





Original article

Improvement of Plant Morphology and Quality in *Ficus Lyrata* Propagation Using Paclobutrazol Under in Vitro Conditions

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Abstract

Ficus lyrata (fiddle-leaf fig), belonging to the Moraceae family, is a plant species of significant commercial value among indoor ornamentals due to its large, glossy leaves and attractive appearance. Its durability and decorative quality have made it highly demanded in both domestic and international markets. However, conventional vegetative propagation methods exhibit low rooting rates, slow multiplication, and considerable morphological variation among the produced plants. Therefore, in vitro propagation through tissue culture offers an important alternative for producing large quantities of genetically uniform and disease-free plant material. One of the common problems observed in *Ficus lyrata* plants propagated under in vitro conditions is the development of thin and weak stems accompanied by excessive elongation, which negatively affects overall plant form, reduces seedling quality, and decreases acclimatization success. To address these issues, this study investigated the dose-dependent effects of paclobutrazol (PBZ), a gibberellin biosynthesis inhibitor, on the morphological quality of *F. lyrata* during both the multiplication and rooting stages under in vitro conditions. Shoot tip explants derived from the 3rd subculture of aseptic stock cultures were cultured on Woody Plant Medium (WPM) supplemented with four PBZ concentrations (0.05, 0.10, 0.25, and 0.50 mg L⁻¹) alongside a control group, with five explants per treatment (n = 5). Cultures were incubated for four weeks at 25 ± 1°C under a 16-hour photoperiod. Shoot length, stem diameter, leaf area, leaf width, and color vividness were evaluated and data were analyzed using one-way ANOVA and Tukey's HSD test (p < 0.05). The 0.10 mg L⁻¹ PBZ treatment produced the most favorable morphological response in both stages, yielding compact shoots with increased stem diameter and expanded leaf area. Higher concentrations (0.25-0.50 mg L⁻¹) significantly suppressed growth, causing leaf deformation and chlorosis, while the lowest dose (0.05 mg L⁻¹) had limited effects. These results indicate that low-dose PBZ application is an effective strategy for enhancing plant quality in the in vitro propagation of *F. lyrata*, offering a valuable biotechnological approach for improving both production efficiency and market quality in the ornamental plant industry.

Keywords: Tissue Culture, *Ficus lyrata*, Micropropagation, Paclobutrazol

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INTRODUCTION

Ficus lyrata is a broad-leaved species belonging to the Moraceae family, originating from West Africa, which can grow both epiphytically and freely. Reaching heights of 15-30 meters in its natural habitat, this species has gained significant importance in the indoor ornamental plant sector as the "violin-leaf rubber plant" due to the lyre-shaped morphology of its broad, leathery leaves (Gilman et al., 2019). The species' natural range encompasses humid tropical biomes stretching from Sierra Leone, Liberia, Ivory Coast, Ghana, and Nigeria to Cameroon and Gabon (Gilman et al., 2019). Anatomically, it possesses a distinct hypodermis, dense palisade parenchyma, and a well-developed vascular system; these structures confer adaptive traits against water and light stresses. The gray-brown bark structure of the trunk and the milky latex exudate that appears after injury are typical characteristics of the Moraceae family and are also prominent in *F. lyrata* (Gilman et al., 2019). Flowering occurs within a closed inflorescence structure called a syconium, and pollination is provided by agaonid fig wasps; however, flower and fruit formation are rarely observed under indoor conditions (Rønsted et al., 2005).

Traditionally, semi-wood cuttings, air layering, and root division techniques have been used in the production of *Ficus lyrata*; however, these methods have limitations such as high rootstock requirements, inability to maintain homogeneity, low rooting rates, and long production times. Therefore, tissue culture has become an increasingly important alternative in the commercial production of this species. The first micropropagation study on the species was conducted by Debergh and De Wael, demonstrating that shoot tip explants could directly form adventitious shoots (Debergh & De Wael, 1977). Jona and Gribaudo confirmed the suitability of the species for organogenesis-based propagation by demonstrating that leaf explants have the capacity to form adventitious buds in cytokinin-rich media (Jona & Gribaudo, 1987). However, various studies have shown that *F. lyrata* explants are prone to phenolic oxidation and that the morphogenic response only occurs under specific hormone balances. This necessitates careful optimization of the culture medium and growth regulators in tissue culture studies of this species.

One of the critical variables affecting success in tissue culture is the mineral composition of the basic nutrient medium. The Murashige and Skoog (MS) medium can cause physiological stress in many woody species due to its high-ion concentration (Murashige & Skoog, 1962). In contrast, the Woody Plant Medium (WPM) developed by Lloyd and McCown offers a nutrient profile more suited to the physiological requirements of woody species due to its lower ammonium and total salt content (Lloyd & McCown, 1980). WPM has been reported to yield more favorable results in *Ficus* species in terms of reducing phenolic oxidation and increasing shoot viability. In this regard, the use of WPM in *Ficus lyrata* cultures, where phenolic oxidation is a significant problem, is considered a rational and scientific medium selection that considers the physiological sensitivities of the species.

Another fundamental problem encountered in *F. lyrata* production is the excessive elongation of plants under indoor conditions, resulting in long internodes and the development of a thin-stemmed form. This problem negatively affects both aesthetic quality and mechanical strength. Paclobutrazol (PBZ), which inhibits the gibberellin biosynthesis pathway, is a growth regulator widely used in the height control of ornamental plants by blocking the ent-kaurene oxidase step and thus limiting cell elongation (Desta & Amare, 2021). Barrett and Nell's study showed that PBZ reduced internode length, shortened plant height, and created a more compact crown structure in *Ficus benjamina* (Barrett & Nell, 1983). Hazarika (2003) reported that plantlets produced under in vitro conditions often exhibit weak structural characteristics, which negatively affect their survival during acclimatization (Hazarika, 2003). The study emphasized that compact and structurally stronger shoots show improved tolerance to environmental stress and higher survival rates during the transition from in vitro to ex vitro conditions.

