

*Full Length Research Article***Anatomical Responses of *Bambusa blumeana* Schult.f. Treated with Alpha-Naphthalene Acetic Acid**

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ABSTRACT

The underlying mechanism of bamboo anatomical responses remains poorly understood when subjected to different levels of Alpha-Naphthalene Acetic Acid (α -NAA) under nursery conditions. In this paper, we aim to examine the leaf and root anatomy of the *Bambusa blumeana* Schult.f., a potential bioremediation alternative species, treated with 0 ppm, 100 ppm, and 200 ppm of α -NAA. The experimentation was executed in a completely randomized design within a 3-month experimentation period. Freehand sectioning was performed to collect the leaf and root transverse sections, and the photomicrographs were taken and analyzed. The results revealed that 200 ppm α -NAA significantly increased the diameters of leaf fusoid cells and xylem. In the roots, the thickness of the rhizodermis, sclerenchymatous layer, cortex, and pericycle, as well as the diameter of the phloem, metaxylem, and pith, were significantly affected by the treatments. Generally, there is a weak to moderate correlation between the leaf and root anatomical structures based on Pearson's correlation and a weak to strong correlation within the organ-specific anatomical structure. Thus, our findings elucidate the potential role of exogenous auxin in inducing anatomical changes that assist the growth and propagation of *B. blumeana*.

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1. Introduction

There is a drastic increase in the appreciation and utilization of non-timber forest products, especially bamboo. In 2020, Asia had the largest bamboo reserves in the world with 24,877,000 ha due to the rapid production in China, India, and Myanmar, followed by South America with 5,389,000 ha and Africa with 4,648,000 ha (FAO 2020; Mishra 2015). They are the tallest perennial grass that belongs to the Poaceae family and have a wide range of economic uses, such as furniture and handicrafts, food, agricultural gears, musical instruments, pulp and paper, and construction materials. Around 2.5 billion people worldwide use bamboo daily, mostly for fiber and food in Asia alone (Borowski 2019). They are also an essential part of the environment due to their high capacity for carbon sequestration in urban areas, plantations, and the wide range of

ecosystems (Heriyanto et al. 2023; Jember et al. 2023). Hence, bamboo has become a part of humans' daily lives for sustainable living and survival.

However, the availability of low-cost and highly efficient propagation methods is still developing in science. Unlike other plants, bamboo has irregular flowering habits, resulting in seed scarcity and affecting their overall reproductive propagation (Gogoi 2022). Even when seeds are available, they have low storage characteristics and viability due to the repercussions of the prevailing biological, climatic, germination medium, and other physiological factors (Renganayaki et al. 2018; Sahoo et al. 2020). Specifically, seven bamboo species in the Philippines (*Schizostachyum lumampao*, *S. brachycladum*, *Gigantochloa levis*, *Dendrocalamus asper*, *D. latiflorus*, *Dinochloa* sp., and *Pseudostachyum polymorphum*) formed flower buds between October and November, but only *G. levis* and *S. lumampao* produced viable seeds (Sinohin 1990). Thus, alternative ways of propagating Kawayang Tinik (*B. blumeana*) were developed through vegetative means, such as culm or branch cuttings using exogenous auxins, layering, marcotting, and tissue culture (Roxas 2012; Tambong 2023). In this way, they have provided solutions for the increasing demand for bamboo worldwide.

Rooting hormones are advisable for the massive reproduction of plants to improve the survival and utilization rate of culm cuttings. The use of Alpha-Naphthalene Acetic Acid (α -NAA) induced the best rooting characteristics in *B. vulgaris*, *B. nutans*, and *Dendrocalamus strictus* by having longer and higher number of adventitious roots compared to the untreated group (Mudoj et al. 2014; Rajput et al. 2019; Tambong 2023). Even in dicots, α -NAA rooting hormone application improved the number of adventitious roots in the stem cuttings of *Mussaenda philippica* (Ogbu et al. 2017), increased the root length of *Andrographis paniculata* (Hossain and Urbi 2016), and enhanced the leaf and root fresh and dry weight biomass in *Morus alba* (Sourati et al. 2022).

The existing literature predominantly focuses on the influence of the α -NAA on the leaf and root morphology of various species, with limited attention to their anatomical responses. Under the controlled conditions, Martins et al. (2018) reported *Aechmea blanchetiana* had an increased root exodermis, root xylem diameter, and xylem vessel numbers, but no changes in root endodermis, and increased leaf xylem vessel number, leaf epidermis, leaf chlorenchyma, leaf sclerenchyma area, and leaf epidermis when exposed to 2 – 6 α -NAA (μ M). In another case, *Alcantarea imperialis* also had an increased root diameter and number of root metaxylem vessels under the α -NAA treatments (Martins et al. 2020). Lastly, the α -NAA induced an observable callus formation and enlarged vascular bundles in *Bougainvillea buttiana* (Huang et al. 2022).

Studying plant anatomy is crucial for understanding plants' adaptive mechanisms and physiological activities in response to environmental conditions. This knowledge is essential for properly maintaining and optimizing plant growth and survival. Despite its importance, there remains a significant gap in the literature regarding the anatomical responses of leaves and roots to α -NAA treatments. In this study, we investigated the leaf and root anatomy of *B. blumeana* using microscopy to determine the effect of α -NAA treatments. The main objectives were to analyze (1) the anatomical changes in the leaves, (2) the anatomical changes in the roots of *B. blumeana*, and (3) provide insights into the correlation of their leaf and root anatomy under different α -NAA treatment conditions.

2. Materials and Methods

2.1. Study Site and Species

The study was conducted in the established field nursery of Zambales Diversified Metals Corporation (ZDMC) in Candelaria, Zambales, Philippines (15°41'16" N 120°2'50" E) situated at 677 m elevation (**Fig. 1**). The Philippine Atmospheric, Geophysical, and Astronomical Services Administration (PAGASA) described the Zambales' climatological normals from 1991 to 2020 as having a 3,551.7 mm annual amount of rainfall, 27.3°C annual mean temperature, and 80% annual relative humidity that fall in Type II climatic type.

