutans*; mineral nutrition; synthetic auxins; asexual propagation

INTRODUCTION

Billbergia spp. are a type of vascular plants which belong to the Bromeliaceae family. One of the most commercialized species is *Billbergia nutans* H. Wendl. ex Regel (Queens Tears plant). This is a monocotyledonous, herbaceous, and perennial plant. In the natural habitat, this genus grows as an epiphyte (Black and Dehgan, 1993), with a rosette growth habit with attractive leaves, and roots that have not only mechanical but also absorptive functions. The plant can grow up to 50 cm in height and about 30 cm in diameter. According to Vervaeke *et al.* (2004), this species is extremely resistant to cold. Flowering occurs in the winter period, in July and August in the southern hemisphere. The flower petals are arched and form clusters of pendulous pink flowers, with pink or purple tepals, and with several colored bracts that initially cover the flower (Reitz, 1983).

The species of the Bromeliaceae family (Bromeliads), are widely marketed as ornamental plants, being used in interior decoration and landscaping projects. Due to the growing demand for these species with ornamental value, illegal extraction in their natural growing habitats has intensified in recent years, leading to some species are at risk of extinction (Cooper and Cave, 2019; Rejane *et al*., 2012).

The market of Bromeliads in Argentina is still incipient. In Buenos Aires state are few specialized plant nurseries, and in Misiones state most of the orchid stores also offer them. The main limitation to the crop of these plants in our country, is the scarce diffusion and bibliography available in Spanish about the management and cultivation of these species. In addition, in Argentina there is limited availability of improved species and hybrids. Growers produce commercial plants of *Billbergia nutans* using asexual propagation, which is the reason that the number of marketable specimens is scarce and ends up having a direct impact on the quantity and cost of these plants. The apical meristem (apical bud) removal technique is used in their formation.

Meristems are composed of actively dividing undifferentiated cells, also called totipotent cells because of their ability to give rise to all plant tissues. Typically, meristematic cells are small, polyhedral, of similar dimensions in all directions (equidimensional). Meristems are classified as primary, located at the apexes of roots and stems, responsible for the growth in length of the plants, and secondary or lateral, which in Bromeliaceae plants are found inside the buds located in the axils of the leaves and distributed throughout the plant, giving rise to a growth in thickness in stems (future offspring) and roots, i.e., secondary growth (Azcón-Bieto and Talón, 2008).

Apical bud removal technique is carried out before flower induction, involving the destruction of the terminal meristem to break the apical dominance, stimulating the sprouting of axillary buds of the stem, and subsequently applying growth regulators that induce sprouting (Py, 1987). Among the apical bud removal alternatives, there are those that remove the 3-4 leaves from the center of the plant (heart) and introduce an implement in the form of a spatula, which is rotated until the apical meristem is destroyed, as cited by Bonilla (1992).

The process of apical bud removal eliminates the apical meristem, the site of production of phytohormones such as auxins. Auxins are found in higher concentrations in the cauline meristems, lateral meristems, active buds, and actively growing organs. They are synthesized in the apical meristems of the stems and are transported to the lower part of the plant through the parenchyma cells surrounding the vascular axes (basipetal transport). Synthetic auxins, naphthalene derivates such as naphthaleneacetic acid (NAA), also undergo polar transport when they are applied to the plant (Azcón-Bieto and Talón, 2008).

Regarding mineral nutrition, Bromeliads are generally slow-growing plants with low nutritional requirements, because species of the Bromeliaceae family and other families grow in habitats that are more deficient in mineral nutrients inhabited by vascular plants (Benzing, 1989; Benzing and Renfrow, 1974). In the production of commercial plants of Bromeliads, mineral nutrition becomes especially relevant, due to the production time is accelerated by the use of controlled-release fertilizer (CRF) or fertilizer applied by foliar application. Fertilization also produces larger plants and better flowers. When the plant is in bloom, abundant fertilization generates more offspring of greater vigor. When the immature inflorescence is cut, it also generates more offspring.

The aim of this work was to develop alternatives for the asexual propagation of *Billbergia nutans*, through the development of a protocol, using the separation of offspring and later, their apical bud removal by destruction of the apical meristem by mechanical methods. In a second stage, we evaluated the effect of mineral nutrition with synthetic auxins on the growth of this species.

