Scientific Research Publishing

American Journal of Plant Sciences, 2018, 9, 1029-1036
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Paclobutrazol on *In Vitro* Growth and Development of *Zygopetalum crinitum* Orchid, and on Seedling Acclimatization

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How to cite this paper: Gimenes, R., Pivetta, K.F.L., Mazzini-Guedes, R.B., Ferraz, M.V., Pereira, S.T.S., Santos, Á.S., de Faria, R.T. and de Almeida, L.C.P. (2018) Paclobutrazol on *In Vitro* Growth and Development of *Zygopetalum crinitum* Orchid, and on Seedling Acclimatization. *American Journal of Plant Sciences*, **9**, 1029-1036.
https://doi.org/10.4236/ajps.2018.95079

Received: December 27, 2017
Accepted: April 17, 2018
Published: April 20, 2018

Abstract

In vitro cultivation is a technique that allows the production of great amount of plants. However, significant losses may occur during the acclimatization period (*ex vitro* stage). The objective of this study was to evaluate the effect of paclobutrazol, in the Murashige and Skoog culture medium, on growth, development, and survival of *Zygopetalum crinitum* plants. The experimental design (both *in vitro* and *ex vitro*) was completely randomized with four treatments: three paclobutrazol concentrations (0.5; 1.0; and 1.5 mg L^{-1} active ingredient) and the control (without PBZ). Morphological changes promoted by the paclobutrazol application were observed in the seedlings; however, it did not result in higher seedling survival rates of the *Zygopetalum crinitum* orchid.

Keywords

Growth Regulator, Growth Retardant, Triazol, Orchidaceae

1. Introduction

Orchids are highlighted as important ornamental and medicinal plants of great economic, ecological, and botanical interests. In Brazil, there is great species diversity of the family Orchidaceae, which is represented by around 200 genera and approximately 2350 species.

The *Zygopetalum* genus comprises 20 orchid species among epiphytes and

terrestrial, all native to South America, occurring in tropical forests, rock outcrops, and forest litter. Flowers are fragrant and, usually, colorful, with inflorescences blooming from autumn to spring [1].

Considering the struggle of orchid sexual propagation in nature due to its dependence on mycorrhizal fungi association, the *in vitro* germination becomes necessary. However, culture mediums must have the required conditions for seedling growth and development [2] [3]. In this sense, the appropriate use of growth regulators along the different stages of such process is essential for seedling development [4].

Paclobutrazol (PBZ) is a plant growth retardant of broad spectrum with great use potential for agronomic and ornamental crops [5] [6]. It acts inhibiting gibberellin synthesis, reducing plant growth, and promoting flower and fruit production [7]. Its mode of action has usually favored orchid acclimatization as it reduces transpiration, plant height, biomass, and leaf area, besides improving plant stomatal resistance; however, further studies on organ formation are still needed [8].

In vitro cultivated plants, when transferred to the *ex vitro* stage, undergo an adaptation process due to factors related to luminosity, photosynthesis, nutrient absorption, and plant health [9]. Such stage may limit the cultivation of some species due to high plant mortality, low growth, and seedling unevenness.

The PBZ used in *in vitro* cultivation may be then an alternative towards greater success in seedling acclimatization, contributing to fast adaptation and high survival rates [10] [11] [12].

Although the orchid species *Zygopetalum crinitum* Lodd. shows great ornamental potential, it is still little studied. Therefore, there is a need for improvements of both *in vitro* and *ex vitro* techniques with the aim to provide great number of high quality seedlings. The objective of this study was to evaluate the effect of paclobutrazol, in the Murashige and Skoog culture medium, on growth, development, and survival of *Zygopetalum crinitum* plants.

2. Materials and Methods

Z. crinitum seeds were inoculated in pre-autoclaved MS medium (121˚C, 1 atm, for 15 minutes) containing 30 g L^{-1} sucrose, 6 g L^{-1} agar and pH adjusted to 5.8. The glass beads with the seeds were kept in a growth room for six months, in incident light intensity of 40 μmol m^{-2} s^{-1}, using 20-watt fluorescent lamps, 16-hour photoperiod, and a controlled temperature of 25˚C ± 1˚C.

Subsequently, seedlings with 1.5 ± 0.2 cm of shoot length were selected for PBZ treatments.

The experimental design was completely randomized with four treatments (three PBZ concentrations: 0.5; 1.0; and 1.5 mg L^{-1}, besides the control with no PBZ) and five replications, with five seedlings per replication, for the *in vitro* stage; for the *ex vitro* stage, there were fifteen replications, with five seedlings per replication, resulting in 75 seedlings per treatment. The doses were adopted

based on researches with plants of the family Orchidaceae [13] [14].