The 25.4 mm section with two-node cuttings of Kawayang Tinik (*B. blumeana*) was used to determine the effects of α -NAA rooting hormones on its leaf and root anatomy. Roxas (2012) discussed that the species is believed to have originated from Sumatra, Java Lesser Sunda Islands, and Borneo. In 1910, it was introduced in the Philippines and naturalized by which they can thrive in areas with low and medium altitudes. The species is well known in the country for its diverse uses, such as food, construction, furniture, and other recreational uses. They are also utilized for soil erosion prevention, as windbreakers, and as living fences for boundary establishment. Currently, *B. blumeana* is employed as a potential bioremediation species for mining areas and tannery sites (Chua et al. 2019; Were et al. 2017).

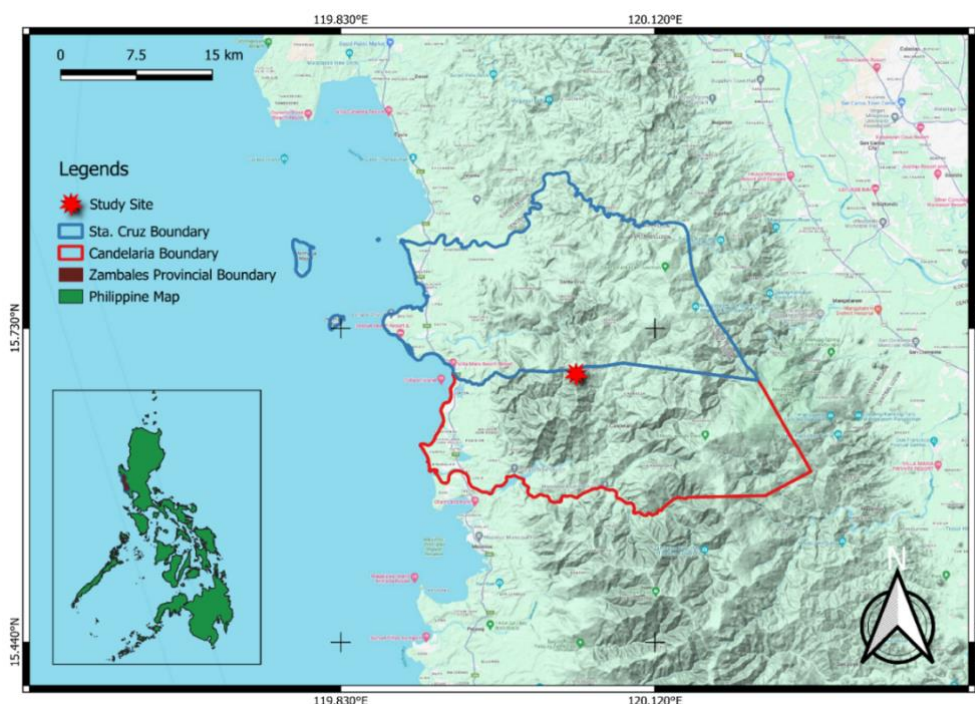


Fig. 1. The study area at the shared boundary between Sta. Cruz and Candelaria, Zambales, Philippines.

2.2. Experimental Design

The cohort of culm cuttings of *B. blumeana* specimens were collected in Brgy. Acoje, Sta. Cruz, Zambales, Philippines. The protocol devised by the Ecosystems Research and Development Bureau (ERDB) of the Department of Environment and Natural Resources for the culm-cutting propagation of bamboo was strictly followed (ERDB 2016). The 1–2-year-old bamboo culms were selected following the criteria: deep green color, healthy bud eye, and no signs of blemishes or diseases. Any culms without at least one criterion were excluded from the experiment. The study

employed a completely randomized design (CRD) with three treatments: T0 (control), T1 (α -NAA at 100 ppm), and T2 (α -NAA at 200 ppm). 150 cuttings from the bamboo culms were used, with 50 cuttings per treatment and five culm replicates within the treatment. A systematic protocol for selecting the cuttings and the sample labeling was employed, as it would rely on the order of nodes in the culm with the basal node as the reference point (ERDB 2016). For instance, at least three two-noded cuttings were sampled from the base, middle, and upper portion of the culm. The three cuttings from each portion were evenly distributed in each treatment, indicating the random set-up. To inhibit further transpiration and promote ideal growth and acclimatization, unnecessary branches and leaves were removed. The specimens for each treatment were immersed in the fungicide mixture constituting a ratio of 50 g of Follicur and 100 L of H₂O for 30 minutes. Then, they were vertically dipped in α -NAA treatments for 24 hours. Subsequently, the treated specimens were planted in polyethylene bags with a mixture of soil and carbonated rice hulls. Each potted specimen was labeled according to its specific treatment. Within three months, they were grown in the nursery of ZDMC Inc. using α -NAA to test its effect on the anatomy. For the destructive sampling analysis, 10 bamboo culms were randomly selected from each treatment group. These selected individuals were carefully extracted from the soil for root (n = 30) anatomy analysis. The leaves (n = 30) were thoroughly washed with running water to prepare for leaf anatomy analysis.

