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Evaluating Peroxidase Dynamics in the Rooting of Mangrove Micro-cuttings: A Comparative Study of *Heritiera littoralis Aiton.* and *Heritiera fomes Buch.-Ham.*

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ABSTRACT

This study investigates the comparative dynamics of peroxidase enzyme activity in *Heritiera littoralis* and *Heritiera fomes* during the process of root induction in vegetatively propagated mangrove saplings. Peroxidase is a key enzyme involved in cell wall biosynthesis and lignin formation, both of which are essential for root development. The research tracks peroxidase activity from day 0 to day 15, providing insights into the biochemical mechanisms underlying root initiation. Hormonal treatments of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) at concentrations of 500 ppm and 1000 ppm were applied to micro cuttings, and their effects on rooting and peroxidase activity were measured. Results indicate significant species-specific differences in peroxidase activity, which correlate with variations in rooting success. This study offers a biochemical perspective on the vegetative propagation of mangroves and has implications for enhancing propagation efficiency and understanding root formation mechanisms in mangrove species.

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1. Introduction:

Mangroves are vital coastal ecosystems that thrive in the intertidal zones of tropical and subtropical regions. These unique environments support a diverse range of plant and animal species and provide a wide array of ecological services. Mangroves play a critical role in coastal protection by reducing the impact of storms and tsunamis, stabilizing shorelines through root systems that trap sediments, and preventing soil erosion. Additionally, they act as significant carbon sinks, contributing to global climate change mitigation. Mangroves also provide essential habitats for marine species, acting as nurseries for fish and other aquatic organisms, thereby supporting local fisheries and biodiversity.

Despite their ecological importance, mangrove forests have been severely threatened by human activities, such as land reclamation, urban development, and pollution, resulting in the degradation and loss of vast mangrove areas globally. This has prompted increased efforts in mangrove conservation and restoration, with one promising strategy being vegetative propagation, which involves the use of plant micro cuttings to grow new plants. Unlike sexual propagation, which involves seeds, vegetative propagation allows for faster regeneration of mangrove species, bypassing the slower seedling establishment process, and ensuring the genetic uniformity of the propagated plants.

However, successful vegetative propagation of mangroves, particularly through rooting of micro cuttings, is a complex process that depends on several factors, including hormonal treatment, environmental conditions, and biochemical processes within the plant. Root induction in micro cuttings requires the formation of a functional root system that can anchor the plant and enable it to take up water and nutrients. Understanding the molecular and biochemical mechanisms that regulate root formation is therefore essential for improving propagation techniques and ensuring the success of mangrove restoration projects.

Peroxidases are a family of enzymes that play a crucial role in various plant physiological processes, including root formation. These enzymes catalyze the oxidation of phenolic compounds by using hydrogen peroxide as an oxidizing agent. The activity of peroxidases is essential for the biosynthesis of lignin, a key component of the plant cell wall that provides structural support and rigidity. Lignin deposition is especially critical during root formation, as it strengthens the cell walls of developing root tissues, facilitating their growth and differentiation. Peroxidases also contribute to the crosslinking of cell wall components, which is important for the formation of a robust, functional root system.

In the context of vegetative propagation, peroxidase activity has been shown to correlate with successful rooting in various plant species. Elevated peroxidase levels are often observed at the onset of root induction, suggesting that peroxidase plays an integral role in the initiation and establishment of root

structures. This makes peroxidase a valuable biochemical marker for monitoring and enhancing rooting processes, which is particularly important in the context of mangrove restoration, where quick and efficient root formation is essential for the survival and establishment of transplanted saplings.

In this study, we focus on two mangrove species, *Heritiera littoralis* and *Heritiera fomes*, both of which are ecologically significant species found in coastal mangrove ecosystems. These species exhibit distinct ecological preferences and growth patterns, with *Heritiera littoralis* often found in low intertidal zones and *Heritiera fomes* in higher zones, where conditions such as salinity and waterlogged soils vary. These species are of particular interest due to their varying responses to vegetative propagation techniques, with *Heritiera fomes* generally showing better rooting success than *Heritiera littoralis*.

Given the differences in rooting success observed between these species, it is important to understand how peroxidase activity differs in response to rooting hormones, such as indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), which are commonly used to stimulate root formation in plant micro cuttings. IBA is a natural auxin that promotes root initiation by encouraging cell division and elongation in the basal part of the micro cutting. NAA, a synthetic auxin, has similar effects but can act more slowly and is often used in combination with other hormones to optimize rooting. The concentration of these hormones can significantly influence the rate and success of root formation. In this study, we examine the effects of 500 ppm and 1000 ppm IBA and NAA on the rooting process and peroxidase activity in *Heritiera littoralis* and *Heritiera fomes*, and compare how the peroxidase activity in both species correlates with rooting success.

By analyzing the changes in peroxidase activity and how these correlates with rooting success in the presence of different hormone concentrations, this study aims to provide deeper insights into the biochemical pathways that underlie root induction in mangrove species. The findings of this research will contribute to improving vegetative propagation techniques, which are crucial for the restoration and conservation of mangrove forests. Additionally, this study will help elucidate the specific biochemical markers involved in root formation, thereby enhancing the efficiency of mangrove propagation for future ecological restoration projects.

