Response of Leaf Soluble Protein and Antioxidant Enzyme Activities to Light Intensity in Seedlings of Eight Mangrove Species: Postprint

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Abstract

The restoration and reconstruction of mangrove wetland ecosystems constitute one of the key research areas in coastal ecological restoration in southern China. However, the artificial restoration and modification of mangrove communities first require attention to the spatial configuration of mangrove species, which necessitates clarifying the physiological and ecological strategies of light adaptation in mangrove plants to provide theoretical guidance for the optimized configuration of artificial mangrove communities. This study selected Sonneratia apetala, Kandelia candel, Bruguiera gymnorrhiza, Aegiceras corniculatum, Acanthus ilicifolius, Acrostichum aureum, Heritiera littoralis, and Hibiscus tiliaceus as research subjects, and through a shading control experiment, investigated the response characteristics of leaf soluble protein content and antioxidant enzyme activities in one-year-old seedlings of these eight mangrove species under different light intensities (100%, 45%, 30%, and 10% of natural light intensity). The results showed that: (1) With decreasing light intensity, the leaf soluble protein content in Bruguiera gymnorrhiza, Acanthus ilicifolius, and Acrostichum aureum was less affected, whereas that in Sonneratia apetala, Kandelia candel, Aegiceras corniculatum, Heritiera littoralis, and Hibiscus tiliaceus exhibited a declining trend. (2) The activities of antioxidant enzymes such as SOD and APX in Bruguiera gymnorrhiza, Acanthus ilicifolius, and Acrostichum aureum under 10% light intensity treatment showed no significant difference compared with the control, while the antioxidant enzyme activities in Sonneratia apetala, Kandelia candel, Aegiceras corniculatum, Heritiera littoralis, and Hibiscus tiliaceus showed an overall decreasing trend. These results indicate that, from the perspective of physiological adaptation to light, Bruguiera gymnorrhiza, Acanthus ilicifolius, and Acrostichum aureum possess certain shade tolerance and are suitable for planting in understory environments with relatively weak light conditions; Sonneratia apetala, Kandelia candel, Aegiceras corniculatum, Heritiera littoralis, and Hibiscus tiliaceus are suitable as upper- or mid-story tree species or for planting in understory environments with lower canopy density.

Full Text

Responses of Leaf Soluble Protein and Antioxidant Enzyme Activities to Light Intensity in Seedlings of Eight Mangrove Species

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Abstract: The restoration and reconstruction of mangrove wetland ecosystems represent a key research priority in coastal ecological restoration in southern China. Optimizing species assembly is critical for successful mangrove forest restoration and management, which requires understanding the physiological and ecological strategies of mangrove species in response to light conditions to provide theoretical guidance for community configuration. This study examined eight mangrove species—*Sonneratia apetala*, *Kandelia candel*, *Bruguiera gymnorrhiza*, *Aegiceras corniculatum*, *Acanthus ilicifolius*, *Acrostichum aureum*, *Heritiera littoralis*, and *Hibiscus tiliaceus*—using a shading control experiment to investigate responses of leaf soluble protein content and antioxidant enzyme activities under four light intensities (100%, 45%, 30%, and 10% of natural sunlight). The results showed: (1) Decreasing light intensity had minimal impact on leaf soluble protein content in *B. gymnorrhiza*, *A. ilicifolius*, and *A. aureum*, whereas the other five species exhibited declining trends. (2) The activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) in *B. gymnorrhiza*, *A. ilicifolius*, and *A. aureum* at 10% light intensity did not differ significantly from the control, while the remaining five species showed overall decreasing antioxidant enzyme activities. These findings indicate that *B. gymnorrhiza*, *A. ilicifolius*, and *A. aureum* possess shade tolerance and are suitable for understory planting in areas with weaker light conditions, whereas *S. apetala*, *K. candel*, *A. corniculatum*, *H. littoralis*, and *H. tiliaceus* are better suited as mid-canopy or upper-layer species or for planting in forests with lower canopy density.