2.3. Sectioning Method

The modified sectioning method was adapted from the freehand sectioning technique (Metusala 2016). The third leaf from newly emerged branches was collected because it is fully developed and not too old or brittle. The intermediate-sized branch roots sampled 5 cm below the root collar area of the newly emerged branch were also collected for analysis. Each leaf from different treatments was sectioned from the middle third of the leaf blade, with segments measuring at least 3 cm in length and 0.5 cm in width. Similarly, each root from different treatments was sectioned to a minimum length of 3 cm. The fixation, dehydration, and freehand sectioning were adapted and modified from Johansen (1940). The leaf and root tissues were embedded in FAA fixative (35:50:5:10 – 40% Formaldehyde, 95% EtOH, Glacial Acetic Acid, and Distilled H₂O) for 24 hours. Following fixation, tissues were washed with 50% EtOH at 1-hour intervals for 3 hours. For dehydration, a series of increasing IPA (70%, 80%, 90%, 95%, 100%) was used to remove remnants of H₂O from the cells at 2-hour intervals. The leaves and root specimens were cut in a transverse orientation using the Gillette stainless blade (Procter and Gamble Philippines, Inc., Philippines). Freshly cut samples were placed in dH₂O and transferred to a small petri dish containing 0.1% Safranin for staining. Microscopic examination was performed using a compound optical microscope (Olympus Model CX23LEDRFS1, Olympus Corporation, Japan) equipped with a Dino-eye Edge camera (Model AM7025X-R9, Aunet Pty Ltd., Perth, Western Australia) at the Plant Systematics Laboratory, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños (CAS-UPLB). Photomicrographs were captured at 40x, 100x, and 400x magnification, corresponding to sight diameters of 5 mm, 2 mm, and 0.45 mm, respectively.

2.4. Anatomical Analysis

The DinoCapture 2.0 software (version 1.5.39.A) was used to measure and analyze the anatomical photomicrographs of the leaves and roots of *B. blumeana*. The scale was calibrated for

each specific magnification used during microscopic investigations. Generally, measurements were taken from five distinct regions of the leaf and root anatomy (**Fig. 2**). The anatomical measurement methods outlined by [Cai et al. \(2017\)](#) were followed. The upper and lower epidermis were sampled and averaged for leaf anatomy to determine the total epidermal thickness. The plicate cells were measured from the upper epidermis down to the boundary of fusoid cells. The mean of the first and second diameters were taken from the fusoid cells, bulliform cells, metaxylem, and phloem. For the root anatomy, measurements included the thickness of the rhizodermis, sclerenchymatous layer, cortex, endodermis, and pericycle. The mean of two diameters was also taken from the phloem, metaxylem, and pith. All measurements were recorded in micrometers (μm).

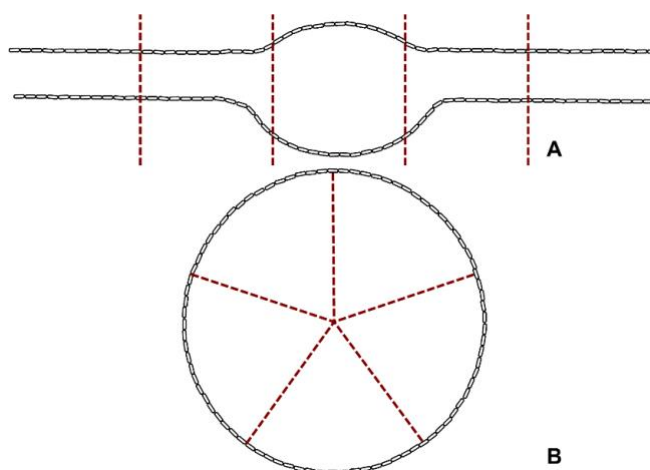


Fig. 2. Five-region measurement sampling in (A) leaf and (B) root anatomy of *B. blumeana*.

2.5. Data Analysis

R software (version 4.2.3) was used to analyze and visualize the data. The Standard Deviation (SD) was computed to assess the data's spread of values. Using the `plot()` function, the Q-Q plot was performed to test the normal distribution of the data set and the Residuals vs Fitted plot for homogeneity. One-way ANOVA was performed to determine the significance of the data at a 95% confidence interval using `aov()` function. For post-hoc analysis, Tukey's Honestly Significant Difference (HSD) test was used for multiple comparisons to identify which treatment pairs showed significant differences employing the `Tukey HSD()` function. Lastly, Pearson's correlation matrix was visualized and interpreted using the transmutation table described by [Schober et al. \(2018\)](#), to determine the relationship between the leaf and root anatomical characters through the `ggcorrplot` R package.

3. Results and Discussion

3.1. Results

3.1.1. Leaf anatomical response

Among the six-leaf anatomical markers, the fusoid cell and metaxylem diameters were the only parameters significantly affected by the α -NAA treatments, at $\alpha = 0.05$ with P-values of $\alpha > 0.0059$ and 0.0271 , respectively (**Table 1** and **Fig. 3**). Specifically, the diameter of fusoid cells at 0 ppm ($21.87 \pm 2.45 \mu\text{m}$) and 200 ppm ($31.82 \pm 9.51 \mu\text{m}$) α -NAA treatments had a significant

difference based on the Tukey's HSD test, while the 0 ppm – 100 ppm and 100 ppm – 200 ppm were found insignificant. There was also a significant difference between the 0 ppm ($24.44 \pm 3.48 \mu\text{m}$) and 200 ppm ($29.63 \pm 5.12 \mu\text{m}$) α -NAA treatments in the metaxylem diameter. In contrast, the other treatment pairs were found to have no significance. Other leaf anatomical characters (total epidermis, plicate cells, bulliform cells, and phloem) have no significant differences from the control treatment attested by the ANOVA (P-value > 0.05).

Table 1. Leaf anatomical response of *B. blumeana* to different treatments of α -NAA

Leaf anatomical measurements	P-values	Mean \pm SD for each Treatment (μm)		
		0 ppm	100 ppm	200 ppm
Thickness of total epidermis	0.0586	11.49 ± 0.94^a	11.05 ± 0.76^a	12.43 ± 1.64^a
Thickness of plicate cells	0.7770	48.72 ± 6.60^a	49.70 ± 10.82^a	46.67 ± 6.25^a
Diameter of fusoid cells	0.0059*	21.87 ± 2.45^a	26.87 ± 6.35^{ab}	31.82 ± 9.51^b
Diameter of bulliform cells	0.2130	54.14 ± 6.53^a	55.11 ± 4.30^a	59.04 ± 7.46^a
Diameter of metaxylem	0.0271*	24.44 ± 3.48^a	27.07 ± 3.41^{ab}	29.63 ± 5.12^b
Diameter of phloem	0.1650	32.47 ± 4.86^a	34.41 ± 4.51^a	38 ± 8.33^a

Notes: * significant at $\alpha = 0.05$; treatments within the row with the same letter are insignificant.