MATERIALS AND METHODS

Plant material and growth conditions:

The experiment was carried out at Rafaela city, Santa Fe, Argentina (31°15'20.1 "S 61°29'49.5 "W), during the months from November 2022 to May 2023. Mother plants of *Billbergia nutans* were produced under a shade house of natural light conditions with a 60% black shading net protection. The technique used of asexual propagation was by clumps division (Fig., 1).

The propagules obtained were transplanted into plastic containers of 12 cm in diameter, 10 cm in height. The substrate used was a mixture of pine bark chips:charcoal:peat in a ratio of 1:1:2 (Fig. 2).

The new plants generated were very similar in their morphological characteristics (length and number of leaves). They were grown for two months, in order to establish them, before the application of the treatments.

Irrigation management of the plants in all trials was the same, being irrigated with a frequency of once a week at the rate of 50 mm per individual.

Fig. 1. Propagules separated from the mother plant.

Treatments and variables measured:

1. Apical bud removal:

The treatments that were applied were defined in accordance with: interviews with farmers of *Billbergia* from Argentina, bibliographic information and the working group's own experience.

A total of 10 seedlings were produced as detailed previously and after 2 months of growth (Fig., 3) were decapitated in order to destroy the cauline apex and the meristematic tissue, breaking the apical dominance and stimulating the sprouting of buds. The method of mechanical apical bud removal was adapted from the methodology previously reported by Bonilla (1992), using an electric drill.

Each pot (Figs., 3 and 4) was firmly held and the center of the rosette was pierced in order to damage the main meristematic tissue. Then, the hole was cleaned of the remains of damaged tissue and sprinkled with cinnamon powder, which has an antifungal effect as reported by Kowalska *et al*. (2020) and Silva-Espinoza *et al*. (2013). At the same time, 10 seedlings were used as control without treatment. Growth conditions were the same as mentioned previously. The treatments were designated as follows: T1 = seedlings subjected to apical bud removal and T0 = controls seedlings without apical bud removal.

Fig. 2. Mixture of substrates used containing pine bark chips:charcoal:river peat.

Fig. 3. Seedlings of *Billbergia* after 2 months of acclimation and growth.

Fig. 4. Application of apical bud removal with electric drill to damage the main rosette (yellow arrow). Yellow labels indicate treated plants (T1) (right).

After 60 days from apical bud removal, the number of offspring emerged, their length (cm) and the average length of leaves (cm) were counted and measured, respectively, in both treatments, T1 and T0 (Figs., 5 and 6).

2. Mineral nutrition with synthetic auxins:

During the second stage of this experiment, 5 seedlings were taken at random from each group evaluated, decapitated (T1) and non-decapitated (T0). To each group, and every 15 days for a period of 60 days, a liquid fertilizer based on auxinic hormones (naphthalene acetic acid, NAA: 155 ppm), and N:P:K liquid fertilizer (18:1:4, Fertifox®), was applied at a dose of 10 ml/l of water. Growing and irrigation conditions are the same as that mentioned previously. Leaf length was measured on the following dates: February 17, March 14, 21 and 28, and April 4, 11, 18, 25. Afterwards, with the data collected, we calculated the relative growth rate (RGR) according to Zhang *et al*. (2015), using the following formula:

$$RGR = \left(\frac{L_T - L_{(T-1)}}{D}\right) \times \left(\frac{1}{L_T}\right) \qquad (1)$$

Where:

RGR = relative growth rate in cm/day × cm^{-1},

L_{T-1} = leaf length measurement in cm at time T-1,

L_T = leaf length measurement in cm at time T,

D = number of days between 2 successive measurements, T and T-1.

During the last sampling date, April 25, the roots of the plants of each treatment were separated from the seedling aerial parts and then were completely washed to remove any substrate residue. They were scanned on an HP Photosmart C4680 flatbed scanner (300 DPI resolution) and saved in JPG format. The images were processed with the free software RhizoVision Explorer (RVE) (©Noble Research Institute, LLC). The parameters analyzed were: total root length (cm), total length of roots smaller than 1 mm (cm) and root area (cm^2) (Fig., 7). This software has been used for a variety of purposes in different crops (Delory *et al.*, 2021; Griffiths *et al*., 2021 a; Griffiths *et al*., 2021 b; Guo *et al*., 2020; Han *et al*., 2020; Seethepalli *et al*., 2021). Also, for this date, the leaf length data and the root to shoot length ratio were analyzed between the two treatments. RVE setting values are presented in Table (1).