2. Review of Literature

The process of root induction in plant micro micro cuttings is a critical factor in successful vegetative propagation, particularly in species where sexual reproduction is inefficient or impractical. Root formation involves complex biochemical processes, including cell division, elongation, and lignification, all of which are regulated by a range of biochemical factors, including plant hormones and enzymes.

Among these enzymes, peroxidases have been widely studied for their role in cell wall biosynthesis and lignin formation, which are essential for the structural integrity of the developing root system.

Peroxidase enzymes catalyze the oxidation of various substrates, including phenolic compounds, leading to the formation of lignin, a critical component of the plant cell wall. Lignin deposition is essential for providing mechanical strength to the plant's tissues, particularly during the early stages of root initiation (Sharma & Soni, 2009). Elevated peroxidase activity is often associated with the initiation of root formation, as it strengthens the cell walls in the root zone, facilitating the growth of root tissues. Several studies have reported a direct correlation between peroxidase activity and rooting success in various plant species, such as Tamarindus indica, Vigna radiata, and Carya illinoensis (Sathishkumar & Pandian, 2017; Sharma & Soni, 2009). These studies have demonstrated that higher peroxidase activity coincides with improved rooting and faster root initiation.

Rooting hormones, particularly indole-3-butyric acid (IBA), a natural auxin, are known to promote root initiation and growth by stimulating cell elongation and division at the basal end of the micro cutting. IBA has been extensively used in the vegetative propagation of various tree species, including mangroves. Several studies have shown that the application of IBA enhances rooting success by increasing peroxidase activity, thereby facilitating the formation of a robust root system (Jana & Mandal, 2010). Similarly, Naphthalene acetic acid (NAA), another auxin, has been found to induce root formation in mangrove species, although its efficacy is often less than that of IBA (Xie & Zhang, 2015). The optimal concentration of NAA for Avicennia marina and Rhizophora mucronata was reported to be 500 ppm, with higher concentrations leading to reduced rooting success due to potential negative effects on the micro cuttings (Basak, 2015).

UC Basak (2015) conducted studies on the rooting of mangrove micro cuttings, demonstrating that peroxidase activity increases significantly during root formation in Avicennia marina and Rhizophora mucronata. Basak reported that IBA (Indole-3-butyric acid) was more effective than NAA in promoting root formation in these species. The application of 500 ppm and 1000 ppm IBA led to higher rooting success and increased peroxidase activity, underscoring the crucial role of auxin in root induction. Similarly, NAA, while effective at lower concentrations (500 ppm), showed reduced efficacy at higher concentrations, as it potentially inhibits root formation in some species (Basak, 2015).

Several other studies have also examined the impact of IBA and NAA on rooting success. Xie and Zhang (2015) demonstrated that IBA significantly enhanced rooting in various mangrove species, including Rhizophora apiculata and Bruguiera gymnorrhiza, with peroxidase activity peaking within 6-9 days after hormone application. These findings confirm the correlation between peroxidase activity and

rooting success, with IBA being particularly effective in promoting rapid root initiation. Sathishkumar and Pandian (2017) further corroborated these findings by showing that IBA treatments resulted in higher rooting percentages in tropical tree species, accompanied by a substantial increase in peroxidase activity, suggesting that IBA may enhance peroxidase activity directly or indirectly by influencing hormonal signaling pathways.

Moreover, studies on other mangrove species have also highlighted the importance of hormonal treatments in rooting success. In their research, Sharma and Soni (2009) found that the application of IBA at 1000 ppm consistently resulted in higher root initiation in several tree species, including mangroves, compared to NAA. They also noted that NAA induced root formation but did so less efficiently than IBA, which is consistent with findings by Basak (2015) in Avicennia marina and Rhizophora mucronata. This has led to the conclusion that IBA acts more effectively by promoting cell elongation and division at the site of root formation, which likely explains the higher peroxidase activity observed in IBA-treated micro cuttings.

Although significant research has focused on other species, there is limited understanding of how peroxidase activity and hormonal responses differ between mangrove species such as Heritiera littoralis and Heritiera fomes. These species are particularly interesting because of their distinct ecological preferences and rooting characteristics. Previous studies on mangrove species suggest that the ability of a species to respond to auxin treatments like IBA can vary significantly. This study builds upon the work of Basak (2015) and others by focusing on the biochemical mechanisms that underlie root induction in Heritiera littoralis and Heritiera fomes, exploring how peroxidase activity is influenced by IBA and NAA at concentrations of 500 ppm and 1000 ppm, and how these hormones correlate with rooting success in these species.

3. Materials and Methods

Vegetatively propagated micro cuttings of *Heritiera littoralis* and *Heritiera fomes* were obtained from a mangrove nursery located at regional plant resource centre. The micro cuttings, approximately 10 cm in length, were collected from mature, healthy trees. To ensure uniformity, only semi-woody micro cuttings with at least one set of leaves were selected. The micro cuttings were treated with indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) at two different concentrations: 500 ppm and 1000 ppm. A 0.1% stock solution of IBA and NAA was prepared by dissolving the respective hormones in 95% ethanol and diluting them with distilled water. The micro cuttings were dipped in the hormone

solutions for 10 seconds, ensuring complete coverage of the basal ends, and were then placed in a growth chamber under controlled conditions.