The most frequently reported adverse effects in tissue culture studies on *Ficus lyrata* are tissue browning due to phenolic oxidation, low shoot viability, thin and weak stem development, excessively elongated internodes, and insufficient structural strength during the acclimatization process (Hazarika, 2003; Jona & Gribaudo, 1987). A significant portion of these problems is related to the species' physiologically long internode structure, high gibberellin activity, and inability to develop effective adaptive morphological responses to stress under in vitro conditions. Paclobutrazol directly limits cell elongation by inhibiting the ent-kaurene oxidase step in the gibberellin biosynthesis pathway; thus, it has the potential to reduce problems specific to *Ficus lyrata*, such as excessive elongation, thin stem form, and weak tissue strength (Desta & Amare, 2021). It has been reported that PBZ reduces internode length in closely related species such as *Ficus benjamina*, resulting in a more compact plant form (Barrett & Nell, 1983). Therefore, PBZ is considered a suitable growth regulator that can be used in *F. lyrata* tissue culture to both improve morphological quality and reduce losses during the acclimatization process.

A general review of the literature reveals two main problems in *F. lyrata* production. First, browning due to phenolic oxidation during the tissue culture stage and the associated low regeneration rates are noteworthy. The second major difficulty is excessive elongation and weak stem form, which are frequently observed in commercial production. Considering the potential of WPM medium to reduce phenolic oxidation due to its low ion content, together with the growth-morphology-regulating effects of paclobutrazol, it is evident that these two approaches could contribute to reducing the problems encountered in *F. lyrata* production. Furthermore, the fact that paclobutrazol has not yet been extensively studied in *F. lyrata* tissue culture systems indicates that studies in this area would fill an important scientific gap. Therefore, this study aims to evaluate the effects of different PBZ concentrations on the morphological quality of *Ficus lyrata* during both the multiplication and rooting stages under in vitro conditions. To our knowledge, this is the first study to systematically investigate PBZ applications in *F.*

lyrata tissue culture, thereby addressing an important gap in the existing literature on ornamental plant micropropagation.

MATERIAL and METHOD

Plant Material

This study covers the development process of *Ficus lyrata* plants, starting from the in vitro propagation stages of previously cultured plants whose production process continues under laboratory conditions, up to the potted production period. The explants used in the study were shoot tip explants obtained from the mother plant, taken from the 3rd subculture of the aseptic cultures currently in propagation, and were selected from healthy shoots showing active growth. Explants were prepared as shoot tips approximately 1.5-2 cm in length.

Within the scope of the study, the effects of different paclobutrazol (PBZ) concentrations on morphological responses during the rooting phase were evaluated. Each treatment consisted of five explants ($n = 5$), and all measurements were based on these replicates. The explants were derived from previously established in vitro stock cultures maintained through regular subculturing. Paclobutrazol applications were conducted separately for the multiplication and rooting stages, and each stage was evaluated using independent experimental sets. Each treatment was arranged in a completely randomized design. The explants used in the experiment were prepared from shoots growing in existing cultures, and all explants were selected to have identical morphological characteristics in terms of meristem content and development level. No material was brought in from the external environment for this study; all samples were taken from plants selected from the existing in vitro propagation system.

Preparation of the Culture Medium

All cultures were prepared based on Woody Plant Medium (WPM). Compared to MS, this medium has a lower total salt and ammonium content, allowing for the preservation of tissue viability and a more stable physiological response in phenol oxidation-prone species such as *Ficus lyrata*. The culture medium was prepared to contain WPM mineral salts, 30 g L⁻¹ sucrose, and 6 g L⁻¹ agar. To support shoot formation during the propagation phase, 0.2 mg L⁻¹ 6-benzylaminopurine (BAP) was added to the medium as a source of cytokinin. During the rooting phase, no exogenous auxin was supplemented to the medium; rooting was achieved solely under the influence of PBZ at the specified concentrations, without the addition of auxin sources such as IBA or NAA. This approach was adopted to isolate the morphogenic effects of PBZ on root organogenesis under auxin-free basal conditions. Different doses to be used in paclobutrazol applications were added directly to the medium composition during preparation, prior to sterilization. PBZ was mixed with the medium components to achieve complete homogeneity at the specified concentrations, the pH of all media was adjusted to 5.78-5.82 prior to autoclaving. All media were dispensed into glass jars and sterilized by autoclaving at 121 °C under 15

psi pressure for 15 minutes. Thus, PBZ remained a constant component of the medium throughout the culture period, ensuring that the explants were exposed to an equal dose of the regulator at every stage.

Paclobutrazol (PBZ) Applications

The PBZ concentrations used in this study (0.05-0.50 mg L⁻¹) were selected based on effective dose ranges reported in in vitro studies of woody plant species and previous studies on closely related *Ficus* species, where low PBZ concentrations have been shown to effectively modulate shoot morphology and improve plantlet quality (Marino, 1988; Barrett & Nell, 1983). Five different PBZ doses were used in the culture medium to evaluate the effects of paclobutrazol on the morphological responses of *Ficus lyrata* shoots during both the multiplication and rooting stages. The PBZ doses used in the propagation stage are denoted by A_x, while the doses used in the rooting stage are denoted by B_x.

PBZ concentrations during the propagation phase were set as A₀(control), A₁(0.05 mg L⁻¹), A₂(0.10 mg L⁻¹), A₃(0.25 mg L⁻¹), and A₄(0.50 mg L⁻¹). During the rooting phase, the same dose range was maintained, and concentrations of B₀(control), B₁(0.05 mg L⁻¹), B₂(0.10 mg L⁻¹), B₃(0.25 mg L⁻¹), and B₄(0.50 mg L⁻¹) were applied.

Preparing separate culture media for each PBZ dose allowed for the evaluation of PBZ responses specific to the development stage of the explants in a dose-dependent, reproducible, and statistically comparable manner. This experimental setup allowed the effects of different PBZ concentrations on shoot multiplication, stem morphogenesis, and rooting to be studied in a highly controlled environment.