Statistical analysis:

Data are presented as means ± SE, and Student's *t*-tests were used to assess statistically significant differences with a significance level of $P<0.05$. Statistical analyses were performed with INFOSTAT software (Di Rienzo *et al*., 2008).

RESULTS AND DISCUSSION

1. Apical bud removal:

Offspring emission was significantly higher in the apical bud removal treatment (p=0.02) (Table, 2).

The length of offspring was higher in plants with treatment (T1) compared with the control (T0) (p=0.03), occurring the opposite for the length of leaves (p=0.003) as shown in Table (2).

Fig. 5. Seedlings of *Billbergia* submitted to the treatment T1 at 30 days (left) and 60 days (right) in two different individuals. The yellow arrows show the offspring.

Fig. 6. Seedlings of *Billbergia* submitted to the treatment T0 at 60 days in two different individuals. The yellow arrows show the offspring.

Fig. 7. Images of whole seedlings of Billbergia from the T1 treatment (left). Scanned roots of seedlings of *Billbergia* (right).

Table 1. RVE setting values.

Thresholding	Filter	Root pruning threshold	Diameter
215	1	1	from 0 to 1 mm

Table 2. Number of offspring in seedlings of *Billbergia* with apical bud removal treatment (T1) and control (T0).

Treatment	Number of offspring	Length of offspring (cm)	Length of leaves (cm)
T1	0.7 ± 0.2 a	11.4 ± 3.6 a	37.7 ± 1.8 a
T0	0.1 ± 0.1 b	2.1 ± 2.1 b	48.4 ± 2.4 b

Mean values (± Standard Error). Different letters indicate statistically significant differences (Student's t test; $P<0.05$).

2. Mineral nutrition with synthetic auxins:

The RGR in seedlings of *Billbergia nutans* H. Wendl. ex Regel under apical bud removal treatment (T1) was significantly higher ($p<0.0001$) than seedlings with the control treatment (T0) during the period under evaluation (Table, 3).

Fig. (8) shows the RGR for the evaluated treatments during the period evaluated. For treatment T1, the RGR was statistically significant for the sampling date of March 21 ($p<0.0001$). However, the RGR was higher for T1 for all sampling dates.

Concerning root analysis, we observed no significant differences between treatments in terms of the parameters evaluated (Table, 4). However, it was observed that the treatment without apical bud removal had the highest values for each parameter evaluated.

For the last sampling date, the treatment without apical bud removal had the greatest leaf length, while in the shoot to root length ratio there were no significant differences between the treatments (Table, 5).

3. Asexual propagation protocol:

a. Seedlings preparation:

Use mother plants of *Billbergia* of good plant health, grown under natural light conditions in a shade house with 60% shading mesh.

Seedlings should be propagated by clump division and transplanted into plastic containers with substrate of pine bark chips:charcoal:river peat in a ratio of 1:1:2.

Acclimatization of seedlings should be carried out for two months.

b. Apical bud removal:

After 2 months of plant growth, the cauline apex should be decapitated using an electric drill, taking special care not to damage the plant.

Each pot should be firmly held and the center of the rosette should be drilled to damage the main meristematic tissue.

The remains of damaged tissue are removed and cinnamon powder is applied to prevent infection (antifungal effect).

c. Mineral nutrition with synthetic auxins:

In order to achieve a good development and growth of seedlings, it is recommended to apply a liquid fertilizer based on auxinic hormones (as naphthalene acetic acid – NAA at 155 ppm) containing N:P:K 18:1:4, at a dosage of 10 ml/l of water, every 15 days.

DISCUSSION

Based on the analysis of other studies (Mason and Maskell, 1928), the decapitated plants prioritized the use of the photoassimilates produced to be translocated and stored in the rhizomes to produce new growth points, which resulted in a greater number of offspring. The rhizomes accumulate reserves, being sinks that then become sources when they must use the photoassimilates for new growth and maintenance of the organs already generated. A reduction in the number of sinks increases the transport of substances to the remaining sinks. Sinks also influence the sources, and an

Table 3. Relative growth rate (RGR) in plants with treatment T1 (apical bud removal + mineral nutrition with synthetic auxins) and T0 (control + mineral nutrition with synthetic auxins).

