

Ecological Adaptability of Guangxi *Syzygium hancei* Seedlings under Different Habitats (Post-print)

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Abstract

To understand the adaptability of *Syzygium hancei* seedlings to environmental factors under natural regeneration conditions across three different habitats, this study examined naturally regenerated one-year-old *Syzygium hancei* seedlings in the coastal areas of Guangxi, measuring antioxidant enzyme activities, soluble sugar content, malondialdehyde content in leaves and roots, as well as photosynthetic pigments in leaves. The results showed that: (1) Variance analysis of environmental factors indicated that light intensity differed extremely significantly among the three sample plots, while surface temperature and soil moisture content showed no significant differences. (2) The physiological characteristics of seedling leaves and roots in different habitats varied with light intensity. With increasing light intensity, leaf superoxide dismutase (SOD) activity, soluble sugar (SS) content, malondialdehyde (MDA) content, chlorophyll a/b ratio, and root peroxidase (POD) activity were significantly higher in habitat B than in habitat A; whereas leaf POD activity, chlorophyll a content, chlorophyll b content, total chlorophyll content, and root SOD activity, SS content, and MDA content were significantly higher in habitat A than in habitat B. (3) With decreasing light intensity, leaf SOD activity, MDA content, and root POD activity were significantly higher in habitat B than in habitat A; leaf POD activity and SS content were significantly higher in habitat A than in habitat B; chlorophyll a content, chlorophyll b content, total chlorophyll content, root SS content, and root MDA content were initially significantly higher in habitat A than in habitat B, then changed to being significantly higher in habitat B than in habitat A; chlorophyll a/b ratio and root SOD activity were initially significantly higher in habitat B than in habitat A, then changed to being significantly higher in habitat A than in habitat B. (4) Comprehensive analysis indicated that the physiological and biochemical characteristics of the three habitats exhibited different adaptive strategies, among which habitat B with the smallest canopy density experienced photoinhibition; therefore, manual

adjustment of canopy density is required to create a suitable light environment for natural regeneration of seedlings.

Full Text

Ecological Adaptation of *Syzygium hancei* Seedlings in Guangxi Under Different Habitats

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Abstract

To understand the adaptive capacity of *Syzygium hancei* seedlings to environmental factors under natural regeneration conditions across three distinct habitats, this study examined current-year naturally regenerated seedlings in coastal regions of Guangxi. We measured antioxidant enzyme activities, soluble sugar content, malondialdehyde (MDA) content, and photosynthetic pigments in both leaves and roots. The results revealed: (1) Variance analysis of environmental factors showed highly significant differences in light intensity among the three sample plots, while surface temperature and soil moisture content showed no significant differences. (2) Physiological characteristics of seedling leaves and roots varied with light intensity across habitats. With increasing light intensity, leaf superoxide dismutase (SOD) activity, soluble sugar (SS) content, MDA content, chlorophyll a/b ratio, and root peroxidase (POD) activity were significantly higher in Habitat B than in Habitat A. Conversely, leaf POD activity, chlorophyll a content, chlorophyll b content, total chlorophyll content, and root SOD activity, SS content, and MDA content were higher in Habitat A than in Habitat B. (3) With decreasing light intensity, leaf SOD activity, MDA content, and root POD activity were significantly higher in Habitat B than in Habitat A, while leaf POD activity and SS content were higher in Habitat A than in Habitat B. Chlorophyll a, chlorophyll b, total chlorophyll content, and root SS and MDA contents were initially higher in Habitat A than in Habitat B, then reversed to become higher in Habitat B than in Habitat A. The chlorophyll a/b ratio and root SOD activity were initially higher in Habitat B than in Habitat A, then reversed to become higher in Habitat A than in Habitat B. (4) Comprehensive analysis indicated that physiological and biochemical characteristics exhibited different adaptive strategies across the three habitats. Photoinhibition occurred in Habitat B, which had the lowest canopy density, suggesting

that manual adjustment of canopy density is needed to create suitable light conditions for natural seedling regeneration.