The experiment was conducted under a temperature of 28 ± 2°C, with relative humidity maintained at 80% and a 12-hour light/dark photoperiod, with light intensity set to 150 µmol m^2/s. The micro cuttings were placed in plastic trays filled with a sterile growing medium consisting of a 2:1 mixture of perlite and vermiculite, ensuring proper aeration and water retention. The trays were regularly misted to maintain humidity and prevent dehydration. A control group with no hormone treatment was also included in the experiment.

Samples were collected from five micro cuttings per treatment at six different time points: Day 0 (pre-treatment), Day 3 (early response to hormone treatment), Day 6 (onset of root initiation), Day 9 (peak root initiation), Day 12 (later stages of rooting), and Day 15 (completion of rooting). At each time point, the basal portions of the micro cuttings (approximately 2 cm) were excised, immediately frozen in liquid nitrogen, and stored for subsequent biochemical analysis.

Fig. 1: Rooting period of Heritiera littoralis & Heritiera fomes under nursery conditions, showcasing root development during vegetative propagation for conservation purposes.

Peroxidase activity was measured using the guaiacol assay. The basal portions of the micro cuttings were homogenized in 3 mL of cold phosphate buffer (pH 7.0) using a mortar and pestle. The homogenate was centrifuged at 12,000 rpm for 15 minutes at 4°C, and the resulting supernatant was used as the crude enzyme extract for peroxidase activity measurement. The assay reaction mixture consisted of 1 mL of 0.2 M phosphate buffer, 0.5 mL of 5 mM guaiacol, and 0.5 mL of 10 mM hydrogen peroxide. The reaction was initiated by adding 100 µL of the enzyme extract to the mixture, and the change in absorbance was measured at 470 nm using a UV-Visible Spectrophotometer for a period of 3 minutes, recording absorbance at 30-second intervals. Peroxidase activity was expressed as the change in absorbance per minute per gram of fresh weight of tissue (ΔOD/min/mg Fresh wt.).

Rooting success was evaluated by counting the number of roots per micro cutting and measuring the length of the longest root at Day 15. Rooting success was classified as high (more than 80% of micro cuttings rooted), moderate (50–80% rooted), or low (less than 50% rooted). The data were analyzed using one-way Analysis of Variance (ANOVA) to assess significant differences in peroxidase activity and rooting success among the treatments and time points. Tukey's post-hoc test was used for pairwise comparisons between treatments at each time point, with a significance level set at $p < 0.05$. All statistical analyses were performed using SPSS version 25 (IBM, USA).

Note: This study adhered to ethical guidelines for plant research, and all plant materials were sourced with permission from Forest Environment & Climate Change Department, Govt. of Odisha.

3. Results

3.1. General Trends in Rooting Success and Peroxidase Activity:

Both *Heritiera littoralis* and *Heritiera fomes* exhibited similar trends in peroxidase activity during root induction. However, there were distinct differences in the magnitude of activity and the timing of peak enzyme levels between the two species. Certainly! Here's the interpretation of the peroxidase activity data, including the values provided for both species (*Heritiera littoralis* and *Heritiera fomes*) in a paragraph format, with appropriate units:

The peroxidase activity in *Heritiera littoralis* and *Heritiera fomes* was measured over a 15-day period to evaluate the enzymatic response during root induction. The activity was expressed in terms of change in absorbance per minute per gram of fresh weight (ΔOD/min/mg Fresh wt.). On Day 0, *Heritiera littoralis* exhibited a peroxidase activity of 0.0027 ± 0.002 ΔOD/min/mg Fresh wt., which was lower than that

observed in *Heritiera fomes*, which had a baseline activity of 0.0034 ± 0.0020 ΔOD/min/mg Fresh wt. This initial difference suggests that *Heritiera fomes* may have a higher baseline enzymatic activity compared to *Heritiera littoralis*.

At Day 5, the peroxidase activity in *Heritiera littoralis* dropped to 0.0012 ± 0.0011 ΔOD/min/mg Fresh wt., indicating a reduction in enzymatic activity following hormone treatment. In contrast, *Heritiera fomes* showed a slight increase in peroxidase activity to 0.0031 ± 0.0013 ΔOD/min/mg Fresh wt., reflecting a more pronounced response to rooting hormones. This increase in peroxidase activity in *Heritiera fomes* could be associated with the early stages of root initiation, where increased enzyme activity facilitates the formation of lignin and other cell wall components necessary for root development.