Keywords: mangrove species, light intensity, antioxidant enzymes, soluble protein, physiological adaptation

Mangrove plants are trees, shrubs, or herbs that grow in tropical and subtropical coastal intertidal zones (Tansley & Fritsch, 1905; Lin, 1987). Known as "coastal guardians,"mangrove forests provide far greater protection against tsunamis and typhoons than engineered structures and deliver multiple ecosystem services (Dasgupta & Shaw, 2017). However, over the past century, intensive human activities such as overexploitation and aquaculture development have caused severe mangrove degradation worldwide (Krauss et al., 2014; Liao & Zhang, 2014; Meng et al., 2016; Lu et al., 2019).

As awareness of mangrove ecological value has deepened, mangrove wetlands have become a conservation priority for biodiversity and wetland protection globally, with increasing emphasis on ecological restoration and reconstruction research (Duke et al., 2007). In recent years, large-scale mangrove afforestation in coastal South China has utilized fast-growing species such as *Sonneratia apetala* for restoration. This tall, rapidly growing species reduces understory light levels, and since light is one of the most critical environmental factors affecting plant growth and survival, its widespread planting inevitably impacts the viability of native understory mangrove species. This necessitates further transformation and optimization of mangrove community structure. To provide theoretical guidance for optimal community configuration and rational stand improvement, we conducted systematic research on the growth, biomass allocation (Tan et al., 2020), and physiological-ecological characteristics of the exotic species *S. apetala* and seven native mangrove species under different light conditions.

Soluble protein content reflects normal plant metabolism. Under stress conditions, plants accumulate water-soluble compounds to protect cellular structures (Zhifang & Loescher, 2003). While the formation of these compounds indicates stress exposure, they also function as osmotic regulators that mitigate stress effects (Yu & Tang, 1999; Huang et al., 2014; Ding et al., 2017). Soluble proteins contain many important enzymes, including RuBP carboxylase, which contributes over 50% to photosynthesis, with other components serving metabolic functions in nitrogen metabolism (Pan, 2006). Consequently, many leaf soluble proteins are regulated by light signals, and their content reflects self-regulation capacity during plant development. Various environmental stresses (e.g., high light intensity, salinity, freezing, nutrient deficiency) can induce increased reactive oxygen species (ROS) concentrations, causing plant damage (McCord & Fridovich, 1969; Gu & Chen, 2006; Xie et al., 2008; Pubu et al., 2019). Through evolutionary processes, plants have developed enzymatic and non-enzymatic protection systems that scavenge ROS and mitigate or prevent cellular damage (Jiang et al., 1994). Among these, antioxidant enzymes have been extensively studied, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR), which work synergistically to remove excess ROS and maintain metabolic balance, thereby enabling plants to resist stress damage (Liang et al., 2003; Chen et al., 2021).

Mangrove plants exhibit varying antioxidant enzyme activities under different habitat conditions. Studies have shown that *Kandelia candel* leaf SOD activity is significantly higher under full light than in forest understory, indicating greater stress under high light conditions (Ye et al., 2001; Diao et al., 2009). High salinity stress causes SOD, POD, and CAT activities in *K. candel* seedlings to initially increase then decrease (Xing et al., 2018), and can increase H_2O_2 content while elevating APX, GR, and SOD activities but significantly decreasing CAT activity in *Bruguiera parviflora* leaves (Zheng & Lin, 1998; Parida et al., 2004). Research by Su et al. (2021) demonstrated that POD activity in mangrove tissues increases during flooding, while CAT, SOD, and APX activities show initial increases followed by decreases, indicating that waterlogging disrupts ROS production-scavenging balance. Previous studies have primarily focused on mangrove responses to high salinity, hypoxia, and other stresses, while systematic research on how light intensity affects mangrove leaf antioxidant enzyme systems remains limited. Therefore, this study selected eight common mangrove species from the Pearl River Delta region, including six true mangroves (*S. apetala*, *K. candel*, *B. gymnorrhiza*, *A. corniculatum*, *A. ilicifolius*, and *A. aureum*) and two semi-mangroves (*H. littoralis* and *H. tiliaceus*). Through comparative analysis of leaf soluble protein content and antioxidant enzyme activities under different light conditions, we explored interspecific differences in physiological-ecological strategies for light adaptation to provide theoretical guidance for optimizing artificial mangrove community configuration and stand improvement, offering scientific references for mangrove conservation and restoration practices.