The culture medium used was a complete MS [15] with 30 g L^{-1} sucrose and 6 g L^{-1} agar, supplemented with the different PBZ concentrations. After pH was adjusted to 5.8 ± 0.1, the medium was distributed in flasks and autoclaved at 121˚C (1 atm) for 20 minutes. The selected seedlings were then placed in the culture medium with PBZ under flow camera conditions. The flasks with the seedlings were maintained in a growth room, for three months, under around 40 μmol m^{-2} s^{-1} luminosity, 16-hour photoperiod, and 25˚C ± 2˚C temperature. After three months, 25 seedlings per treatment were used for the destructive analyses, while 75 seedlings per treatment were taken to acclimatization (*ex vitro* stage).

During the acclimatization, which began in November, the seedlings were kept in a greenhouse coated with a black net of 70% shading on the top and sides. Plastic trays were used, which had 98 cells (3.5 × 3.5 × 5.0 cm) with holes in the bottom surface. Sphagnum, which was also previously autoclaved, was used as a substrate. The water replenishment was performed daily, so that the substrate was maintained at 100% of its water retention capacity due to the need for high humidity at this stage. The mean temperature in the *ex vitro* phase of the experiment was 26˚C ± 3˚C and air humidity 46% to 68%. The seedlings were evaluated after 60 days of transplantation.

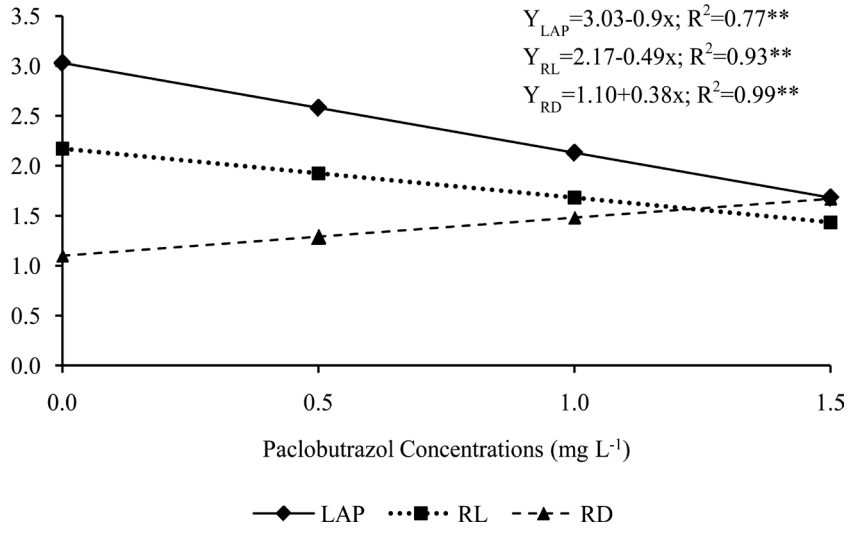
Evaluated characteristics, for both *in vitro* and *ex vitro* stages, were: leaf area, with the help of a digital area meter; number of roots and leaves; length of roots and aerial part; root diameter, with the help of a digital caliper; and dry matter of roots and aerial part. Data of number of roots and leaves were transformed into $(x + 1)^{1/2}$. Polynomial regression analysis was performed to verify variable behavior according to the PBZ concentrations. For the *ex vitro* experiment, the survival rate was also evaluated at 15, 30, 45, and 60 days after transplant, which data were transformed into arc sine $(x/100)^{1/2}$.

3. Results and Discussion

For the *in vitro* experiment, both length of aerial part and roots decreased according to the increment in PBZ concentrations (**Figure 1**); at 1.5 mg L^{-1} PBZ, the length of aerial part and roots reduced 44.6% and 33.8%, respectively, in comparison with the control (PBZ absence).

The PBZ application increases the endogenous cytokinin, aiding at cell formation, and inhibits gibberellin production, preventing cell expansion, what results in minor development of the aerial part [16], as happened in this study. Similar results were found for other orchids also cultivated *in vitro* and submitted to different PBZ concentrations, such as *Cattleya mossiae* [14], *Dendrobium* sp. [8], and *Cattleya labiata* [17].

For *C. mossiae*, for instance, there was a width reduction in both leaf epidermis and blade when seedlings were submitted to PBZ, what increased plant capacity for water loss [14]. Although leaf reduction is an important factor regarding



**Significant at $p \leq 0.01$.

Figure 1. Length of aerial part (LAP) (cm), root length (RL) (cm), and root diameter (RD) (mm) of *Zygopetalum crinitum* seedlings after 90 days of *in vitro* cultivation in the Murashige and Skoog medium supplemented with paclobutrazol.

water loss by transpiration, there are other factors to be considered in seedling survival during the *ex vitro* stage, such as morphological changes promoted by the growth regulator and the environmental conditions.