Culture Conditions

Explants transferred to culture media containing paclobutrazol and prepared as control groups were incubated under controlled environmental conditions accepted as standard for plant tissue culture. The culture process was carried out at a temperature of 25 ± 1°C, with a photoperiod of 16 hours of light and 8 hours of darkness. Lighting was provided by cool white LED light sources providing a light intensity of 40-50 μmol m⁻² s⁻¹. Throughout the culture period, the explants' phenolic browning tendencies, tissue integrity, shoot development, and viability were observed and recorded at weekly intervals. These conditions are critical for monitoring shoot quality and evaluating PBZ-dose-dependent morphological changes in phenol oxidation-prone species such as *Ficus lyrata*.

Morphological Measurements

At the end of the four-week culture period, five key parameters were evaluated to determine the morphological responses of the explants to PBZ. Stem length was determined by measuring the distance from the apical tip of the shoot to the base point on millimeter paper. Stem thickness was recorded by measuring the widest diameter value taken at the point closest to the shoot base on millimeter paper. Leaf width was determined by tracing the contour of the widest leaf developed on the explant on

millimeter paper and reading the width value in millimeters. Color vividness was evaluated comparatively using visual observation.

Leaf area was included as a key morphological parameter since it directly reflects the photosynthetic capacity of the plantlet and has been reported to be significantly affected by PBZ application in ornamental plant species (Desta & Amare, 2021). Leaf area was determined using digital image analysis, in which photographs were captured against a millimeter-scaled reference background and imported into Adobe Photoshop for pixel-based area quantification. Pixel counts were subsequently converted to mm² based on the known reference dimensions. This approach is consistent with previously validated digital image-based methods reporting high accuracy and reproducibility in leaf area measurement (Xiao et al., 2005).

These measurement methods are compatible with techniques commonly used in literature for the morphological evaluation of in vitro ornamental plants and have been validated in numerous studies as reliable and reproducible approaches for assessing plant quality under controlled culture conditions.

Statistical Analysis

Statistical analyses were performed to evaluate the responses of the morphological data obtained in this study to paclobutrazol (PBZ) applications. Each treatment consisted of five biological replicates (n = 5), and all measurements were based on these replicates. Measurement values related to the propagation (A series) and rooting (B series) stages were evaluated using one-way analysis of variance (ANOVA) to examine the differences between PBZ concentrations. In the analyses, the statistical significance level was set at $p < 0.05$; the Tukey HSD multiple comparison test was applied to determine the differences between groups for parameters with significant differences. All statistical procedures were performed using IBM SPSS Statistics 25.0 software, and the findings were reported in detail in the relevant sections.

RESULTS and DISCUSSION

This section presents the evaluation of the morphological responses of *Ficus lyrata* to different paclobutrazol (PBZ) doses during the in vitro propagation (A_x Series) and rooting (B_x Series) stages. The findings show that PBZ exhibits a dose-dependent effect profile and significantly modulates both shoot and root morphology. The evaluations were interpreted separately for the two series, as the physiological dynamics of the propagation and rooting stages are different.

In vitro Propagation Stage

Morphological Observations

The general shoot morphology of *Ficus lyrata* explants propagated at different PBZ doses clearly demonstrated the dose-dependent effect of PBZ. While the control group (A₀) had long internodes and a weaker stem structure, the PBZ-treated groups showed shoots that gradually became more compact in form. Specifically, the 0.1 mg L⁻¹ PBZ application (A₂) exhibited the most balanced morphological appearance during propagation, characterized by broad leaf plates, vivid green pigmentation, and thick stem tissue. At higher PBZ doses (A₃-A₄), growth inhibition became more pronounced, leaf size and shoot length decreased, and plant height between nodes decreased. These findings indicate that PBZ is a critical regulator shaping morphological quality during the propagation phase.

Explants without paclobutrazol typically exhibited longer internodes, relatively thin stems, and limited leaf width. Elongation growth specific to the propagation phase is dominant, and compactness is low (Figure 1). At low PBZ doses, slight compaction is observed in shoot architecture, and stem thickness begins to increase compared to the control group. A noticeable but limited increase in leaf area is observed compared to the control group (Figure 2). This dose provided the highest morphological quality during the propagation phase. Leaf area increased significantly, stem thickness reached its maximum level, and shoots gained a compact yet healthy form. Homogeneous development in plants is noteworthy (Figure 3). It is observed that growth is significantly suppressed with increasing PBZ concentration. Leaves have shrunk, shoot length has shortened, and a decrease in overall plant vigor has occurred (Figure 4). Growth inhibition is most pronounced at the highest PBZ dose in the series. Shoots are quite short, leaves are small, and stem development is limited. It is clear that the excessive dose negatively affects morphological quality (Figure 5). Comprehensive assessment of morphological changes related to PBZ dose during the propagation phase. With an increasing dose, compactness increased, leaf area reached a maximum at the medium dose (A₂), and growth inhibition became pronounced at high doses. The A₂ series represents the optimal morphological response (Figure 6).

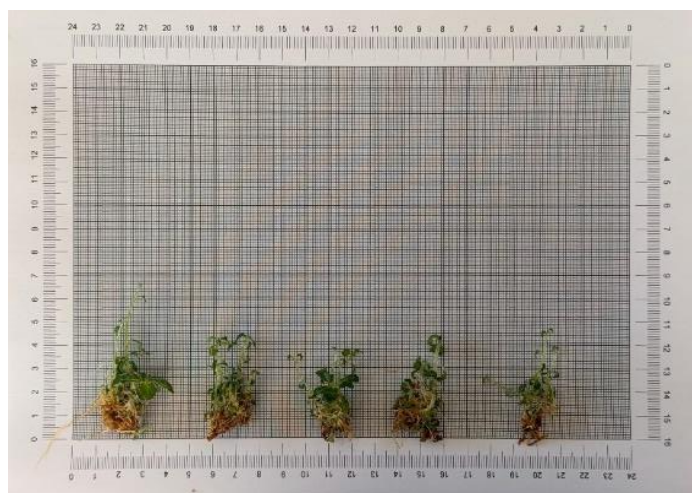


Figure 1. Morphological appearance of control group (A₀)