1.1 Study Site and Seed Source

The shading experiment was conducted from October 2013 to October 2014 at the Comprehensive Experimental Ecology Station of South China Botanical Garden, Chinese Academy of Sciences, in Guangzhou, Guangdong Province (23°10 42.79 N, 113°21 25.28 E, altitude 40 m). The region has a south subtropical maritime monsoon climate with an annual mean temperature of 20–22°C, average relative humidity of 77%, and mean annual precipitation of 1,982.7 mm.

Mangrove seedlings were purchased from a nursery near the Qi’ ao Mangrove Nature Reserve in Zhuhai, Guangdong. The reserve is located at 113°36 40 –113°39 15 E, 22°23 40 –22°27 38 N, with a south subtropical maritime monsoon climate (Liao et al., 2008). The area has a mean annual temperature of 22.2°C, essentially frost-free conditions, and annual rainfall of 1,875.7 mm, with 84% occurring from April to October (Liao et al., 2006). Influenced by rainfall, river runoff, and tides, seawater salinity ranges from 3.31‰ to 7.05‰, and the tidal regime is irregular semidiurnal (Cai et al., 2016).

In August 2013, one-year-old seedlings of eight mangrove species with uniform growth were selected from the nursery near Qi’ ao Island Mangrove Nature Reserve. *Sonneratia apetala* is an exotic species native to Bangladesh that shows strong ecological adaptability to China’ s coastal environment and has been extensively planted on Qi’ ao Island. The other seven species are native, including five true mangroves (*K. candel*, *B. gymnorrhiza*, *A. corniculatum*, *A. ilicifolius*, and *A. aureum*) and two semi-mangroves (*H. littoralis* and *H. tiliaceus*). Initial growth parameters of the eight species are shown in Table 1 .

Table 1 Initial values of seedling growth parameters of eight mangrove species tested in the present study (Mean±SE, n=5) (adapted from Tan et al., 2020)

Species (Abbreviation)	Family	Latin Name	Plant Height (cm)	Basal Diameter (mm)
Sonneratia apetala (Sa)	Sonneratiaceae	*Sonneratia apetala*	111.1\$±7.6|13.58±1.1||∗ *Kandeliacandel*∗ (*Kc*)|*Rhizophoraceae*|∗ *Kandeliacandel*∗ |74.4±8.6|18.69±1.36||∗ *Bruguieragymnorrhiza*∗ (*Bg*)|*Rhizophoraceae*|∗ *Bruguieragymnorrhiza*∗ |35.8±2.4|13.65±0.37||∗ *Aegicerascorniculatum*∗ (*Ac*)|*Myrsinaceae*|∗ *Aegicerascorniculatum*∗ |82.7±1.4|14.21±1.04||∗ *Acanthusilicifolius*∗ (*Ai*)|*Acanthaceae*|∗ *Acanthusilicifolius*∗ |58.1±2.0|21.36±1.33||∗ *Acrostichumaureum*∗ (*Aa*)|*Acrostichaceae*|∗ *Acrostichumaureum*∗ |43.3±2.8|11.93±1.32||∗ *Heritieralittoralis*∗ (*Hl*)|*Sterculiaceae*|∗ *Heritieralittoralis*∗ |103.3±14.7|15.2±2.8||∗ *Hibiscustiliaceus*∗ (*Ht*)|*Malvaceae*|∗ *Hibiscustiliaceus*∗ |57.6±6.9|18.52±\$2.34	

Note: T. True mangrove; S. Semi-mangrove.

1.3 Experimental Design

Four light intensity gradients were established: 100% (T0, control), 45% (T1), 30% (T2), and 10% (T3) of natural sunlight (Huang & Zhan, 2003). Different light transmittance levels were achieved using black nylon nets of varying density. During the initial shading period on clear days, photosynthetic photon flux density was measured at 9:00, 12:00, and 15:00 daily for three consecutive days using a Li-250A light meter (LI-COR, Inc, USA). The measured relative light intensities for the four treatments are shown in Table 2 .