Other experiments with growth regulators presented similar results for the aerial part variable. Studies with PBZ applied via orchid soil of the *Epidendrum radicans* species to obtain smaller plants, an important characteristic for potted plants, presented a 50% reduction in shoot height in relation to the control plant when applied 5 mg L^{-1} of the regulator every fortnight for 5 months [18]. Experiment similar to that testing increasing doses of PBZ in the same orchid species obtained better results when applied to 20 mg L^{-1} of the growth regulator once a month for five months [19].

The reduction in root length observed in seedlings treated with PBZ may also be explained by the inhibition of gibberellin biosynthesis, as such plant regulator interferes on root growth, promoting long and fine roots; its absence may also reduce root elongation [20]. The PBZ may then be considered an antagonist of gibberellins, but its effects on plant development are different according to the plant part [21], so future studies are still needed to establish the PBZ role in, at least, root development. There are reports attesting that PBZ promotes rooting but, at the same time, controls shoot growth [6]. Corroborating this study, there was a decrease in root length of *C. mossiae* seedlings treated with PBZ [14].

The increase in PBZ concentrations promoted a 51.9% increment in root diameter of *Z. crinitum* seedlings (**Figure 1**), when compared with the control. Root thickening is promoted by radial cell divisions over cell elongation; although PBZ inhibits cell elongation, it does not prevent cell division [21].

Studies on asparagus showed that the culture medium supplemented with triazol, which is structurally similar to PBZ, promoted root thickening, growth re-

duction of the aerial part, and seedling vigor increase [22]. Analogous results were reported for *Lilium* sp. submitted to PBZ, resulting in an increment in root diameter [11]. Therefore, the culture medium supplemented with PBZ for *Lilium* sp. cultivation [11] and, also, chrysanthemum [23], improves plant survival along the *ex vitro* stage. Such increase in root diameter may be a good characteristic for seedling acclimatization, as at such stage, roots are fragile and need fast adaptation to reduce mortality. Similar results were also observed for *Dendrobium* sp. [8].

The growth regulator promoted morphoanatomic changes in the seedlings, as shown in **Figure 2**, for *in vitro* cultivation, however, there was no regression adjustment for leaf area, root number and shoot dry matter and roots.

For the acclimatization stage, there was a 49.7% decrease in the length of aerial part promoted by the highest PBZ concentration, in comparison with the control (**Figure 3**). This result corroborates the *in vitro* cultivation, as such variable had already shown a reduction at that stage. The reduced leaf area does present a decrease in photosynthesis but, on the other hand, implies a mechanism of drought stress prevention [24]. Furthermore, the plant total leaf area is related to the potential for water loss by transpiration [25]. However, in this study, the reduction in the aerial part was not enough to decrease plant losses during acclimatization.

The PBZ increment in the culture medium promoted a linear increase in root diameter at the *in vitro* stage, what was maintained along acclimatization (**Figure 3**).

The acclimatization process is usually impaired by high rates of seedling mortality due to excessive water loss and root fragility. Root thickness may then be a good characteristic towards seedling adaptation during the *ex vitro* stage. Thus, the PBZ application provides higher resistance to desiccation and, consequently, greater seedling survival [20].

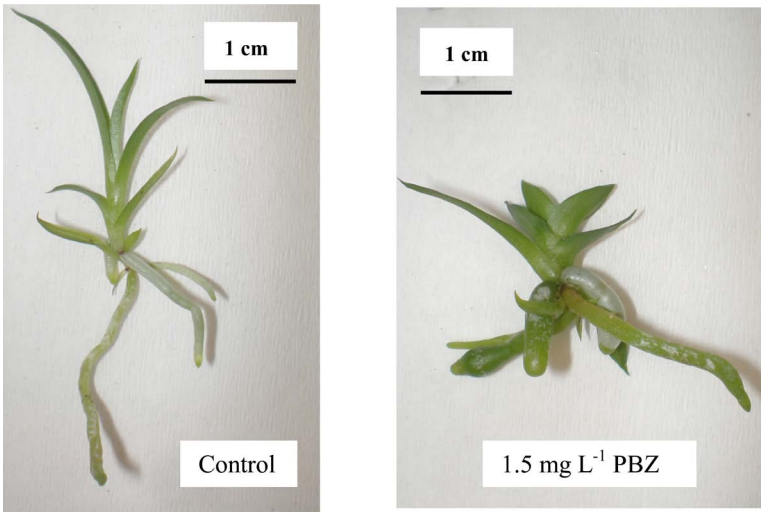
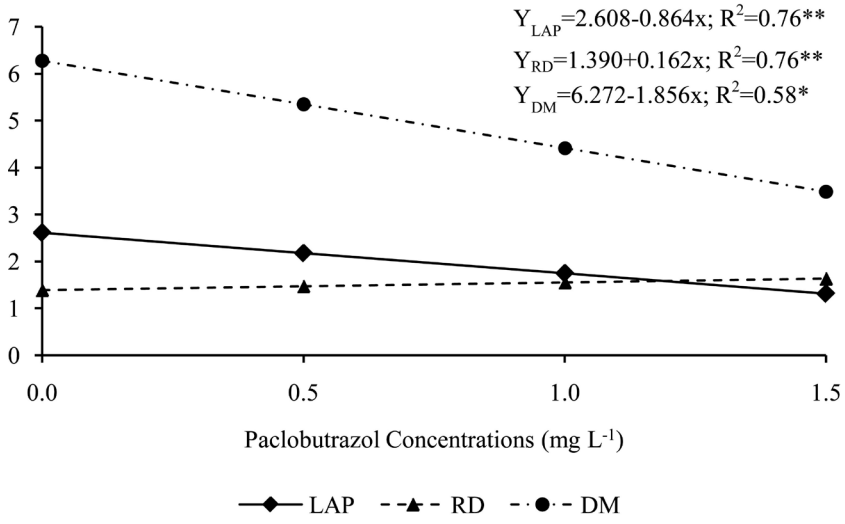