Treatment	Relative Growth Rate (RGR)
T1	0.0100 ± 0.0017 a
T0	0.0039 ± 0.0004 b

Mean values (± Standard Error) Different letters indicate statistically significant differences (Student's t test; P<0.05).

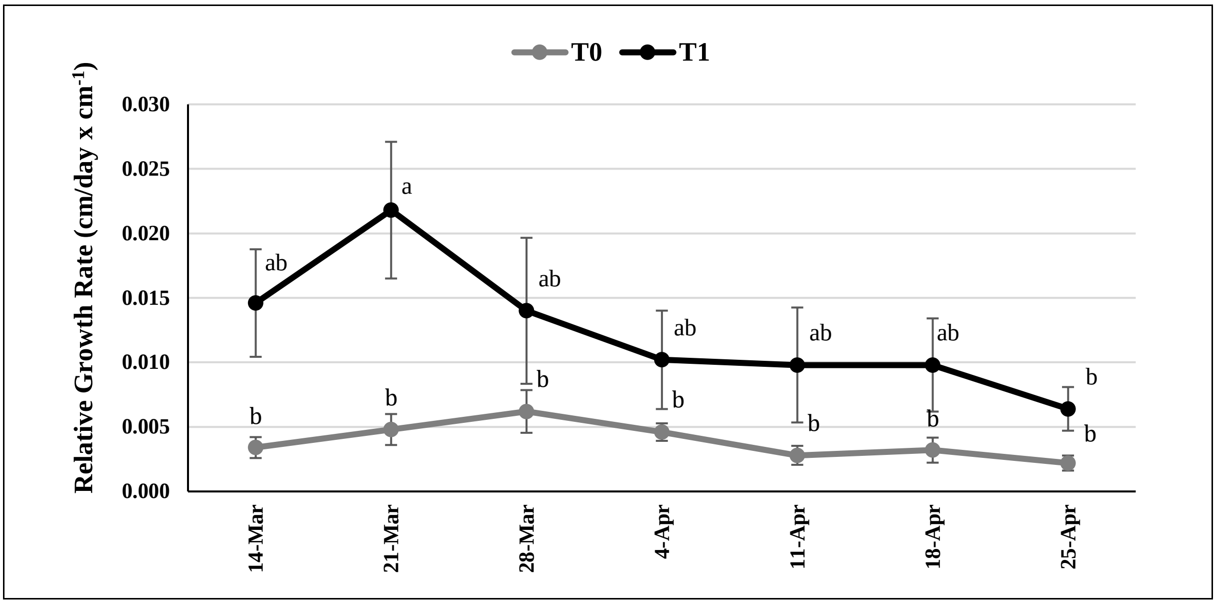

Fig. 8. Relative growth rate (RGR) for treatments T1 (apical bud removal + mineral nutrition with auxins) and T0 (control + mineral nutrition with auxins) during the sampling period. Different letters indicate significant differences (Mean values ± Standard Error; n=70; p<0.05, Student's t test).

Table 4. Total root length (cm), total root length less than 1 mm (cm) and root area (cm^2) in plants with treatment T1 (apical bud removal + mineral nutrition with auxins) and T0 (control + mineral nutrition with auxins).

Treatment	Total root length (cm)	Total root length less than 1	Root area (cm^2)
T0	6.03 ± 1.59 a	2.51 ± 0.65 a	1258.78 ± 665.57 a
T1	4.70 ± 1.17 a	1.89 ± 0.22 a	699.82 ± 353.78 a

Mean values (± Standard Error). Different letters indicate statistically significant differences (Student's t test; P<0.05).

Table 5. Length of leaves (cm) and Shoot/Root length ratio in plants with treatment T1 (apical bud removal + mineral nutrition with auxins) and T0 (control + mineral nutrition with auxins).