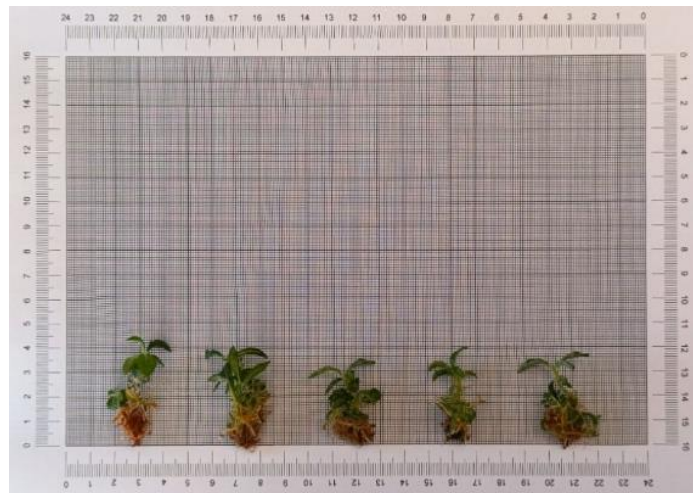


Figure 2. Appearance of explants treated with 0.05 mg L⁻¹ PBZ (A₁).



Figure 3. Appearance of explants treated with 0.1 mg L⁻¹ PBZ (A₂)

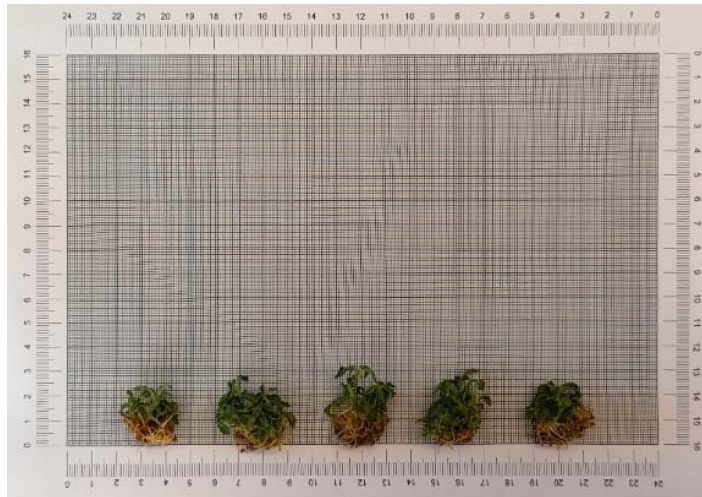


Figure 4. Appearance of explants treated with 0.25 mg L⁻¹ PBZ (A₃)

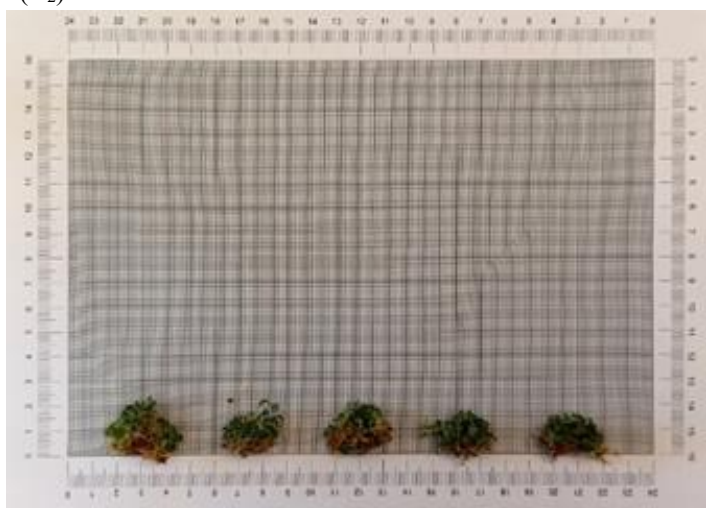


Figure 5. Appearance of explants treated with 0.5 mg L⁻¹ (A₄)



Figure 6. Comparative morphological appearance of PBZ treatments (From left to right: A₀, A₁, A₂, A₃, A₄).

Shoot Length

The effect of PBZ doses on shoot length is pronounced and statistically significant ($F_{4, 20} = 15.20$; $p < 0.001$). The control group (A_0) produced the longest shoots with an average of 4.84 cm, and shoot length gradually decreased with PBZ application. Growth inhibition became particularly pronounced at high doses (A_3 - A_4), decreasing to an average of 2.02 cm in the A_4 group (Figure 7). These results showed the physiological mechanism whereby PBZ inhibits elongation growth by inhibiting the steps of gibberellin biosynthesis. The difference was found to be statistically significant in the ANOVA analysis ($p < 0.001$), and the Tukey test determined that the A_0 group was significantly longer than A_3 and A_4 .