Figure 2. *Zygopetalum crinitum* seedlings after 60 days of *in vitro* cultivation in MS medium either with no paclobutrazol or supplemented with 1.5 mg L^{-1} paclobutrazol.

When seedlings were cultivated in the greenhouse, the dry matter of aerial part was greater under PBZ absence (Figure 3); seedlings had a 44.4% increment promoted by the control treatment in comparison with the highest PBZ concentration. Such result may be explained by the minor length of aerial part, verified at the *in vitro* stage.

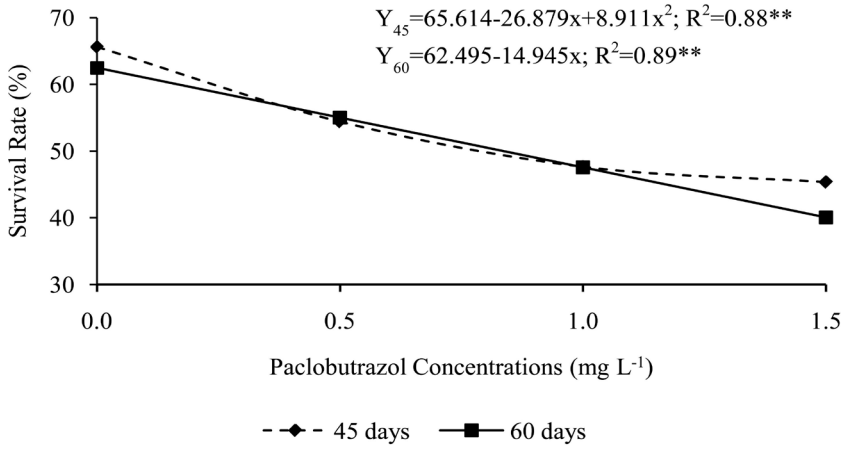
For the acclimatization stage, there was no regression adjustment for the variables leaf area, leaf number, root number and length, and dry matter of roots.

For the other variables, there were no regression adjustments at 15 and 30 days after transplant either. However, at 45 and 60 days of transplanting, seedling survival was lower when PBZ was used, in comparison with the control (Figure 4). For both evaluations, seedling survival was lower than 50% when submitted to the highest PBZ concentration, that is, 1.5 mg L^{-1}.



*Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$.

Figure 3. Length of aerial part (LAP) (cm), root diameter (RD) (mm), and dry matter of aerial part (DM) (mg) of *Zygopetalum crinitum* seedlings after 60 days of acclimatization.



**Significant at $p \leq 0.01$.

Figure 4. Survival rate of *Zygopetalum crinitum* seedlings after 45 and 60 days of acclimatization.

However, PBZ did improve the survival rate of *Dendrobium nobile* orchids during acclimatization and, yet, promoted shorter but stronger plantlets [20]. There are also other reports of fast adaptation and high survival in plant acclimatization with the use of PBZ at the *in vitro* cultivation stage [10] [11]. Such achievement was not verified in this study probably due to PBZ high concentrations, which did not promote the necessary seedling changes towards major resistance to the conditions of *ex vitro* cultivation.

4. Conclusion

For *in vitro* cultivation of *Zygopetalum crinitum* seedlings, the Murashige and Skoog culture medium, supplemented with paclobutrazol, promoted root thickening and a reduction in the length of aerial part and roots. At the acclimatization stage, there was also root thickening, but minor length and dry matter of seedling aerial part. The morphological changes observed in the seedlings caused by the paclobutrazol application did not imply superior survival of the orchid *Zygopetalum crinitum*.

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