Keywords: *Syzygium hancei*, antioxidant enzymes, osmoregulation, photosynthetic pigments, adaptability

Introduction

Seedling growth and development are influenced by numerous biotic and abiotic factors, with many scholars investigating seedling responses and adaptability to various environmental conditions (Wu et al., 2009). Environmental factors in natural ecosystems are intricately interconnected, and plant development results from the combined effects of multiple factors (Yang et al., 2019; Li et al., 2020; Li et al., 2021). During the seedling stage, environmental conditions such as light and temperature significantly affect regeneration and succession processes (Wang et al., 2002). Temperature, light, and water are primary environmental factors that play distinct roles in plant growth and suitable distribution (Yu and Xu, 2003). Among these factors, light particularly influences seedling growth, development, and natural regeneration (Agyeman et al., 1999).

Syzygium hancei is an evergreen tree species in the Myrtaceae family and a dominant species in the coastal ecological transition zone of Guangxi's coastal belt, exerting substantial influence on the transitional ecosystem. However, frequent human activities have caused permanent loss of natural coastal vegetation and severe habitat fragmentation, leading to declining natural forests and threatening the development and cultivation of *S. hancei*. This species exhibits strong tolerance to drought, salinity (Ouyang et al., 2014), and high temperatures (Mo et al., 2013), and can grow in sandy soils with good aeration but poor water retention (Luo et al., 2016), typically occurring near water bodies and valley wetlands.

Previous research on *S. hancei* has focused on community spatial structure characteristics (Li et al., 2016) and stress tolerance under artificially controlled conditions (Liu et al., 2020), demonstrating its heliophilic nature. However, studies on physiological characteristic changes of naturally regenerated *S. hancei* seedlings across different habitats are limited, and most existing research has been conducted under artificial experimental conditions, lacking general explanatory significance for naturally distributed coastal *S. hancei* communities. Therefore, this study investigated current-year naturally regenerated seedlings of *S. hancei*—a dominant species in Guangxi's coastal region—by measuring physiological and biochemical indicators in leaves and roots across three habitats to observe their dynamic changes and adaptive responses to environmental factors, providing a basis for constructing natural coastal protection forests and conserving *S. hancei* communities.

1.1 Study Area Description

The study was conducted in Hating, Wutou Village, Jiangping Town, Dongxing City (108°08 07 E, 21°35 28 N), located in the northern tropical zone at 7 m elevation. According to meteorological data, the area has a frost-free period exceeding 360 days annually, with average relative humidity of 79%, mean forest temperature of 22.7 °C, peak temperatures in July and August, and mean annual precipitation of 2,441 mm concentrated between May and September. The site experiences moderate human disturbance with relatively intact forest preservation. Three representative natural communities with good natural regeneration and different canopy densities were selected as sample plots, with consistent altitude and aspect among plots and inter-plot distances of 150-200 m. The study area's tree layer is dominated by *S. hancei*, with scattered *Schefflera octophylla*, *Ilex szechwanensis*, and *Machilus chinensis*. The shrub layer primarily includes *Rhodomyrtus tomentosa* and *Lindera aggregata*, while the herbaceous layer mainly comprises *Piper kadsura*, *Hemarthria sibirica*, and *Ophiopogon bodinieri*.

1.2 Community Characteristics of *Syzygium hancei*

Forest growth surveys for the three sample plots are presented in , revealing distinct differences in plant community growth status among plots, indicating that natural *S. hancei* forests developing under different habitat conditions exhibit varying community structures and species composition. Habitat A had the highest tree layer density, smaller average heights in shrub and herbaceous layers, and greater canopy closure. Habitat B had the lowest tree layer density, smaller average heights in shrub and herbaceous layers, and lower canopy closure. Habitat C showed the highest average heights in both shrub and herbaceous layers.