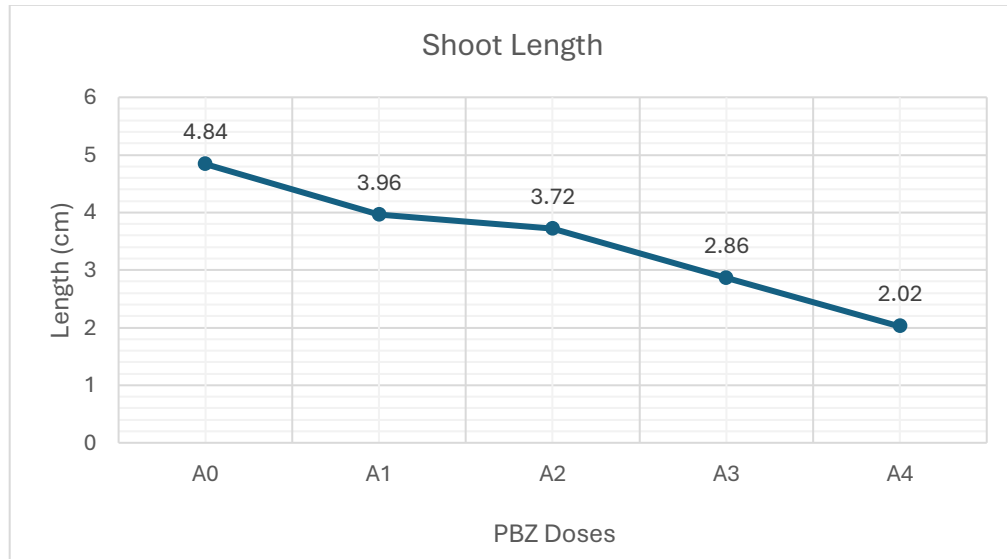


Figure 7. Effect of paclobutrazol on shoot length during the multiplication phase

Leaf Area

Leaf area analyses show that PBZ modulates the growth response during the multiplication phase not only by shortening the stem but also by affecting photosynthetic surface development. In the A series, the highest leaf area was obtained in the A_2 group treated with 0.1 mg L^{-1} PBZ; this dose significantly promoted leaf expansion in the plants. While more limited leaf development was observed in the control and low PBZ doses, significant reductions in leaf area occurred at high PBZ levels due to excessive suppression of growth. This morphological trend is clearly visible in the distribution graph presented in Figure 8, where the A_2 group is distinctly separated from all other treatments.

ANOVA analysis provided a high level of explanatory power for this parameter ($F_{4,20} = 13.35$; $p < 0.001$); the calculated effect size ($\eta^2 = 0.73$) confirms the strong and biologically meaningful effect of PBZ on leaf development.

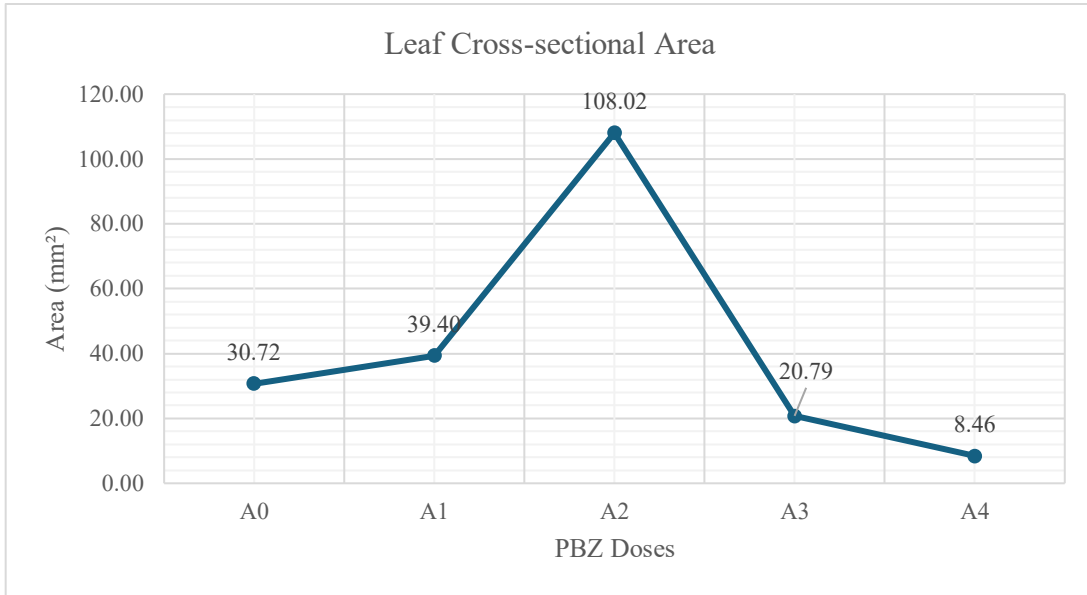


Figure 8. Effect of paclobutrazol on leaf cross-sectional area during the multiplication phase

Stem Diameter

Stem diameter is a critical parameter in terms of plant resilience and acclimatization readiness during the propagation phase. The average stem diameter measured in group A2 was significantly higher than both the control and low-dose PBZ applications ($F_{4,20} = 11.89$; $p < 0.001$). It was observed that low doses of PBZ created a compact and resistant structure in the stem tissue, while high doses led to stem thinning due to excessive growth suppression (Figure 9). This data indicate that the A2 dose produces the optimal physiological response for the propagation stage. The mean values of all morphological parameters measured in the propagation stage, calculated as mean \pm SD from five explants per treatment, are summarized in Table 1.

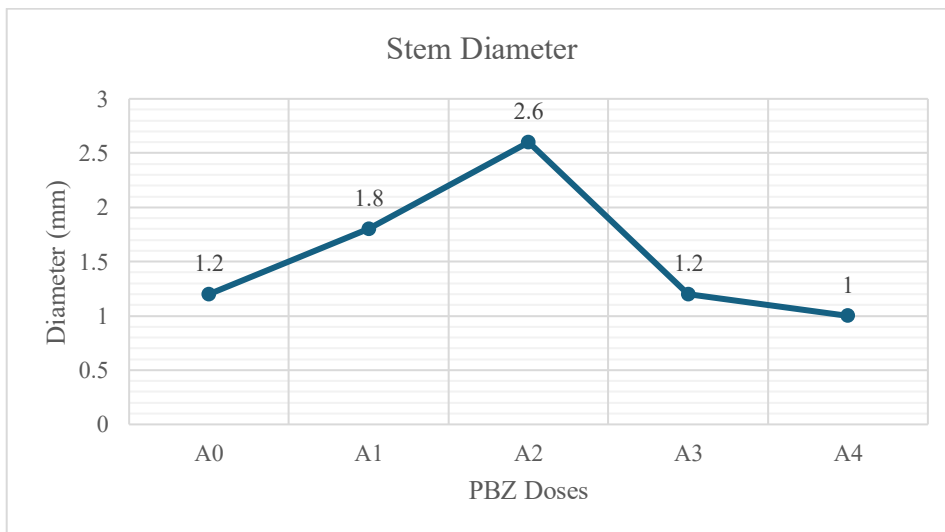


Figure 9. Effect of PBZ doses on stem diameter during the multiplication phase

Table 1. Mean values of morphological parameters obtained from five explants per treatment during the in vitro multiplication stage of *Ficus lyrata*