Table 2 Relative light intensity measured in the four light treatments (adapted from Tan et al., 2020)

Treatment	9:00	12:00	15:00
T0 (100%)	100%	100%	100%
T1 (45%)	45%	45%	45%
T2 (30%)	30%	30%	30%
T3 (10%)	10%	10%	10%

Labeled seedlings were placed in the four light treatment zones, with 10–12 seedlings per species per treatment. Seedlings were planted individually in 13.4 L pots (30 cm top diameter, 21 cm bottom diameter, 26 cm height) filled with sea mud from Qi'ao Island (salinity 8.3%, water content 42.9%, total nitrogen 1.61%, organic carbon 1.85%). Pots were placed in plastic trays (27.5 cm diameter, 10.5 cm height) containing artificial seawater to simulate the average salinity (6‰) of nearshore waters at Qi' ao Island. Salinity was monitored throughout the experiment, and artificial seawater was replenished as needed. The shading treatment lasted for one year.

1.4 Determination of Leaf Soluble Protein Content and Antioxidant Enzyme Activities

After one year of shading treatment, five seedlings per species were selected from each treatment. Six to ten fresh mature leaves were randomly collected from each seedling for determination of soluble protein content and antioxidant enzyme activities.

Fresh leaves (approximately 0.3 g) were weighed using an FA1104 electronic balance (precision 0.0001 g) and ground in 1.8 mL ice-cold extraction buffer (0.05 $\text{mol} \cdot \text{L}^{-1}$ phosphate buffer pH 7.8, 1 $\text{mmol} \cdot \text{L}^{-1}$ EDTA-Na_2, 1% PVP) in an ice bath. The homogenate was centrifuged at 16,000×g for 20 min at 4°C, and the supernatant was used for soluble protein and enzyme activity assays. Soluble protein content was determined using the Coomassie brilliant blue staining method (Bradford, 1976) with bovine serum albumin as the standard.

Superoxide dismutase (SOD) activity was measured according to Giannopolitis & Ries (1977), with 0.05 $\text{mol} \cdot \text{L}^{-1}$ phosphate buffer pH 7.8 replacing the enzyme solution as a blank. One enzyme activity unit (U) was defined as the amount inhibiting nitroblue tetrazolium photoreduction by 50%, expressed as $\text{U} \cdot \text{g}^{-1}$ fresh weight (FW). Catalase (CAT) activity was determined following Chance &

Maehly (1955), with one U defined as the amount causing a 0.01 change in OD_{240} per minute, expressed as $U \cdot g^{-1}$ $FW \cdot min^{-1}$. Peroxidase (POD) activity was measured according to Chen & Wang (1989), with one U defined as the amount causing a 0.01 change in OD_{470} per minute, expressed as $U \cdot g^{-1}$ $FW \cdot min^{-1}$. Ascorbate peroxidase (APX) activity was assayed following Nakano & Asada (1981), with one U defined as the amount oxidizing 1 mol AsA per minute, expressed as mol $AsA \cdot g^{-1}$ $FW \cdot min^{-1}$. Glutathione reductase (GR) activity was determined according to Foyer & Halliwell (1976), with one U defined as the change in OD_{340} over 3 minutes, expressed as $U \cdot g^{-1}$ $FW \cdot min^{-1}$.

1.5 Data Analysis

All data were processed using Microsoft Excel 2013 to calculate means and standard errors. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA), with $P < 0.05$ considered statistically significant. Differences in soluble protein content and antioxidant enzyme activities among light treatments were tested using one-way ANOVA for each species. When significant differences were detected, Tukey post hoc comparisons were conducted.

2.1 Soluble Protein Content

The effect of light intensity on leaf soluble protein content varied among mangrove species (Table 3). Shading significantly affected soluble protein content in *S. apetala*, *K. candel*, *A. corniculatum*, *A. ilicifolius*, *H. tiliaceus*, and *H. littoralis* seedlings, but not in *B. gymnorrhiza* and *A. aureum* seedlings. Under shading treatments, soluble protein content in leaves of *S. apetala*, *K. candel*, *A. corniculatum*, and *A. ilicifolius* seedlings was significantly lower than in the control, with no significant differences among low-light treatments. *Heritiera littoralis* and *H. tiliaceus* showed no significant differences among 100%, 45%, and 30% light treatments, but soluble protein content decreased significantly at 10% light intensity (Figure 1 [Figure 1: see original paper]).