Treatment	Length of leaves (cm)	Shoot/root length ratio
T0	58.40 ± 3.50 a	12.01 ± 2.58 a
T1	33.00 ± 2.83 b	8.25 ± 1.32 a

Mean values (± Standard Error). Different letters indicate statistically significant differences (Student's t test; P<0.05).

increase in the demand for photoassimilates increases photosynthesis in leaves and mobilization of reserves, while elimination of sinks has the opposite effect (Bera *et al*., 2022; Paul and Foyer, 2001; Shakya and Lal, 2018).

Jiménez Díaz (1999), using the same apical bud removal treatments, but on pineapple plants (*Ananas comosus* L.), found that the growth of the offspring was more accelerated when the terminal meristem was destroyed. Bonilla (1992), working with Bromeliads, reported that the apical bud removal of the plant is to break its dominance, stimulating the sprouting of axillary buds, which guarantees the obtaining of offspring in the shortest possible time and in greater quantity, obtaining 4 to 6 offspring per mother plant.

These results are correlated with the studies carried out by Bidwell (1993) and Lafont Rapnouil *et al.* (2023) in Bromeliaceae, in which they mentioned that the application of auxins to the medium affected directly the number of offspring and their growth, because when there is a balance of cytokinin - auxin, it causes the stimulation of sprouting and growth of shoots, while there is no balance of the hormones, the growth of a plant is lower or deficient.

Primary root growth is largely indeterminate, while lateral roots and tertiary roots show determinate growth. Most of the total length of the root system is made up of fine roots, first and second order lateral roots, counted from the outside to the inside (Eissenstat and Yanai, 1997). Fine roots (less than 1 mm in diameter) have an important role in water and nutrient uptake, and their amount and functionality are mainly affected by the presence of nutrients in the soil solution (Jones *et al*., 2009; Roumet *et al*., 2016). Also, seedling roots can grow in smaller soil pores or growing substrates, giving a larger root surface area in contact with the soil solution, increasing the total uptake of water (Macfall *et al*., 1991) and nutrients such as N (Jiang *et al*., 2000) and P (Santner *et al*., 2012).

One of the main objectives of nurserymen is to achieve more balanced seedlings between the distribution of the aerial parts and roots. On the one hand, regardless of the size of the container used to produce commercial seedlings, which is important to optimize the available space in the nursery, a great development of the roots is not an indicator that the majority of them are active, and in many occasions it happens that roots are on the periphery in contact with the surface of the container are more likely become to die and necrotic, ceasing to be active and decreasing their capacity to absorb water and nutrients, which is supported by the hypothesis of balanced growth (Shipley and Meziane, 2002; Xie *et al*., 2012). The above becomes relevant due to the time that the seedlings must endure until sale and definitive transplant. On the other hand, seedlings that were not decapitated had a longer leaf length, and, therefore, greater maintenance respiration of the leaf area, greater water needs due to the greater transpiration surface, and a greater nutritional needs. Roots rely upon plant aerial portions for photosynthates and various hormones, while plant aerial portions rely on the roots for water, nutrients, support, and hormones. The delicate balance between roots and shoots can be upset when the root system is restricted in a small rooting volume, which in the short term will affect growth (Nesmith and Duval, 1998).

Finally, two situations must be analyzed, first, that the results found allow us to verify that apical bud removal did not considerably affect the development of the root system with respect to the control seedlings, and second, apical bud removal affected favorably the conformation of the seedlings for its sale.

CONCLUSION

The proposed apical bud removal treatment promoted the development of new propagules in *Billbergia nutans*. It was possible to detect that the length of the offspring was greater in the plants with apical bud removal treatment compared to those that had not received treatment.

Mineral nutrition and the application of synthetic auxins stimulated the growth of leaves and shoots in both treatments. It was observed that decapitated plants were the first to react to these chemical stimuli.

It was proven that the proposed protocol for the production of *Billbergia nutans* plants was effective for commercial purposes, in which the plants produced have had a good development of the aerial and root parts without them having been affected by the apical bud removal treatment.

Data availability:

The data that support the findings of this study are available on request from the corresponding author.

Conflict of interest:

The authors declare that there is no conflict of interest.

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Author contributions:

F. Durán: Investigation, Resources, Methodology, Writing – original draft, Software.

G.I. Perez: Conceptualization, Data curation, Formal analysis, Validation, Supervision, Visualization, Writing review & editing, Software.

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