Treatment	PBZ Dose (mg L ⁻¹)	n	Shoot Length (cm)	Leaf Area (mm ²)	Stem Diameter (mm)
A0	0.00	5	4.84 ± 1.08	30.72 ± 24.76	1.20 ± 0.45
A1	0.05	5	3.96 ± 0.36	39.40 ± 11.89	1.80 ± 0.45
A2	0.10	5	3.72 ± 0.64	108.02 ± 45.07	2.60 ± 0.55
A3	0.25	5	2.86 ± 0.30	20.79 ± 6.17	1.20 ± 0.45
A4	0.50	5	2.02 ± 0.35	8.46 ± 3.98	1.00 ± 0.00

Values represent the mean ± SD of five explants per treatment. The data presented correspond to the graphical results shown in Figures 7-9.

In vitro Rooting Phase

Morphological Observations

The effects of PBZ during the rooting phase were not limited to shoot morphology but directly affected the root-shoot balance. While control shoots exhibited a long but thin stem structure, PBZ application increased shoot compactness and stem strength. Specifically, the 0.1 mg L⁻¹ PBZ application (B₂) showed the highest quality at this stage, with a large leaf area, pronounced stem thickness, and dense root development. At high PBZ doses, root and shoot development were suppressed, and plants exhibited weaker morphological characteristics.

Explants not treated with PBZ during the rooting phase exhibited long shoots but a weak structure in terms of stem and root tissue. The leaf area was quite small (Figure 10). At low PBZ doses, shoot compactness began to increase, and the leaf area expanded significantly. Root development was more intense and robust compared to the control group (Figure 11). The most superior morphological response during the rooting phase was obtained at this dose. Leaf area was at its maximum level, the stem was noticeably thickened, and a dense, healthy root system had formed. Group B₂ showed ideal performance in terms of root-shoot balance (Figure 12). At the medium dose of PBZ, shoot length was significantly shortened, leaf area decreased, and root development was weaker than at the optimal dose (Figure 13). Growth inhibition is pronounced at the highest dose in the series. Shoots are short, leaves are small, stem development is limited, and root architecture is sparse and weak (Figure 14). Collective evaluation of morphological responses to PBZ dose during the rooting phase. The best development was observed in B₂; low and high doses failed to provide optimal root-shoot balance. The B₄ group, where growth was excessively suppressed, showed the weakest morphological response in the series (Figure 15).

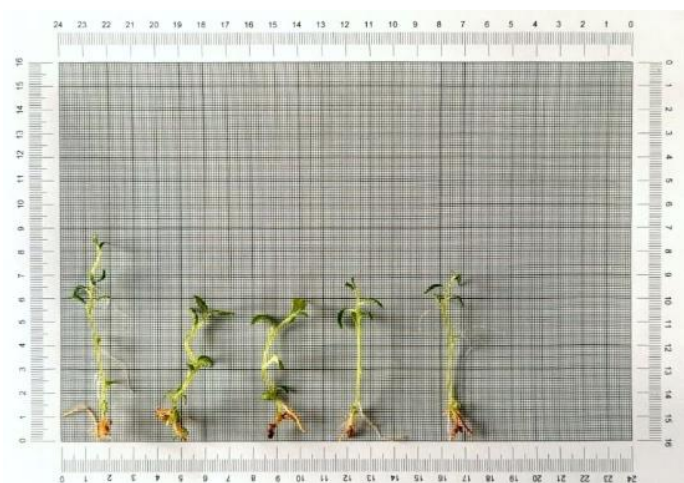


Figure 10. Morphological appearance of control group (B₀) explants.



Figure 11. Morphological appearance of 0.05 mg L⁻¹PBZ (B₁) Explants.



Figure 12. Morphological appearance of 0.1 mg L⁻¹PBZ (B₂) explants.



Figure 13. Morphological appearance of 0.25 mg L⁻¹PBZ (B₃) explants.



Figure 14. Morphological appearance of 0.5 mg L⁻¹PBZ explants.



Figure 15. Comparative morphological appearance of PBZ (B₀ to B₄) doses during the rooting stage. (From left to right: B₀, B₁, B₂, B₃, B₄).

Shoot Length

It was determined that PBZ applications during the rooting phase had a significant and statistically meaningful effect on shoot length ($F_{4,20} = 11.43$; $p < 0.001$). The control group (B_0) produced the longest shoots, with an average of 7.00 cm, while shoot length decreased steadily with increasing PBZ dose. Growth inhibition became more pronounced at medium and high doses; shoot length decreased to an average of 3.16 cm, particularly in the B_4 group (Figure 16). These results indicate that PBZ effectively limits GA-dependent elongation growth even during the rooting stage.