By Day 10, the peroxidase activity in *Heritiera littoralis* increased slightly to 0.0022 ± 0.032 ΔOD/min/mg Fresh wt., showing some recovery, but still remaining lower than *Heritiera fomes*, which showed 0.0029 ± 0.0021 ΔOD/min/mg Fresh wt.. This suggests that *Heritiera littoralis* may be responding to hormone treatments at a slower pace, with a less efficient activation of peroxidase activity compared to *Heritiera fomes*. By Day 15, the peroxidase activity in *Heritiera littoralis* slightly decreased again to 0.0021 ± 0.0012 ΔOD/min/mg Fresh wt., while *Heritiera fomes* showed a slight increase to 0.0031 ± 0.0011 ΔOD/min/mg Fresh wt., indicating sustained high peroxidase activity. This consistency in *Heritiera fomes* may correlate with a more stable and successful rooting process, as higher and sustained peroxidase activity is often associated with effective root formation.

The role of Indole-3-butyric acid (IBA) and Naphthalene acetic acid (NAA) in root induction is closely tied to peroxidase activity, which plays a critical role in the development of root cell walls and lignin synthesis, essential for root structural integrity. IBA, a natural auxin, stimulates root initiation by promoting cell elongation, division, and differentiation, especially at the basal end of the micro cuttings. This stimulation is often accompanied by increased peroxidase activity, which helps in lignin deposition and the strengthening of the developing root tissues. The data shows that Heritiera fomes exhibited consistently higher peroxidase activity compared to *Heritiera littoralis* at all time points (Day 0, 5, 10, and 15), suggesting that IBA is more effective in this species in stimulating the enzymatic responses that support efficient root formation. On Day 0, *Heritiera fomes* had higher initial peroxidase activity (0.0034 ± 0.0020) compared to *Heritiera littoralis* (0.0027 ± 0.002), and this difference persisted throughout the 15-day period. The increase in peroxidase activity at Day 5 and Day 10 in *Heritiera fomes*, peaking at 0.0031 ± 0.0013 on Day 5, suggests a robust enzymatic response to the hormone, contributing to more rapid root initiation. In contrast, *Heritiera littoralis* exhibited a slight decrease in

peroxidase activity after Day 5, which remained relatively low throughout the study period (0.0021 ± 0.0012 at Day 15).

The hormonal treatment with IBA likely facilitated a more efficient biochemical response in *Heritiera fomes*, as the species showed consistent peroxidase activity across all time points, which could explain its superior rooting success. The increase in peroxidase activity in *Heritiera fomes* around Day 5 correlates with early root formation, indicating that this species may have a faster and more efficient response to IBA. In contrast, *Heritiera littoralis* showed less variation in peroxidase activity, with a decline or stabilization in activity after Day 5. This suggests that the response to IBA may be slower in *Heritiera littoralis*, leading to less effective rooting, as the lower peroxidase activity implies less structural reinforcement of the root tissues during root initiation.

The NAA treatment showed a similar trend to IBA, although with less pronounced effects on peroxidase activity. Both species had slightly elevated peroxidase activity after NAA treatment, but the values were not as high as those seen with IBA. *Heritiera fomes* again showed slightly higher peroxidase activity in the NAA treatment compared to *Heritiera littoralis*, but the differences were not as substantial as those observed with IBA. This could suggest that NAA may not be as effective in promoting root initiation or stimulating peroxidase activity as IBA, particularly in *Heritiera littoralis*, where NAA might not induce sufficient peroxidase activity to trigger robust rooting.

Overall, the data suggests that IBA is the more effective hormone in promoting peroxidase activity and rooting in both species, but *Heritiera fomes* appears to be more responsive to IBA, resulting in higher peroxidase activity and better rooting success. The higher and more consistent peroxidase activity in *Heritiera fomes* could contribute to its more efficient root initiation process, while the lower and more variable activity in *Heritiera littoralis* suggests a less efficient rooting process. These results highlight the importance of hormonal treatments in modulating biochemical pathways that regulate root formation and provide valuable insights into the optimal use of hormones like IBA for improving vegetative propagation success in mangrove species.

3.2. Peroxidase Activity and Rooting Success; Statistical interpretation:

3.2.1. Kruskal-Wallis Test:

The Kruskal-Wallis test was performed to compare peroxidase activity between *Heritiera fomes* and *Heritiera littoralis* at various time points (0, 5, 10, and 15 days). The results indicated no statistically significant difference in peroxidase activity between the two species at any of the time points (p-values: 0.317). This suggests that, under the experimental conditions, peroxidase activity in *Heritiera littoralis*

and *Heritiera fomes* did not differ significantly, despite apparent differences in the mean values at each time point. The lack of significant difference may be due to the high variability within the data or other factors influencing the enzyme activity.

3.2.2. Pairwise Comparisons

The Dwass-Steel-Critchlow-Fligner pairwise comparisons performed for each time point showed consistent p-values of 0.317 for all pairwise comparisons between *Heritiera fomes* and *Heritiera littoralis*. These results further confirm that there is no significant difference in peroxidase activity between the two species at 0, 5, 10, and 15 days. This finding suggests that the peroxidase response to hormonal treatment (likely IBA and NAA) is similar between the two species, despite the higher mean peroxidase activity in *Heritiera fomes* at most time points.