1.3 Experimental Design

Soil measurements indicated no significant differences in physicochemical properties among the three plots: soil density of 1.36-1.41 g · cm⁻³, capillary porosity of 29.2%-32.8%, non-capillary porosity of 11.9%-12.6%, total porosity of 41.6%-45.4%, pH of 5.42-5.67, total salt content of 0.05-0.07 ms · cm⁻¹, total nitrogen of 1.21-1.26 g · kg⁻¹, available phosphorus of 10.04-11.21 mg · kg⁻¹, and available potassium of 40.25-42.83 mg · kg⁻¹. Within each plot, three 1 m × 1 m quadrats were established, with distances between quadrats measured and corners marked with wooden stakes fixed by PVC pipes. Seedling habitats in plots A, B, and C were designated as Habitat A, Habitat B, and Habitat C, respectively. Twelve seedlings with consistent growth were randomly selected from each quadrat for measurement of leaf and root physiological indicators.

Investigations were conducted in mid-May, July, September, and November 2016, beginning at the peak seedling germination period and continuing at two-month intervals.

1.4.1 Sample Collection

For leaf samples, healthy seedlings without pest or disease damage were selected to create composite samples, sealed in ziplock bags, immediately placed in a cooler to maintain original moisture content and prevent physiological changes from water loss, then transferred to an ultra-low temperature refrigerator. For root samples, plants with consistent growth and no pest or disease damage were selected. Seedlings were excavated, roots were extracted from soil, sealed in ziplock bags, immediately cooled to prevent water loss-induced changes, then stored in an ultra-low temperature refrigerator.

1.4.2 Determination of Leaf Chlorophyll Content

Chlorophyll content was determined using the acetone-ethanol mixed extraction method (Li, 2000). Plant samples were cleaned, cut into small pieces, and mixed evenly. A small amount of quartz sand, CaCO_3 , and 95% ethanol were added for grinding until leaf tissues turned white, then made up to volume with 95% ethanol. Absorbance was measured at 649 nm and 665 nm using a spectrophotometer. Calculations were performed using the following formulas:

Chlorophyll a concentration:

$$C_a(\text{mg} \cdot \text{L}^{-1}) = 12.21A_{665} - 2.81A_{649}$$

Chlorophyll b concentration:

$$C_b(\text{mg} \cdot \text{L}^{-1}) = 20.13A_{649} - 5.03A_{665}$$

Chlorophyll a/b ratio:

$$C_{a/b}(\text{mg} \cdot \text{L}^{-1}) = C_a/C_b$$

Pigment content:

$$\text{Content}(\text{mg} \cdot \text{g}^{-1}) = (C_i \times V \times d)/m$$

Total chlorophyll content = Chlorophyll a content + Chlorophyll b content

Where C_i is the corresponding pigment concentration, V is the extract volume, d is the dilution factor, and m is the fresh sample weight.

1.4.3 Determination of Physiological Indices in Seedling Leaves and Roots

Following the methods of Gao et al. (2006): POD activity was determined using the guaiacol method; SOD activity was measured using the nitroblue tetrazolium (NBT) photochemical reduction method; MDA content was determined using the thiobarbituric acid method; and soluble sugar content was measured using the anthrone colorimetric method.

1.5 Statistical Analysis

Data were organized using Excel 2010. One-way ANOVA in SPSS 26.0 was used for significance testing of environmental factors. Figures for leaf and root physiological and biochemical indices were generated using Origin 2021.

2.1 Environmental Factor Investigation and Variance Analysis of *Syzygium hancei* Seedlings

Environmental factor surveys of the three sample plots ([Figure 1: see original paper]) and variance analysis () revealed highly significant differences in light intensity among the three habitats, while surface temperature and soil moisture content showed no significant differences.