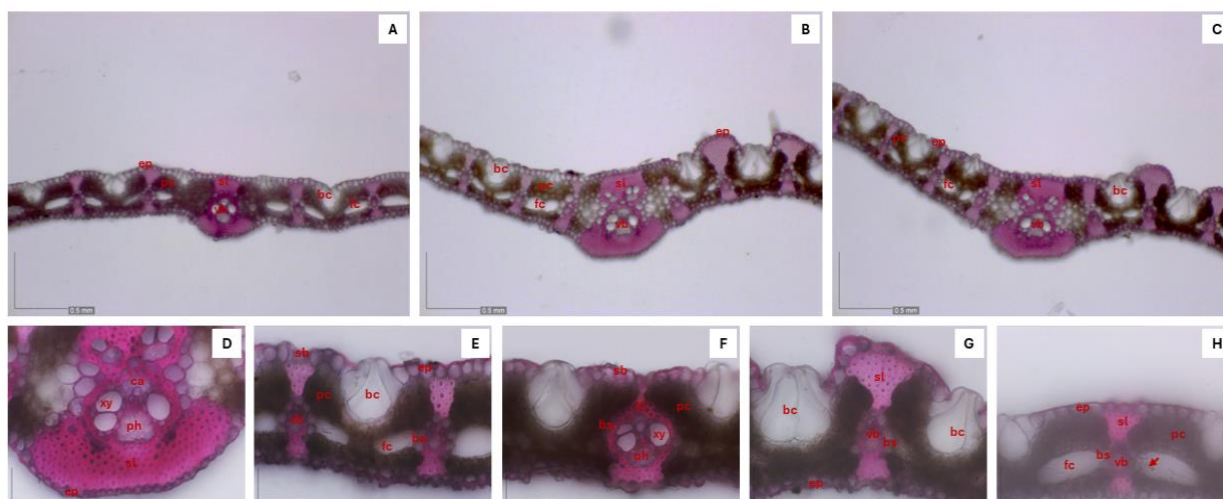


Fig. 3. Representative transverse sections of *B. blumeana* leaves with different α -NAA treatments: (A) 0 ppm, (B) 100 ppm, (C) 200 ppm, (D) magnified midrib, (E) Kranz anatomy of *B. blumeana*, (F) secondary vascular bundle, (G) bulliform cells, (H) fusoid cells, the arrow represents the starch reserves (bc – bulliform cells, bs – bundle sheath, ca – cavity, ep – epidermis, fc – fusoid cells, pc – plicate cells, ph – phloem, sb – silica bodies, sl – sclerenchyma, vb – vascular bundle, xy – xylem).

3.1.2. Root anatomical response

Seven of eight root anatomical markers showed a significantly increased measurement (P-value < 0.05) in response to the application of α -NAA (Table 2 and Fig. 4). The rhizodermis ($17.75 \pm 1.95 \mu\text{m}$) and sclerenchymatous layer ($24.50 \pm 4.33 \mu\text{m}$) were thickest at 200 ppm. Only the 0–100 ppm pair showed an insignificant post-hoc test (P-value > 0.05). In the cortical region, the cortex ($396.52 \pm 32.42 \mu\text{m}$) from the sclerenchymatous layer to the boundaries of endodermis at 200 ppm had the thickest measurement among the treatments, and the pair of 0 ppm – 100 ppm

has no significance. Interestingly, the α -NAA treatments did not influence the thickness of the innermost single-layer cells in the cortex ($P = 0.0575 > 0.05$). Within the stele region, the thickest pericycle was significantly observed (P -value < 0.05) at 200 ppm ($15.75 \pm 1.97 \mu\text{m}$) compared with 0 ppm and 100 ppm. Only the 0 ppm – 100 ppm pair had no significant difference in pericycle thickness (P -value > 0.05). The widest structure in the stele was the pith at 200 ppm ($354.29 \pm 50.60 \mu\text{m}$), significant at 0.05. In vascular tissues, the diameters of phloem and metaxylem were also influenced by α -NAA treatments, showing significant differences among treatments ($P = < 0.0001 < 0.05$). Both tissues were widest at 200 ppm of α -NAA ($22.41 \pm 2.33 \mu\text{m}$ and $65.28 \pm 5.94 \mu\text{m}$, respectively). The 0 ppm – 100 ppm in the pith and phloem diameters had no significant difference, and the pair of 100 ppm – 200 ppm in metaxylem diameter (P -value > 0.05) had no significant difference.

Table 2. Root anatomical response of *B. blumeana* to different treatments of α -NAA

Root anatomical measurements	P-values	Mean \pm SD for each Treatment (μm)		
		0 ppm	100 ppm	200 ppm
Thickness of rhizodermis	0.0098*	14.99 ± 2.52^a	14.64 ± 1.98^a	17.75 ± 1.95^b
Thickness of the sclerenchymatous layer	$< 0.0001^*$	16.32 ± 3.11^a	16.04 ± 3.81^a	24.50 ± 4.33^b
Thickness of cortex	$< 0.0001^*$	312.4 ± 41.37^a	308.72 ± 14.32^a	396.52 ± 32.42^b
Thickness of endodermis	0.0575	11.96 ± 1.88^a	12.37 ± 1.97^a	13.95 ± 1.71^a
Thickness of pericycle	0.0008*	13.01 ± 1.80^a	12.50 ± 1.57^a	15.75 ± 1.97^b
Diameter of phloem	$< 0.0001^*$	15.95 ± 2.28^a	17.87 ± 2.22^a	22.41 ± 2.33^b
Diameter of metaxylem	$< 0.0001^*$	44.12 ± 9.60^a	59.12 ± 11.66^b	65.28 ± 5.94^b
Diameter of pith	$< 0.0001^*$	209.77 ± 18.51^a	223.53 ± 27.07^a	354.29 ± 50.60^b

Notes: * significant at $\alpha = 0.05$; treatments within the same row with the same letter are insignificant.