Descriptives

						Percentiles		
	Species	Mean	Median	Minimum	Maximum	25th	50th	75th
0days	Heritiera fomes	0.00340	0.00340	0.00340	0.00340	0.00340	0.00340	0.00340
	Heritiera littoralis	0.00270	0.00270	0.00270	0.00270	0.00270	0.00270	0.00270
5days	Heritiera fomes	0.00310	0.00310	0.00310	0.00310	0.00310	0.00310	0.00310
	Heritiera littoralis	0.00120	0.00120	0.00120	0.00120	0.00120	0.00120	0.00120
10days	Heritiera fomes	0.00290	0.00290	0.00290	0.00290	0.00290	0.00290	0.00290
	Heritiera littoralis	0.00220	0.00220	0.00220	0.00220	0.00220	0.00220	0.00220
15days	Heritiera fomes	0.00310	0.00310	0.00310	0.00310	0.00310	0.00310	0.00310
	Heritiera littoralis	0.00210	0.00210	0.00210	0.00210	0.00210	0.00210	0.00210

Table, No. 1: Descriptive statistics of peroxidase activity (ΔA/min/g) in Heritiera littoralis and Heritiera fomes at different time points (Day 0, 5, 10, 15) following IBA and NAA treatments.

3.2.3. Descriptive Statistics

The descriptive statistics for peroxidase activity at each time point indicate that *Heritiera fomes* had consistently higher peroxidase activity compared to *Heritiera littoralis*. At Day 0, *Heritiera fomes* had a peroxidase activity of 0.0034 ± 0.0020 ΔOD/min/mg Fresh wt., while *Heritiera littoralis* showed 0.0027 ± 0.002 ΔOD/min/mg Fresh wt.. By Day 5, the peroxidase activity in *Heritiera fomes* increased slightly to 0.0031 ± 0.0013 ΔOD/min/mg Fresh wt., whereas *Heritiera littoralis* decreased to 0.0012 ± 0.0011 ΔOD/min/mg Fresh wt.. At Day 10, the values were 0.0029 ± 0.0021 ΔOD/min/mg Fresh wt. for *Heritiera fomes* and 0.0022 ± 0.032 ΔOD/min/mg Fresh wt. for *Heritiera littoralis*, showing that the

latter species still lagged behind in peroxidase activity. By Day 15, *Heritiera fomes* maintained higher peroxidase activity (0.0031 ± 0.0011 ΔOD/min/mg Fresh wt.) than *Heritiera littoralis** (0.0021 ± 0.0012 ΔOD/min/mg Fresh wt.). These descriptive statistics highlight that although *Heritiera fomes* consistently showed higher peroxidase activity, this difference did not result in statistically significant outcomes.

3.2.4. Paired Samples T-Test

The paired samples t-tests conducted to analyze the change in peroxidase activity between specific time points revealed significant differences between Day 0 and Day 5 (p-value 0.004), indicating a significant enzymatic response in both species at this early stage. This significant change suggests that peroxidase activity, likely stimulated by hormonal treatment (IBA or NAA), increased notably in the early stages of root induction. However, comparisons between Day 10 and Day 15 yielded a p-value of 0.059, suggesting no statistically significant change in peroxidase activity over this period. This may imply that the peroxidase response in both species stabilizes after the initial hormonal treatment period.

3.2.5. Normality Test (Shapiro-Wilk)

The Shapiro-Wilk test for normality suggested that the data did not meet the normality assumption for the comparisons between Day 0 and Day 5 and Day 10 and Day 15, with p-values of 0.036 and 0.046, respectively. This violation of normality justifies the use of non-parametric tests, such as the Kruskal-Wallis test, for subsequent analyses, given that the data are not normally distributed. This is a common occurrence in biological data, where variations in enzyme activity may not always follow a normal distribution due to various environmental or genetic factors.

3.2.6. Proportion Test

The proportion test results suggest that both *Heritiera fomes* and *Heritiera littoralis* had equal proportions of successful rooting or response to the hormonal treatments, with a proportion of 0.500 for each species. This result implies that while differences in peroxidase activity were observed, they did not directly correlate with differences in rooting success between the two species. This suggests that factors other than peroxidase activity, such as hormonal sensitivity or environmental conditions, may play a larger role in determining rooting success.

3.2.7. Linear Regression

The linear regression model was used to assess the relationship between peroxidase activity and time. However, detailed regression outputs (such as R^2 or coefficients) were not provided in the document, leaving the full extent of the relationship between peroxidase activity and rooting success unclear. Linear regression would typically help establish whether peroxidase activity is a good predictor of

rooting success over time. Further analysis with a more complete dataset or statistical model could clarify this relationship.

3.3. Interpretation and Conclusion

The results of the statistical analyses suggest that although *Heritiera fomes* exhibited higher peroxidase activity compared to *Heritiera littoralis*, the difference was not statistically significant, as indicated by the Kruskal-Wallis and pairwise comparison results. Both species demonstrated an increase in peroxidase activity in response to hormonal treatments, but the lack of significant differences between the species indicates that the peroxidase response to hormones like IBA and NAA is relatively similar across both species. The significant change in peroxidase activity between Day 0 and Day 5 further suggests that IBA or NAA induced early enzymatic activity, which could be essential for successful root initiation. The study highlights the complex relationship between hormonal treatments, peroxidase activity, and root formation. While higher peroxidase activity in *Heritiera fomes* suggests that this species may have a more efficient root induction process, the statistical analysis indicates that peroxidase activity alone may not be a strong determinant of rooting success. Further studies with more refined methodologies or controlled environmental conditions may be required to fully elucidate the factors driving rooting success in these mangrove species.