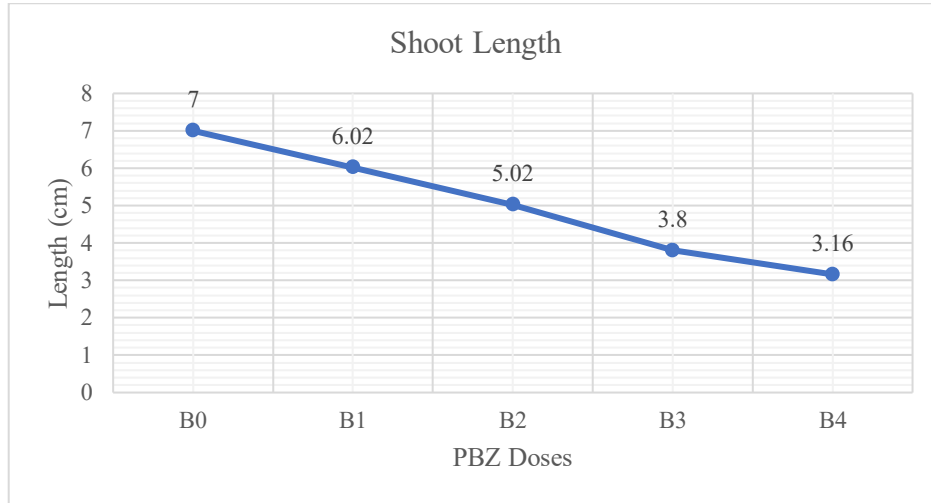


Figure 16. Effect of paclobutrazol on shoot length during the rooting phase

Leaf Area

During the rooting phase, PBZ was found to have a significant and strong regulatory effect on leaf area. The highest leaf area was recorded in the B_2 group containing 0.1 mg L^{-1} PBZ, and this value was statistically significantly higher than all control (B_0), low dose (B_1), and high dose (B_3 - B_4) groups ($F_{4,20} = 8.96$; $p < 0.001$; $\eta^2 = 0.64$). This dramatic increase indicates that PBZ at low doses positively affects both shoot vigor and potential carbon acquisition by enhancing photosynthetic surface development during the rooting stage. At high PBZ doses, however, a reduction in leaf area was observed due to excessive suppression of growth. These results are clearly evident in the leaf area distribution presented in Figure 17 and confirm that the B_2 dose represents the optimal morphological response for the rooting stage.

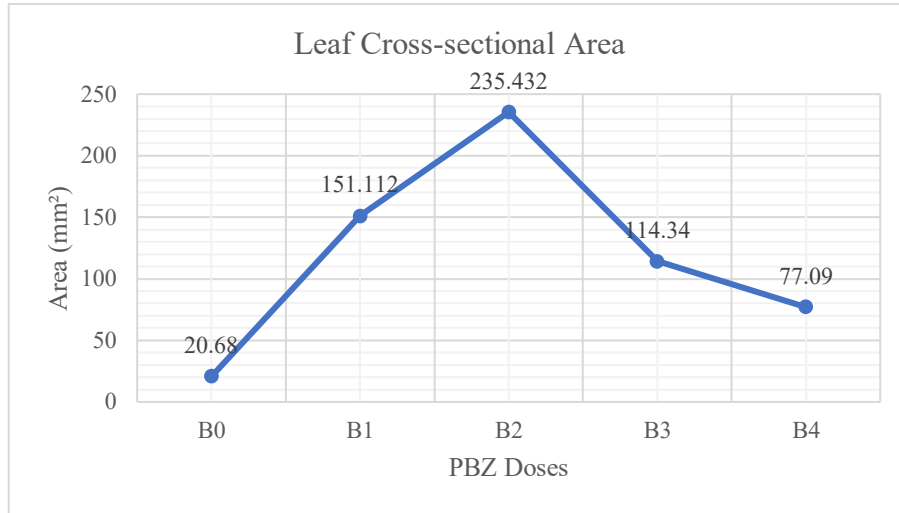


Figure 17. Effect of paclobutrazol on leaf area during the rooting stage

Stem Diameter

Stem diameter is an important indicator of seedling vigor and acclimatization success during the rooting stage. The highest stem diameter value among the PBZ applications was recorded in the B₂ group containing 0.1 mg L⁻¹ PBZ, and this group showed significantly higher values than all other doses ($F_{4,20} = 5.19$; $p < 0.01$). Medium doses of PBZ increased the compactness of the stem tissue; at high doses (B₃-B₄), stem thickness decreased significantly due to the effect of growth inhibition. This trend indicates that PBZ strengthened shoot mechanical resistance at low doses, while physiological suppression, which reduced sensitivity to growth regulators in shoot tissues, increased at high doses, and consequently, stem thickening was overshadowed. The stem diameter distribution presented in Figure 18 clearly shows that the B₂ group provided the highest morphological quality for the rooting stage. The mean values of all morphological parameters measured in the rooting stage, calculated as mean \pm SD from five explants per treatment, are summarized in Table 2.

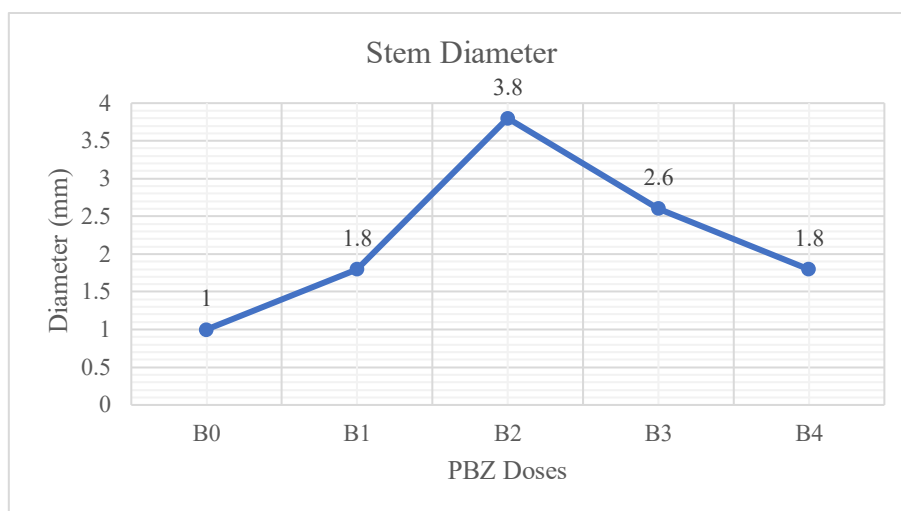


Figure 18. Effect of PBZ doses on stem diameter during the rooting phase

Table 2. Mean values of morphological parameters obtained from five explants per treatment during the in vitro rooting stage of *Ficus lyrata*

Treatment	PBZ Dose (mg L ⁻¹)	n	Shoot Length (cm)	Leaf Area (mm ²)	Stem Diameter (mm)
B0	0.00	5	7.00 ± 1.07	20.68 ± 8.85	1.00 ± 0.00
B1	0.05	5	6.02 ± 0.68	151.11 ± 73.34	1.80 ± 0.45
B2	0.10	5	5.02 ± 1.73	235.43 ± 91.21	3.80 ± 2.05
B3	0.25	5	3.80 ± 0.84	114.34 ± 57.94	2.60 ± 0.55
B4	0.50	5	3.16 ± 0.29	77.09 ± 31.94	1.80 ± 0.84

Values represent the mean ± SD of five explants per treatment. The data presented correspond to the graphical results shown in Figures 16-18.