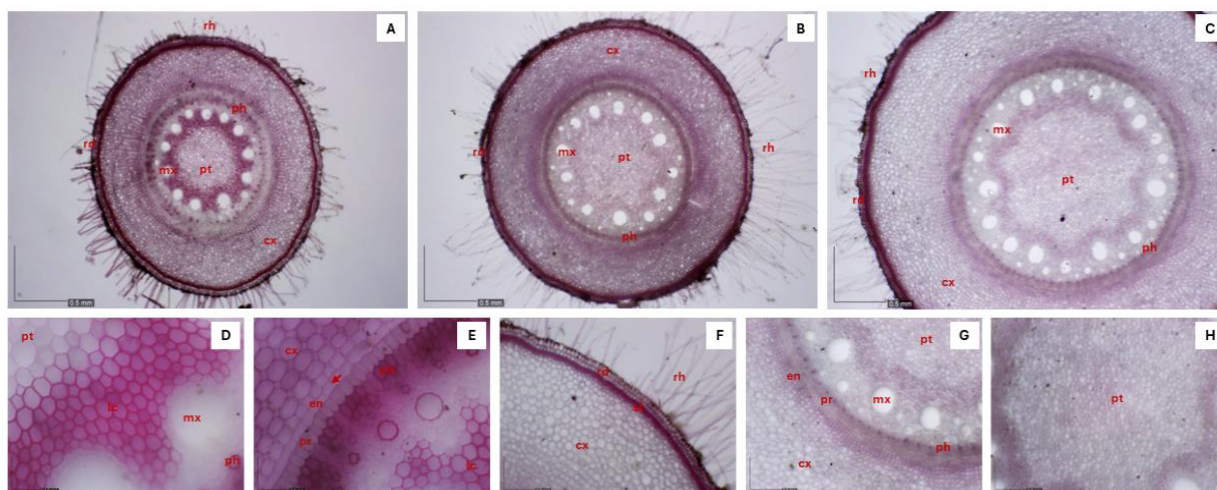


Fig. 4. Representative transverse sections of *B. blumeana* roots with different α -NAA treatments: (A) 0 ppm, (B) 100 ppm, (C) 200 ppm, (D–E) magnified anatomical structures of roots in control treatment, (D) lignified cells in the pith region, (E) cortex and stele region, the arrow represents developing Casparian strips, (F–H) magnified anatomical structure of roots with α -NAA treatments, (F) root hairs and the sclerenchymatous layer, (G) cortex and stele region, and (H) pith (cx – cortex, en – endodermis, lc – lignified cells, mx – metaxylem, ph – phloem, pr – pericycle, pt – pith, rd – rhizodermis, rh – root hairs, sl – sclerenchymatous layer).

3.1.3. Correlation of leaf and root anatomy

The existing ρ = correlation coefficients in Pearson’s correlation matrix (**Fig. 5**) were significant at $\alpha = 0.05$. The BDL had a weak to moderate positive correlation to FDL, PTL, and ETR. Meanwhile, the XDL showed a weak positive correlation to PTL ($\rho = 0.38$) and a moderate positive correlation to BDL and FDL. Interestingly, the PDL had a strong positive correlation to XDL ($\rho = 0.74$) and a moderate positive correlation to BDL and FDL. On the other hand, the PTL showed a negatively moderate correlation to STR ($\rho = -0.46$) and the FDL ($\rho = -0.42$). At the same time, the STR had a positive weak correlation to PTR and a positive moderate correlation to PiDR, FDL, CTR, and PDR. Specifically, the PTR was moderately positively correlated to EnTR and PiDR, while the same applies to the XDR for FDL, PiDR, and PTR. The diameter of root pith (PiDR) had the most number of correlated variables with positively moderate correlation ranging from $\rho = 0.41$ to $\rho = 0.58$ (TEL, XDL, FDL, PTR, ETR, EnTR, CTR, PDR, XDR, and STR). In CTR, a weak positive correlation was recorded with ETR ($\rho = 0.37$), while the TEL, PDR, STR, and PiDR had a moderate positive correlation ($\rho = 0.45 - 0.69$). Lastly, the PDR had a moderately positive correlation to CTR, STR, PiDR, XDR, and FDL. Overall, the influence of α -NAA treatments resulted in a weak to moderately positive correlation between the significant parameters in leaf and root anatomy ($\rho = 0.39$ to 0.52). The correlation within the leaf parameters ranged from weak to strong ($\rho = 0.36$ to 0.74), while a weak to intermediate correlation was recorded within the root parameters ($\rho = 0.36$ to 0.69).