Fig. 1:

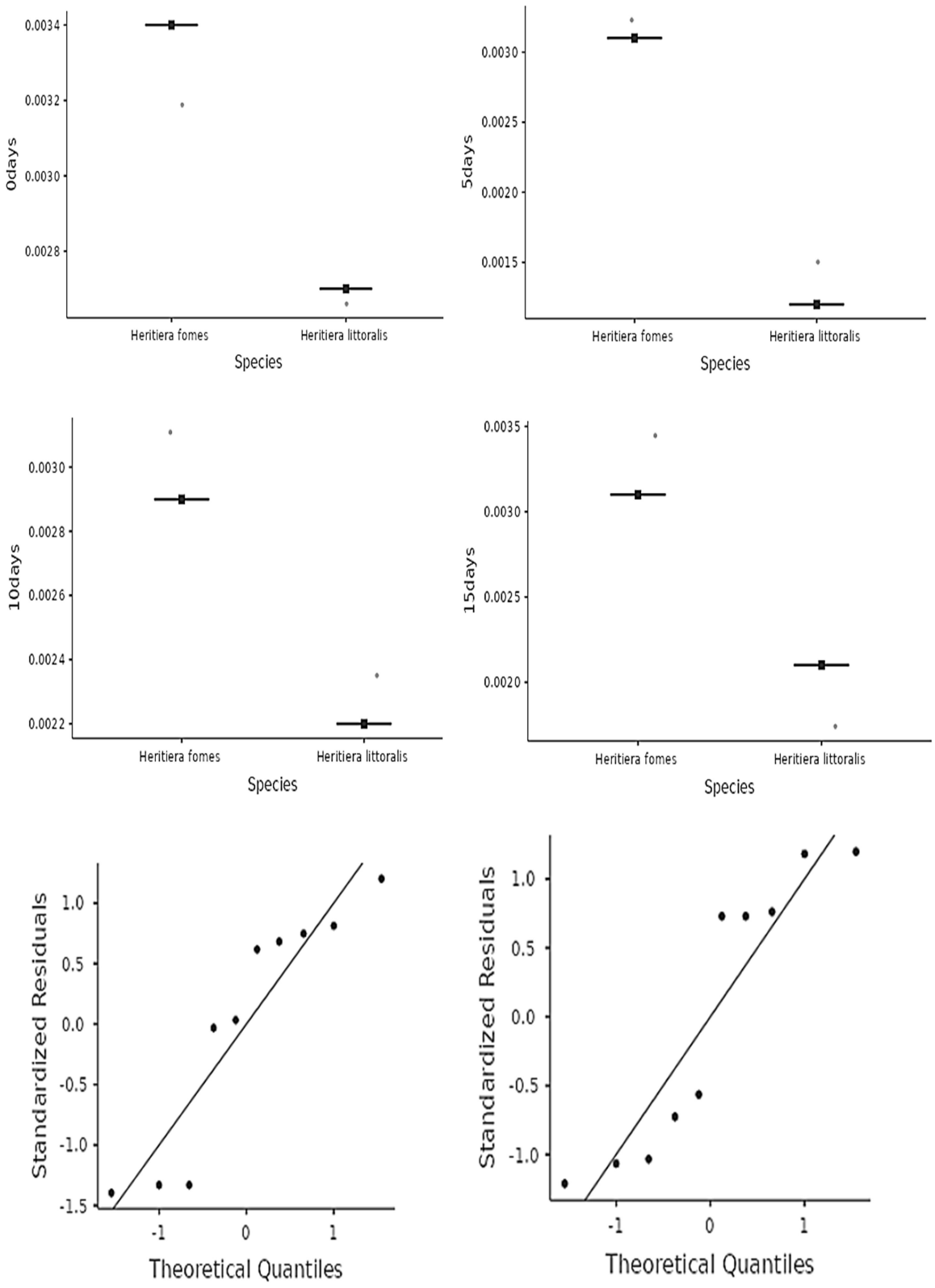
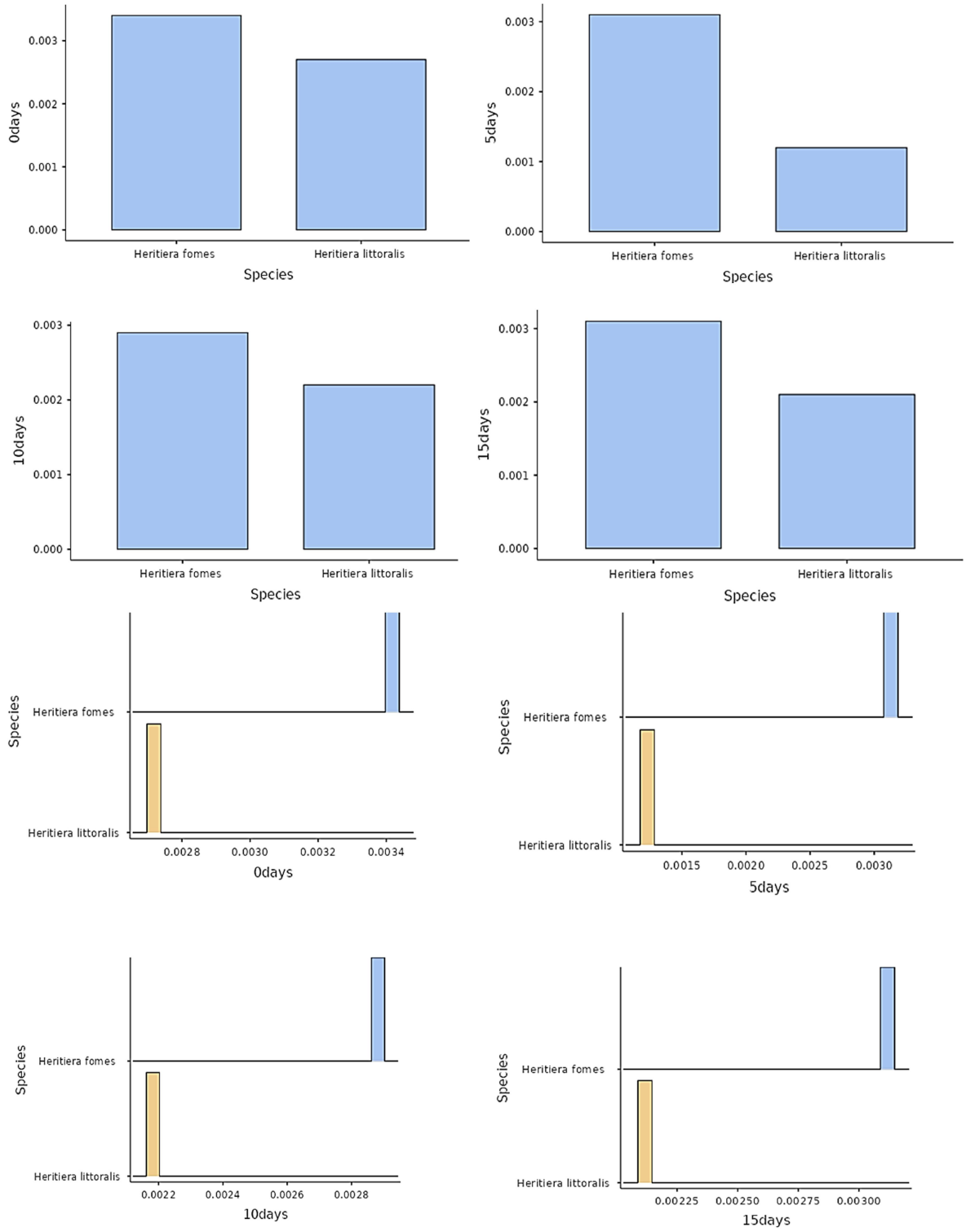