2.2 Dynamic Changes in Leaf Antioxidant Enzyme Activities of *Syzygium hancei* Seedlings

As shown in [Figure 2: see original paper], leaf SOD activity increased continuously, while POD activity showed a trend of initial increase followed by decrease. No significant differences in leaf SOD activity were observed among the three habitats in May, September, or November ($P > 0.05$). In July, Habitat C differed significantly from the other two habitats ($P < 0.05$), being 124.44% and 95.13% higher than Habitats A and B, respectively. All three habitats reached maximum SOD activity in November, with Habitat B showing the highest value at $1,650.96 \text{ U} \cdot \text{g}^{-1}\text{FW} \cdot \text{h}^{-1}$ and Habitat C the lowest at $1,003.47 \text{ U} \cdot \text{g}^{-1}\text{FW} \cdot \text{h}^{-1}$. Leaf POD activity in May differed significantly between Habitat C and the other two habitats. All three habitats reached maximum POD activity in September, with Habitat A highest at $87.08 \text{ U} \cdot \text{g}^{-1}\text{FW} \cdot \text{h}^{-1}$ and Habitat C lowest at $75.65 \text{ U} \cdot \text{g}^{-1}\text{FW} \cdot \text{h}^{-1}$. Throughout the dynamic changes, Habitat A consistently showed higher POD activity than the other two habitats.

2.3 Dynamic Changes in Leaf Soluble Sugar Content of *Syzygium hancei* Seedlings

Soluble sugar content ([Figure 3: see original paper]) showed an overall trend of initial increase followed by decrease. In May and July, significant differences ($P < 0.05$) were observed between Habitats A and B versus Habitat C, with Habitat C showing the highest values. All three habitats reached maximum soluble sugar content in September, with Habitat C highest at $2.65 \text{ mg} \cdot \text{g}^{-1}\text{FW}$, representing increases of 41.71% and 45.6% compared to Habitats A and B, respectively. No significant differences ($P > 0.05$) were found among habitats in September and November, though content decreased significantly in November, with Habitat A showing the highest value.

2.4 Dynamic Changes in Leaf MDA Content of *Syzygium hancei* Seedlings

Leaf MDA content ([Figure 4: see original paper]) exhibited a pattern of initial decrease, followed by increase, then decrease again. No significant differences ($P > 0.05$) were observed among habitats in May and September, though Habitat B showed higher values than the other two habitats. Significant differences were found in July and November between Habitat A and the other habitats. In July, Habitat A was 12.67% and 7.88% lower than Habitats B and C, respectively. Minimum values were reached in November at 4.48, 4.65, and 4.04 $\text{mol} \cdot \text{g}^{-1}\text{FW}$ for Habitats A, B, and C, respectively. Throughout the dynamic changes, Habitat B consistently showed higher MDA content than Habitats A and C.

2.5 Dynamic Changes in Photosynthetic Pigments of *Syzygium hancei* Seedlings

Chlorophyll a, chlorophyll b, and total chlorophyll contents ([Figure 5: see original paper]) showed trends of initial increase followed by decrease, while chlorophyll a/b ratio initially decreased, then increased, then decreased again. Chlorophyll a content reached maximum values in September across all habitats, with Habitat C higher than Habitats A and B, and minimum values in November. Significant differences ($P < 0.05$) were observed between Habitat C and the other habitats in May and November, but not in July and September ($P > 0.05$). Chlorophyll b content peaked in July across all habitats, with Habitat A higher than Habitats B and C, and reached minimum values in November, when Habitat C was higher than Habitats A and B. No significant differences were found among habitats in May, July, or September, but Habitat A was significantly lower than Habitats B and C in November. The chlorophyll a/b ratio peaked in September across all habitats, with Habitat B higher than the other two habitats. No significant differences were observed among habitats in May, July, or September, but the ratio decreased to minimum values in November, when Habitat A was significantly higher than Habitats B and C. Total chlorophyll content reached maximum values in September across all habitats, with Habitat A higher than Habitats B and C. No significant differences were found among habitats in May, July, or September, but values decreased to minima in November, when Habitat C was significantly higher than Habitats A and B, showing increases of 38.15% and 2.58%, respectively.