Table 3 Results from one-way ANOVA (F value) for light treatment effects on leaf total soluble protein content and SOD, CAT, POD, APX, and GR activities for seedlings of the eight mangrove species

Species	Soluble Protein Content	SOD Activity	CAT Activity	POD Activity	APX Activity	GR Activity
Sa	49.21***	7.57**	23.17***	91.26***	204.04***	171.72***
Kc	11.24***	2.50ns	53.32***	50.78***	38.34***	48.04***
Bg	1.47ns	6.21*	27.59***	18.09***	24.76***	22.31***
Ac	20.02***	1.53ns	25.84***	15.20***	11.84***	163.68***
Ai	8.11**	13.42***	17.87***	21.28***	18.46***	30.26***
Aa	2.77ns	1.92ns	27.08***	3.87*	5.15*	31.41***
Hl	7.24**	4.63*	36.14***	5.88**	19.22***	1.31ns

Species	Soluble Protein Content	SOD Activity	CAT Activity	POD Activity	APX Activity	GR Activity
Ht	6.03**	8.42**	30.12***	29.15***	55.60***	1.31ns

Note: Species abbreviations are shown in Table 1. Significance levels: ns indicates $P > 0.05$, * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$.

Figure 1 Soluble protein contents in leaves of seedlings of the eight mangrove species under different light treatments. Different letters for each species indicate significant differences between light treatments ($P < 0.05$); ns indicates no significant differences ($P > 0.05$). Species abbreviations are shown in Table 1. The same below.

2.2 Antioxidant Enzyme Activities

One-way ANOVA results (Table 3) showed that shading significantly affected leaf SOD activity in three true mangrove species (*S. apetala*, *B. gymnorrhiza*, and *A. ilicifolius*) and two semi-mangrove species (*H. littoralis* and *H. tiliaceus*), but not in *K. candel*, *A. corniculatum*, and *A. aureum*. As shown in Figure 2 [Figure 2: see original paper], leaf SOD activity in seedlings exhibited an initial increase followed by a decrease with declining light intensity. *Sonneratia apetala* and *A. ilicifolius* showed no significant differences in SOD activity among 100%, 45%, and 30% light treatments, but activity decreased significantly at 10% light intensity. *Bruguiera gymnorrhiza*, *H. tiliaceus*, and *H. littoralis* had higher SOD activity under 45% and 30% light treatments, significantly exceeding that under 100% and 10% light treatments.

Shading significantly affected leaf CAT and POD activities in all eight mangrove species. Catalase activity in *S. apetala*, *A. ilicifolius*, and *H. littoralis* decreased significantly with declining light intensity. In contrast, CAT activity in *K. candel*, *B. gymnorrhiza*, *A. corniculatum*, and *A. aureum* showed an initial increase followed by a decrease; except for *A. corniculatum*, these species exhibited significantly higher CAT activity under 45% light than other treatments. *Hibiscus tiliaceus* showed significantly higher CAT activity under shading treatments compared to the control, with minimal differences among the three shading levels (Figure 2 [Figure 2: see original paper]).

Peroxidase activity in *S. apetala*, *K. candel*, *B. gymnorrhiza*, *A. corniculatum*, *H. littoralis*, and *H. tiliaceus* increased significantly with decreasing light intensity, reaching maximum values at 10% light and significantly exceeding other treatments. In contrast, POD activity in *A. ilicifolius* and *A. aureum* decreased significantly under low-light treatments (30% and 10% light) compared to the control. Substantial interspecific differences in leaf POD activity were observed among the eight mangrove species, with true mangroves (*S. apetala*, *B. gymnorrhiza*, and *A. corniculatum*) showing significantly lower POD activity than semi-mangroves (*H. littoralis* and *H. tiliaceus*). Semi-mangrove leaf POD activity ranged approximately 426.3–1,643.6 $\mathrm{U \cdot g^{-1}\ FW \cdot min^{-1}}$, while true mangrove activity ranged 1.4–724.0 $\mathrm{U \cdot g^{-1}\ FW \cdot min^{-1}}$.