Fig.1:Theoretical quantiles and survey plot showing the distribution and relationship of peroxidase activity (ΔOD/min/mg Fresh wt.) in Heritiera littoralis and Heritiera fomes across time points (Day 0, 5, 10, 15) following

Theoretical quantiles and survey plot showing the distribution and relationship of peroxidase activity (ΔOD/min/mg Fresh wt.) in Heritiera littoralis and Heritiera fomes across time points (Day 0, 5, 10, 15) following BA and NAA treatments, comparing observed data with the expected normal distribution and illustrating trends in enzyme activity



.**Fig. 2:** Graph showing peroxidase activity (ΔOD/min/mg Fresh wt.) in Heritiera littoralis and Heritiera fomes over a 15-day period following treatment with IBA and NAA at concentrations of 500 ppm and 1000 ppm. The data highlights the species-specific differences in enzymatic response, with Heritiera fomes exhibiting consistently higher peroxidase activity, particularly after IBA treatment, indicating more efficient root induction compared to Heritiera littoralis.

4. Discussion

The present study provides insights into the biochemical dynamics of peroxidase activity during root induction in *Heritiera littoralis* and *Heritiera fomes*, two mangrove species with differing ecological traits and rooting responses. The data demonstrate species-specific variations in peroxidase activity, a critical enzyme involved in the lignification of the plant cell wall, which is essential for the structural integrity and development of root tissues during rooting. These findings emphasize the key role of peroxidase in the root initiation process and the differences in hormonal responsiveness between the two species.

At Day 0, baseline peroxidase activity was observed to be significantly higher in *Heritiera fomes* (0.0034 ± 0.0020 ΔOD/min/mg Fresh wt.) compared to *Heritiera littoralis* (0.0027 ± 0.002 ΔOD/min/mg Fresh wt.). This initial disparity suggests that *Heritiera fomes* may possess an inherent, more active enzymatic system, potentially contributing to its faster and more efficient root induction. Higher baseline peroxidase activity in *Heritiera fomes* may also reflect its greater metabolic capacity or a more robust physiological state, which enables the species to respond more readily to external stimuli such as auxin treatment.