DISCUSSION

This study thoroughly evaluated the effects of paclobutrazol (PBZ) on morphological quality during the in vitro propagation and rooting stages of *Ficus lyrata* and revealed the dose-response relationship of PBZ with high resolution. The results obtained show that PBZ significantly modulates the growth architecture of *F. lyrata* in both the propagation and rooting stages and that the optimal dose is 0.1 mg L⁻¹. This finding is consistent with previous studies indicating that GA biosynthesis inhibitors promote compact plant form in ornamental plants (Desta & Amare, 2021).

The findings of the present study are consistent with previous reports on closely related *Ficus* species, particularly *Ficus benjamina*. In this species, paclobutrazol application has been shown to significantly reduce plant height and internode length while promoting a more compact and structurally stable plant form (LeCain et al., 1986). Similar morphological responses observed in *F. lyrata* in the present study suggest that the growth-regulating effects of PBZ are conserved across *Ficus* species. These effects are primarily associated with the inhibition of gibberellin biosynthesis, which limits cell

elongation and redirects growth toward increased tissue density and structural integrity (Desta & Amare, 2021). In addition, improved plant architecture resulting from PBZ application is associated with enhanced acclimatization performance, as compact and mechanically stronger shoots exhibit higher survival rates during the transition from in vitro to ex vitro conditions (Hazarika, 2003). Therefore, the results of this study not only align with existing findings in related species but also reinforce the broader applicability of PBZ as an effective growth regulator in woody ornamental plant tissue culture systems.

During the propagation stage, PBZ was found to have a particularly strong regulatory effect on leaf area and stem thickness. The large leaf area and increased stem thickness obtained at the A₂ dose (0.1 mg L⁻¹) indicate that PBZ does not completely suppress cell expansion at low-to-medium doses; rather, shoot-leaf balance is optimized within physiological limits. This is consistent with mechanistic explanations that PBZ inhibits only elongation growth (GA-dependent process) while allowing lateral tissue development. The fact that A₂ provides the ideal balance in this respect can be considered the most important effect that could increase commercial quality under in vitro conditions.

The effects of PBZ were even more pronounced during the rooting phase. In particular, the dramatic increase in leaf area in the B₂ (0.1 mg L⁻¹) group suggests an increase in photosynthetic capacity and, possibly, carbon acquisition. The positive structural characteristics observed in root development indicate that the plant resource-consumer balance may have been improved by increasing shoot compactness. This situation shows that GA inhibition plays an important role not only in shoot architecture but also in root-shoot coordination.

At high PBZ doses (A₃-A₄ and B₃-B₄), however, significant declines in growth parameters were observed. These results indicate that PBZ applications exceeding the optimal dose range can cause adverse effects such as inhibition of meristem activity, decline in leaf differentiation, and decrease in shoot vigor. Therefore, this study confirms that PBZ is a potent growth regulator but emphasizes the critical importance of carefully optimizing the dose.

The findings are consistent with the literature, but the simultaneous evaluation of PBZ during propagation and rooting stages specifically for *Ficus lyrata* has not been comprehensively conducted before. In this respect, the study fills an important gap in both in vitro culture literature and commercial ornamental plant production. Furthermore, considering that PBZ may potentially enhance not only morphological quality but also acclimatization success, this study provides a strong foundation for future physiological, metabolic, and stress tolerance research.

Conclusion

This study comprehensively demonstrated the regulatory effects of paclobutrazol on morphological quality during the in vitro propagation and rooting stages of *Ficus lyrata*. The findings indicate that PBZ significantly shapes the growth architecture of the species and that the 0.1 mg L⁻¹ dose produces the most balanced and commercially desirable structure in both shoot and root development. At this dose level, the leaf surface area expanded, the stem thickness increased, and the shoot tissue gained a more compact form; thus, morphologically superior plant material was obtained in both the propagation and rooting stages. It was also clearly observed that high doses of PBZ suppressed growth and limited meristem activity; this situation demonstrated that the precise dosage adjustment of the growth regulator is a critical parameter in *Ficus lyrata* micropropagation protocols.

The results of the study indicate that PBZ not only regulates shoot morphology under in vitro culture conditions but may also potentially affect root-shoot coordination, tissue resistance, and acclimatization success. The comprehensive structure of this study, which evaluates both the propagation and rooting stages together, fills an important gap in the existing literature on *Ficus lyrata* and provides a scientific basis for optimizing tissue culture-based commercial production processes for the species. Future studies examining the physiological and biochemical effects of PBZ in greater detail and evaluating parameters such as hormone dynamics, carbon metabolism, stress tolerance, and acclimatization performance will contribute to a more comprehensive understanding of the application of growth regulators in *F. lyrata* and similar woody ornamental plants.

Additional Declaration

Author Contributions

In this study, the contribution of the authors was equal; both authors contributed equally to the development of the research ideas, data analysis, writing and proofreading stages.

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Responsible Artificial Intelligence Statement

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Conference Presentation

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Conflicts of Interest

The authors declare that there are no conflicts of interest related to the publication of this study.

Ethics Approval

This study does not require ethics committee approval as it does not involve any direct application on human or animal subjects.

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