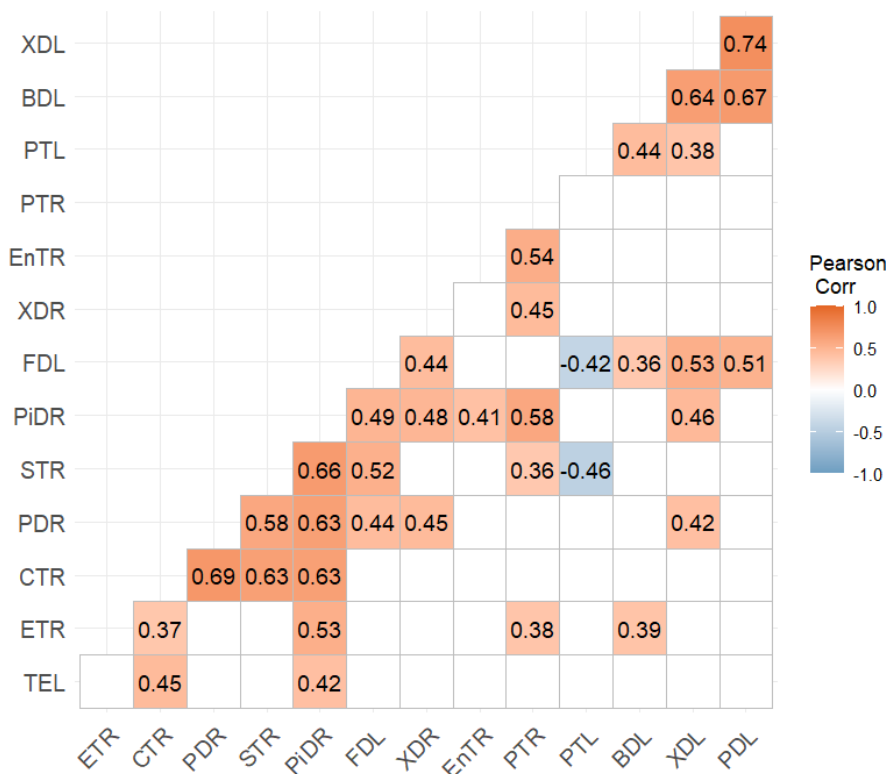


Fig. 5. Pearson’s correlation matrix of the leaf and root anatomical parameters (TEL = total epidermis thickness leaf, FDL = fusoid cells diameter leaf, PTL = plicate cells thickness leaf, BDL = bulliform cells diameter leaf, XDL = metaxylem diameter leaf, PDL = phloem diameter leaf, ETR = epidermis thickness root, STR = sclerenchymatous layer thickness root, CTR = cortex thickness root, EnTR = endodermis thickness root, PTR = pericycle thickness root, PDR = phloem diameter root, XDR = metaxylem diameter root, PiDR = pith diameter root).

3.2. Discussion

3.2.1. Leaf anatomical response

The α -NAA is one of the commonly used synthetic auxins at a substantial to relatively low amount to promote auxin-mediated root initiation, leaf development, fruiting, and biomass production, among others (Awan et al. 2015; Basnet et al. 2021; Yan et al. 2014;). Specifically, the fusoid cells in diverse genera under the subfamily Bambusoideae (Poaceae) have numerous acclaimed functions – absorption and redistribution of light within the leaf in shaded areas (March and Clark 2011), structural support in mature leaf (Vega et al. 2016), nutrient transportation, and water balance (Wang et al. 2016), and for synthesis and storage of starch (Leandro et al. 2018). Our results revealed that 100 or 200 ppm of α -NAA was the most effective treatment, increasing the fusoid cell diameter compared with 0 ppm. This result was aligned with the study of Chen et al. (2022) and Siddik et al. (2015), where α -NAA application increased plants' photosynthetic activity, which may related to the enhanced light distribution in the leaf due to the development of fusoid cells. Increased photosynthetic activity also increases glucose production, which leads to higher starch reserves. Exogenous application of auxins, such as α -NAA, stimulates starch hydrolysis into sugar, elevating the available sugar in the plant system (Leakey 2014; Zheng et al. 2020). Hence, the subsidiary starch was stored in fusoid cells for later usage, expanding their diameter.

Our results also suggest that only 0 and 200 ppm had significantly different xylem diameters, while the two treatment pairs had stable diameters. The leaf xylem tissues function mostly for passive water and nutrient transport and mechanical support by maintaining leaf architecture (Sakurai and Miklavcic 2021). They are associated with transpiration-related water hydraulics (Venturas et al. 2017). Hagen-Poiseuille law states that the xylem vessel's water flow rate is proportional to the fourth power of the vessel's radius. Thus, α -NAA improves leaf water and nutrient translocation, as Martins et al. (2020) reported. However, Aloni (2010) contradicted our findings because α -NAA treatment with auxin inhibitor (2,3,5-triiodobenzoic acid, TIBA) in *A. thaliana* resulted in narrow vascular bundles with few vessels, and α -NAA alone did not induce any changes in the xylem. This implies that even the naturally occurring auxin in the plant is enough for vascular bundle formation.

From the leaf morphological point of view, the application of α -NAA enhanced the leaf length and number in *Spinacia oleracea* (Awan et al. 2015) and *Bambusa vulgaris* (Tambong 2023), the elongation of leaf strips in *Phaseolus vulgaris* (Keller 2017), and the leaf blade area in *Arabidopsis thaliana* (Kalve et al. 2020). We hypothesize that the increased diameters of fusoid cells and xylem vessels of *B. blumeana* may contribute to morphological changes caused by α -NAA treatments. On the other side, cytokinins are synthesized and naturally occur in roots. Still, their function is mostly manifested in shoots, such as for shoot initiation, leading to leaf epidermal development, mesophyll cell enlargement, or bulliform cell growth (Wu et al. 2021; Zuch et al. 2022). These insignificant findings in the thickness of the total epidermis and plicate cells, as well as the diameter of bulliform cells and phloem, may be regulated by either antagonistic interactions or synergistic inputs of auxins and cytokinins (Yang et al. 2017; Zhao et al. 2010). This would only mean there was inhibition of leaf cell expansion, which explains the insignificant results across various treatments of α -NAA.

3.2.2. Root anatomical response

The use of α -NAA has been proven to induce lateral roots, root cell division, and elongation in various floral species. Our results suggest that the α -NAA effectively increased the thickness of the rhizodermis (0–200 ppm and 100–200 ppm). The exogenous (NAA) and endogenous (IAA) auxins were responsible for increasing the number of epidermal outgrowths or root hairs that form in the trichoblasts (Zhang et al. 2018). This resulted in increased root hair density, root organ growth, and more allocation of epidermal cells, affecting the overall thickness (Löffke et al. 2015; Niu et al. 2011). Hence, exogenous auxin application signals epidermal cells to initiate more root hairs, resulting in increased epidermal thickness in our study. In contrast, Alarcón et al. (2014) and Alarcón et al. (2019) reported alternative results that α -NAA decreased the thickness of the rhizodermis due to hormone-specific action on cell division.

Our results also signify the development of the sclerenchymatous layer, often called cortical sclerenchyma, between the rhizodermis and cortex in response to α -NAA application. It appears that *B. blumeana* invested in developing the root sclerenchymatous layer, which provides mechanical support and assists in water and nutrient transport (Martins et al. 2018; Schneider 2022). Additionally, the root sclerenchymatous layer improves the penetration of monocot roots in compacted soils (Schneider et al. 2021), and α -NAA application to *B. blumeana* may have a direct association with this phenomenon, as shown in the results.