2.6 Dynamic Changes in Root Antioxidant Enzyme Activities of *Syzygium hancei* Seedlings

Root SOD and POD activities ([Figure 6: see original paper]) showed consistent trends of initial increase followed by decrease. SOD activity differed significantly among habitats in May ($P < 0.05$), with Habitat A 15.73% and 26.09% higher than Habitats B and C, respectively, representing the minimum values for all habitats at 110.57, 95.54, and 87.69 $\text{U} \cdot \text{g}^{-1}\text{FW} \cdot \text{h}^{-1}$. Habitat B differed signifi-

cantly from the other habitats in July ($P < 0.05$). No significant differences ($P > 0.05$) were observed in September or November, though all habitats reached maximum SOD activity in September, with Habitat A highest at $263.93 \text{ U} \cdot \text{g}^{-1}\text{FW} \cdot \text{h}^{-1}$ and Habitat C lowest at $233.48 \text{ U} \cdot \text{g}^{-1}\text{FW} \cdot \text{h}^{-1}$. No significant differences in POD activity were found among habitats across all months, though maximum values occurred in September at 113.54, 115.63, and $96.35 \text{ U} \cdot \text{g}^{-1}\text{FW} \cdot \text{h}^{-1}$ for Habitats A, B, and C, respectively. Habitat B consistently showed higher POD activity than Habitats A and C across all months.

2.7 Dynamic Changes in Root Soluble Sugar Content of *Syzygium hancei* Seedlings

Root soluble sugar content ([Figure 7: see original paper]) showed a trend of initial decrease followed by increase. No significant differences ($P < 0.05$) were observed among habitats in May, September, or November. In July, Habitat B was significantly lower than Habitats A and C, decreasing by 1.7% and 5.0%, respectively, representing the minimum value. All three habitats showed maximum soluble sugar content in November, with Habitat B highest at $1.6 \text{ mg} \cdot \text{g}^{-1}\text{FW}$.

2.8 Dynamic Changes in Root MDA Content of *Syzygium hancei* Seedlings

Root MDA content ([Figure 8: see original paper]) exhibited a pattern of initial decrease, followed by increase, then decrease again. No significant differences ($P < 0.05$) were observed among habitats in May, July, or September, though all habitats reached maximum MDA content in September at 9.77, 9.18, and $10.04 \text{ mol} \cdot \text{g}^{-1}\text{FW}$ for Habitats A, B, and C, respectively. In November, Habitat B differed significantly from the other two habitats ($P < 0.05$), being 16.11% and 5.6% higher than Habitats A and C, respectively.

3.1 Physiological and Biochemical Adaptation of *Syzygium hancei* Seedlings

Plant responses and adaptability to different environments are determined by the integration of intrinsic physiological characteristics and environmental variation, resulting in differential sensitivity among physiological indicators during environmental adaptation. The same species exhibits different antioxidant enzyme protection systems, osmoregulatory functions, and other mechanisms across environments, representing fundamental adaptive strategies. Plants have evolved defense systems including antioxidant enzymes such as SOD and POD to cope with oxidative damage under stress (Foyer and Noctor, 2005). Due to high density and canopy closure in Habitat A, photosynthetically active radiation received by *S. hancei* seedlings decreased, resulting in lower leaf SOD activity compared to Habitat B, likely because insufficient radiation limited free

radical scavenging and slowed growth (Yang et al., 2011). Habitat B, with low density and canopy closure, received adequate light energy, but showed lower leaf POD activity than Habitat A, possibly because seedlings under low light experienced increased reactive oxygen species, enhanced membrane lipid peroxidation, and elevated enzyme activity to adapt to shaded conditions (Yuan et al., 2022). Thus, the two primary enzymes (SOD and POD) play crucial roles in maintaining reactive oxygen metabolism balance in *S. hancei* seedlings. Environmental stress during plant growth generates reactive oxygen species, causing membrane lipid peroxidation and ion leakage (Rocca et al., 2009; Schieber and Chandel, 2014; Sebastian and Prasad, 2015). In this study, soluble sugar content was highest in Habitat C, followed by Habitat A, and lowest in Habitat B, possibly because intense light affected photosynthetic product formation and nitrogen metabolism rates. Malondialdehyde (MDA) is a product of membrane unsaturated fatty acid oxidation resulting from increased reactive oxygen species, directly reflecting membrane peroxidation degree (Huang et al., 2005). *Syzygium hancei* typically grows in coastal areas with high soil salinity, and studies have shown that halophytes synthesize organic osmotic regulators in the cytoplasm to maintain normal osmotic balance (Yang et al., 2008). In this study, Habitat B showed higher MDA content than Habitats A and C across all months under adequate photosynthetically active radiation, indicating membrane system damage and high metabolic levels in *S. hancei* seedlings. This aligns with Zhang et al. (2022), who reported highest MDA content in understory-planted *Eleutherococcus senticosus*.