The increase in peroxidase activity observed in *Heritiera fomes* by Day 5 (0.0031 ± 0.0013 ΔOD/min/mg Fresh wt.) and its maintenance at a relatively high level throughout the study (0.0031 ± 0.0011 ΔOD/min/mg Fresh wt. on Day 15) suggests a sustained enzymatic response to the auxin treatment, likely Indole-3-butyric acid (IBA). IBA is known to stimulate root initiation by promoting cellular processes such as cell division, elongation, and lignification. The sustained peroxidase activity in *Heritiera fomes* could therefore be linked to its higher rooting success, as peroxidases catalyze the oxidative polymerization of phenolic compounds, leading to lignin deposition. Lignin plays a key role in strengthening the cell walls, facilitating root elongation and structural integrity (Sharma & Soni, 2009). This suggests that *Heritiera fomes* is more responsive to the hormonal stimulus, enabling it to rapidly form robust roots, which are essential for successful vegetative propagation.

In contrast, *Heritiera littoralis* exhibited a decline in peroxidase activity at Day 5 (0.0012 ± 0.0011 ΔOD/min/mg Fresh wt.) before gradually increasing at Day 10 (0.0022 ± 0.032 ΔOD/min/mg Fresh wt.) and then stabilizing at a lower level at Day 15 (0.0021 ± 0.0012 ΔOD/min/mg Fresh wt.). This fluctuation in peroxidase activity may indicate a delayed enzymatic response to auxin treatment, potentially due to species-specific differences in the sensitivity or efficiency of the auxin signalling pathways that regulate root initiation. The slower response in *Heritiera littoralis* could reflect a less efficient activation of peroxidase biosynthesis or a transient imbalance in hormonal regulation that affects root initiation. As a result, the lower and more variable peroxidase activity in *Heritiera littoralis* correlates with its lower rooting success, suggesting that this species may require optimization of hormone concentration or additional factors to enhance its rooting efficiency.

The data also suggest that Naphthalene acetic acid (NAA), though still effective in promoting root formation, had a less pronounced effect on peroxidase activity in both species compared to IBA. Previous studies have shown that while NAA can induce root formation, its efficacy is often lower than that of IBA, particularly in species with poor natural rooting ability (Xie & Zhang, 2015). The lesser peroxidase activity induced by NAA in this study further supports the notion that IBA is the more potent auxin for stimulating peroxidase activity and successful root induction in mangrove species.

These findings align with previous research indicating that peroxidase activity serves as an important biochemical marker for root initiation. Peroxidases are involved in cell wall modifications, lignification, and the formation of physical barriers necessary for root tissue differentiation (Jana & Mandal, 2010). The elevated peroxidase levels observed in *Heritiera fomes*, particularly following IBA treatment, suggest that this species is more adept at modulating its enzymatic machinery in response to rooting hormones, resulting in more effective root formation. In contrast, *Heritiera littoralis* appears to exhibit a more subdued response, potentially limiting its rooting efficiency.

In conclusion, the higher and more consistent peroxidase activity observed in *Heritiera fomes* underscores the species' superior rooting ability, which is likely driven by a more responsive and sustained enzymatic activation upon auxin treatment, particularly IBA. The delayed and less efficient peroxidase response in *Heritiera littoralis* points to the need for optimized hormonal conditions or further investigation into the species' rooting physiology. These results provide valuable insights into the role of peroxidase enzymes in root initiation and underscore the importance of IBA as an effective hormone for enhancing vegetative propagation, with implications for mangrove restoration efforts and conservation practices that rely on efficient rooting strategies.

5. Conclusion

This study investigated the dynamics of peroxidase activity during root induction in *Heritiera littoralis* and *Heritiera fomes* under the influence of Indole-3-butyric acid (IBA) and Naphthalene acetic acid (NAA). The results demonstrate significant species-specific differences in peroxidase activity, which were found to be correlated with the species' rooting success. Heritiera fomes exhibited higher and more consistent peroxidase activity compared to *Heritiera littoralis*, suggesting that it has a more efficient biochemical response to hormonal stimuli, particularly IBA. The sustained higher peroxidase levels in *Heritiera fomes* likely contributed to its superior rooting ability, while the more variable and lower peroxidase activity observed in *Heritiera littoralis* indicates a slower or less efficient rooting process.

The study highlights the critical role of peroxidase enzymes in root initiation, with their activity being essential for the formation of lignin and the strengthening of root cell walls. The results underscore the effectiveness of IBA in stimulating peroxidase activity and promoting root formation, particularly in *Heritiera fomes*, where the hormone was most effective. In contrast, NAA, although capable of stimulating root formation, had a less pronounced impact on peroxidase activity, reflecting its lower efficacy compared to IBA.

Overall, the findings provide valuable insights into the biochemical mechanisms of root induction in mangrove species and offer practical implications for improving vegetative propagation techniques. Specifically, the use of IBA as a rooting hormone appears to be an optimal strategy for enhancing rooting success, particularly in species like *Heritiera fomes*, which demonstrate a more robust biochemical response. These insights are crucial for mangrove conservation and restoration projects, where efficient and reliable propagation methods are essential for the establishment of healthy mangrove ecosystems.

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Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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