The cortex serves as the storage region for reserve materials and other nutrients and aids in the transportation mechanism of water and nutrients throughout the plant system (Melo et al. 2014). In this study, the thickest cortex was recorded in 200 ppm α -NAA, implying the activation of cell division in the region. Monder et al. (2021) agreed with the result of this study that the cortex grows intensively after applying α -NAA treatments using *Rosa* “Hurdal”. Hence, α -NAA treatments expounded the root storage capacity of *B. blumeana* and efficiently improved its water and nutrient radial translocation. Alternatively, Alarcón et al. (2019) and Kollárová et al. (2012) consistently revealed that the α -NAA reduced the length, area, and number of cortical cells.

Interestingly, the endodermal thickness had no significant differences, suggesting consistent thickness in the control and experimental units. Seo et al. (2021) used N-1-naphthylphthalamic acid (NPA), an auxin transport inhibitor, resulting in the accumulation of auxin in endodermis, leading to endodermal cell divisions. However, α -NAA is not considered an auxin transport inhibitor, but the introduction of exogenous auxin possibly surpassed the optimum auxin accumulation in the roots, leading to unchanged endodermal thickness. This result raises important discussions about the interplay of α -NAA with other plant hormones within the root system.

The four anatomical structures that made up the stele (pericycle, vascular tissue, and pith) showed increased diameters under α -NAA treatments. Various studies proved that α -NAA induced lateral roots in bamboo and trees (Mudoj et al. 2014; Rajput et al. 2019; Tambong 2023). Specifically, the pericycle consists of the single-layer cells after the endodermis and the outermost area of the stele, which are responsible for lateral root initiation. Due to the accumulated auxins, some of these pericycle cells obtained the status of being founder cells, resulting in asymmetrical cell division that induced high production of lateral root primordia (Alarcón et al. 2019; Marhavý et al. 2013; Teixeira and ten Tusscher 2019). Thus, pericycle cells are expected to be thicker under α -NAA treatments as they were directly related to lateral root density.

The vascular tissues in the roots also responded well to α -NAA treatments. Because the effect of natural auxin is manifested mainly in roots, we assume the case of α -NAA also triggers

vascular differentiation and development in *B. blumeana*, as shown in our results. This study agrees with the results of [Martins et al. \(2018\)](#) and [Martins et al. \(2020\)](#), where increased α -NAA concentration resulted in increased xylem number and diameter in roots. However, one prominent study in phloem revealed that α -NAA competes with the naturally occurring auxin, Indole acetic acid (IAA), leading to its transport inhibition ([Tamas and Davies 2016](#)). This instance may be observed, yet requires further studies in the *B. blumeana* to ensure the interaction of α -NAA with other hormones translocated within the phloem. Like the xylem diameter in leaves, the root xylem and phloem had wider diameters, indicating a higher hydraulic conductivity and nutrient transport throughout the plant.

Lastly, pith functions for retaining water and other photosynthates within their parenchymatous cells, facilitating the distribution of these materials and providing structural support to roots ([Fujimoto et al. 2018](#)). Our results show the improved pith diameter, the centermost parenchymatous ground tissue, under α -NAA treatments, typically unexplored in anatomical studies. In some research, α -NAA increased the diameter of the vascular cylinder by which pith occupies a huge area ([Martins et al. 2018](#); [Martins et al. 2020](#)). Thus, our findings suggest that *B. blumeana* had wider food storage, efficient materials translocation, and stronger structural roots. However, when α -NAA was combined with IAA, a significant decrease in the diameters of the stele region, xylem, and pith was observed, but the phloem diameter increased ([El-Dengawy et al. 2017](#)). It shows that the effect of α -NAA alone in the pith diameter is still developing, and our findings contribute to further studies.

3.2.3. Correlation of leaf and root anatomy

In this study, we primarily attempted to correlate the leaf and root anatomical responses of *B. blumeana* treated with α -NAA. We found a weak to moderate positive correlation of BDL to PTL, ETR, and FDL within the leaf tissue. Bulliform cells are an essential part of foliar epidermal cells in monocots that become larger when filled with water and smaller when flaccid, allowing the rolling of leaves for water conservation during transpiration ([Chen et al. 2020](#); [Matschi et al. 2020](#)). [Nedukha \(2022\)](#) reported that some water in the bulliform cells had a symplastic pathway to the mesophyll cells. Since the fusoid cells of *B. blumeana* were positioned in the lateral area of vascular bundles, [Vega et al. \(2016\)](#) argued that the water from these cells could have a symplastic pathway from the mesophyll cells where plicate cells are found. Thus, the bulliform cells, plicate cells (as part of mesophyll cells), and fusoid cells have a complex hydraulic relationship. These findings imply that as BDL increases, the ETR and FDL increase under α -NAA treatments.

Additionally, since the xylem is also an essential structure for leaf water distribution ([Sakurai and Miklavcic 2021](#); [Venturas et al. 2017](#)), its diameter also had a positive correlation with PTL (weak) and FDL (moderate), suggesting a consistent correlation as also shown in BDL (moderate). Even when treated with α -NAA, our result is coherent with xylem tissues' function for water transport in other leaf anatomical structures that support leaf hydraulics. We hypothesized that α -NAA contributes to the hydraulic dynamics in the bulliform, plicate, and fusoid cells as these anatomical structures have similar water relation functions. Lastly, the PDL had a moderate positive correlation with BDL and FDL as the phloem also transports water and other minerals ([Katifori 2018](#)). Our results implied that phloem was possibly linked with the bulliform and fusoid cells as they served to store water and starch granules. The strong positive correlation of XDL and PDL was a logical result, as vascular differentiation is mediated by auxin. However, our findings

contradict the findings of Aloni (2010) because α -NAA did not affect vascular differentiation. Beyond these, FDL and PTL have a moderate negative correlation, suggesting that the starch from the fusoid cells may be deposited to plicate cells for photosynthesis, leading to their inverse parametric findings (Liana et al. 2019).