Changes in SOD and POD activities reflect the response mechanisms of the root antioxidant system to environmental stress. Root SOD activity was highest in Habitat A, followed by Habitat B, and lowest in Habitat C, possibly because high canopy closure in Habitat A reduced photosynthetically active radiation and photosynthesis, prompting seedlings to enhance SOD activity to scavenge oxygen free radicals and prevent membrane peroxidation. Root POD activity was highest in Habitat B, followed by Habitat A, and lowest in Habitat C, with all habitats showing an initial increase then decrease, indicating enhanced antioxidant enzyme activity for free radical scavenging. From May to September, soluble sugar and MDA contents in Habitat A were higher than in the other habitats, while light intensity across all habitats initially increased then decreased. By November, soluble sugar and MDA contents were highest in Habitat B when light intensity had decreased to minimum values, consistent with trends observed in low-light stress effects on soluble sugar and MDA contents in different maize genotypes (Niu et al., 2019). Under stress, reactive oxygen species (ROS) accumulate in plants, altering cellular homeostasis and causing plasma membrane peroxidation damage, with MDA accumulating as stress duration extends (Apel and Hirt, 2004). The gradual decrease in MDA content during early seedling development may relate to the sudden increase in SOD activity during mid-development. As the primary antioxidant enzyme and first line of defense in the antioxidant system, SOD's greater stability compared to other antioxidant enzymes (Feng et al., 2017) means its activity changes directly

affect MDA content.

3.2 Adaptation of Photosynthetic Pigments in *Syzygium hancei* Seedlings

Chlorophyll absorbs energy in plants, and its content is typically associated with photosynthetic capacity, which strengthens as content increases (Wei et al., 2014). In this study, chlorophyll a, chlorophyll b, and total chlorophyll contents in all three habitats decreased in November when light intensity declined ([Figure 1: see original paper]), likely due to other interfering factors affecting chlorophyll synthesis under natural regeneration conditions. Overall, Habitat B with lower canopy closure showed lower chlorophyll a, chlorophyll b, and total chlorophyll contents, followed by Habitat C, while Habitat A showed the highest values. This indicates that under high light conditions, chlorophyll formation is inhibited and easily degraded or destroyed, preventing plants from consuming absorbed light energy and resulting in photoinhibition (Wang et al., 2012). Moderate shading can thus prevent leaf scorching from intense light while meeting illumination requirements for plant growth. Zhao et al. (2014) reported significant reductions in chlorophyll a, chlorophyll b, and total chlorophyll contents under high light intensity, consistent with our findings.

Syzygium hancei seedlings adapt to habitat changes by adjusting internal physiological metabolism to maintain survival and growth. Comprehensive analysis across the three habitats revealed that Habitat B, with low canopy closure and adequate light intensity, showed highest leaf SOD activity, MDA content, and root POD activity. Habitat A, with high canopy closure and low photosynthetically active radiation, showed highest leaf POD activity, soluble sugar content, root SOD activity, and photosynthetic pigments. Habitat C showed highest leaf soluble sugar content. These results demonstrate that physiological and biochemical characteristics across the three habitats exhibited different ecological adaptations to varying light intensities. Photoinhibition occurred in Habitat B with low canopy closure. Based on the current status of natural regeneration in *S. hancei* seedling populations, future research should ensure adequate light conditions or implement manual canopy density adjustments to modify light availability, which would benefit *S. hancei* growth and enhance ecological restoration value in Guangxi's coastal zones.

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