Within the root tissue, the PiDR had a moderate positive correlation to STR, ETR, EnTR, PTR, XDR, CTR, and PDR. Specifically, our findings elucidate the increasing pith diameter resulted in increasing sclerenchymatous layer thickness under α -NAA treatment. Both structures were known to provide structural or mechanical support (Lux et al. 2004; Martins et al. 2018). We assume that α -NAA enhances the support mechanism of the pith and sclerenchymatous layer; however, the role of α -NAA in forming sclerenchymatous tissues remains unexplored and needs further elaboration. Our results also reported that *B. blumeana* invested in STR development under α -NAA treatments instead of alleviating the lignified cells in the pith as shown in the control. This may explain the inhibition of lignification in the pith of treated roots, as Xu et al. (2023) had a clear investigation regarding this phenomenon in *Pyrus* spp. The PiDR was still increasing due to the continuous cell division stimulated by α -NAA within the region as the STR also increased.

On the other hand, the stimulation of lateral roots by α -NAA widened the root area for efficient water absorption in the soil. Thus, it seems the larger root anatomical structures enhanced the water pathway within the root system. Barberon and Geldner (2014) discussed that the root hairs absorb the soil water, pass through the cortex, endodermis, and pericycle, and finally reach the vascular tissues and pith for storage. These soil waters may be transported radially through apoplastic, symplastic, or coupled transcellular pathways (Barberon and Geldner 2014; Zarebanadkouki et al. 2019), which explains the positive correlation of most of these root anatomical structures. Like the PiDR, the root vascular tissues were directly correlated to the root anatomical structures associated with water or nutrient transport. The increasing PDR, PiDR, and PTR increased XDR. The same case was observed in the PDR to XDR, STR, PiDR, and CTR. These anatomical structures were the initial pathway of water and nutrients absorbed in the rhizodermis (Barberon and Geldner 2014; Zarebanadkouki et al. 2019).

Our result about the intermediate positive correlation of PTR and EnTR raises additional perspective in lateral root formation under α -NAA treatments. In grasses, the evolutionary evidence revealed that the lateral root was derived from the cell division of the pericycle and endodermis, while in some cases, the cortex was also included (Singh et al. 2023; Xiao et al. 2019). In this study, the direct correlation of endodermis and pericycle thickness of *B. blumeana* challenges the common perception that lateral roots are only derived in the pericycle. Robbins et al. (2014) argued that the pericycle can perceive and gauge the mechanical resistance of the endodermis to pericycle swelling; thus, the endodermis responds by adjusting morphologically for the developing primordia. Marhavý et al. (2013) added that the cell division in the pericycle exhorted the neighboring root structures (endodermis and cortex) to adapt to the arising lateral root primordia. Hence, our results suggest that the endodermis regulates auxin accumulation and signals the pericycle founder cell to initiate lateral root. On the other hand, our findings also reveal the direct correlation between the PTR and STR. Schneider et al. (2021) substantiated that the sclerenchyma layer and the pericycle in Corn and Rice had similar cellular components (pectin/lignin). These similarities in the two root structures may explain their direct responses when exogenous auxin was applied. As the sclerenchymatous layer becomes thicker, the roots may penetrate deeper into the soil, and the plant may induce various lateral roots for a wider area of water and nutrient absorption (Rogers and Benfey 2015; Schneider et al. 2021).

Between the leaf and root anatomical correlation in response to α -NAA, our results have consistent findings on the weak to intermediate positive correlation of PiDR to FDL, TEL, and XDL, considering its water and nutrient transport function (Fujimoto et al. 2018). Through debates and discussions regarding the origin of the pith, whether from the ground meristem or provascular tissue, many botanists found evidence that as the root becomes older, the pith at the most basal area in the root differentiates into protoxylem (Tomescu and McQueen 2022). The involvement of the pith in the axial transport of materials was modeled by Lynch et al. (2021), supporting the narrative pith's functional traits, which also explain its positive correlation with the anatomical structures under α -NAA. A direct correlation between root pith diameter and the foliar epidermis thickness and diameters of xylem and fusoid cells in the leaf was due to the activity of pith in axial material transport.

The root vascular tissue had an intermediate positive correlation to the FDL under the α -NAA treatments. The xylem transports water and other substances from the roots to the shoots, while the phloem transports photosynthates and other materials from the shoots to the roots and *vice versa* (Pittermann 2010; Schenk et al. 2021). We assume that those water, nutrients, and other substances absorbed in the roots were translocated by these vascular tissues and stored in the leaf fusoid cells, resulting in their direct correlation. However, the mechanism behind the moderate positive correlation between the TEL and PiDR or CTR remains unexplored. Interestingly, we found a compelling moderately negative correlation between the STR and PTL. No studies have attempted to correlate the two structures' responses to α -NAA. The starch produced in the plicate cells was possibly allocated to the sclerenchymatous layer in the roots. Regardless, the responses and interrelation of root and leaf anatomical structures towards α -NAA treatments seemed consistent with their specific functions.

4. Conclusions

This study described and analyzed the leaf and root anatomical responses of *B. blumeana* treated with α -NAA. The 200 ppm of α -NAA increased bulliform cells' diameters and the leaf's metaxylem. A similar case was observed as the 200 ppm enhanced the root anatomical structures except for the endodermis thickness. Interestingly, it appears that α -NAA contributed significantly to the cell division and elongation of the leaf and root system within 3 months, which may justify the morphological changes reported by various researchers in the field. The correlation findings also suggest insights into enhancing water and nutrient transport, lateral root initiation, and auxin-mediated functions. However, we strongly recommend, based on these findings, further look into the unexplored anatomical structures (e.g., plicate cells, pith, sclerenchymatous layer, etc.), signaling interplay of α -NAA with other plant growth hormones, and the chemical and molecular mechanism behind these anatomical changes induced by α -NAA.

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