Leaf APX and GR activities under different light conditions are shown in Figure 3 [Figure 3: see original paper]. One-way ANOVA results (Table 3) indicated that shading significantly affected APX activity in all eight mangrove species. Ascorbate peroxidase activity in *S. apetala* and *A. corniculatum* decreased significantly with declining light intensity, being significantly lower than the control under low-light treatments. *Kandelia candel*, *B. gymnorrhiza*, *A. ilicifolius*, *A. aureum*, and *H. littoralis* showed maximum APX activity under 45% light, while *H. tiliaceus* had maximum activity at 30% light and minimum at 10% light. Shading significantly affected GR activity in *S. apetala*, *K. candel*, *B. gymnorrhiza*, *A. corniculatum*, *A. ilicifolius*, *A. aureum*, and *H. littoralis*, but not in *H. tiliaceus*. Glutathione reductase activity in *S. apetala*, *K. candel*, *B. gymnorrhiza*, *A. corniculatum*, *A. aureum*, and *H. littoralis* decreased significantly with declining light intensity, though no significant differences existed between low-light treatments (30% and 10% light). *Acanthus ilicifolius* showed substantial GR activity reduction under shading, with significant differences among all four light treatments.

Figure 2 Activities of SOD, CAT, and POD in leaves of seedlings of the eight mangrove species under different light treatments.

Figure 3 APX and GR activities in leaves of seedlings of the eight mangrove species under different light treatments.

3.1 Soluble Protein Content

Previous studies have shown that reduced soluble protein content indicates stress damage in plants (Huang et al., 2014; Li et al., 2019). Insufficient light decreases soluble protein content and accelerates plant senescence (Zhao et al., 2022), whereas shade-tolerant plants can maintain soluble protein content under shading (Deng et al., 2012). This study found that leaf soluble protein content in *B. gymnorrhiza* and *A. aureum* seedlings did not differ significantly between shading treatments and the control, indicating effective regulation of leaf soluble protein content under varying light intensities to mitigate low-light effects (Annicchiarico et al., 2013). *Acanthus ilicifolius*, a major naturally regenerated species in Qi' ao Island mangroves, maintained high soluble protein content even at 10% light intensity, demonstrating strong shade tolerance and suitability for understory planting. In contrast, *S. apetala*, *H. littoralis*, and *H. tiliaceus* could not effectively regulate soluble protein content to alleviate low-light stress, making them unsuitable for planting in high-canopy-density mangrove communities.

3.2 Antioxidant Enzyme Activities

Among the eight mangrove species studied, SOD activity showed an initial increase followed by a decrease with declining light intensity, consistent with patterns observed in mangroves under stress (Li et al., 2014; Wang et al., 2014; Zhao et al., 2014; Su et al., 2021). This indicates that mangrove seedlings' capacity to scavenge ROS decreased under shading, limiting their growth (Tan et al., 2020). Zhao et al. (2022) similarly found that *Bhesa robusta*, an important coastal transitional zone species, exhibited reduced antioxidant enzyme activity under chronic light deficiency, resulting in growth inhibition.

Peroxidase catalyzes H_2O_2 decomposition, preventing cellular toxicity from peroxide accumulation. In this study, six species (*S. apetala*, *K. candel*, *B. gymnorrhiza*, *A. corniculatum*, *H. littoralis*, and *H. tiliaceus*) showed increased POD activity under shading, suggesting they could upregulate POD to alleviate H_2O_2 accumulation caused by low-light stress (Zhang et al., 2021). Conversely, *A. ilicifolius* and *A. aureum*, as shade-tolerant species, experienced high light as a stress condition, exhibiting higher POD activity under strong light. Elevated POD activity under high-light stress can mitigate oxidative damage (Sano et al., 2020). The two semi-mangrove species in this study showed substantially higher POD activity than true mangroves, possibly reflecting physiological differences between these groups (Huang et al., 2020).

Catalase decomposes H_2O_2 into water and oxygen, protecting plants from H_2O_2 toxicity (Wang et al., 2017; Aires et al., 2021). Low-light stress typically inhibits CAT synthesis, causing activity decline (Zhao et al., 2022). This study found that CAT activity in *S. apetala*, *A. ilicifolius*, and *H. littoralis* decreased with light intensity, while *K. candel*, *B. gymnorrhiza*, *A. corniculatum*, and *A. aureum* showed initial increases followed by decreases, indicating low-light stress reduced H_2O_2 conversion capacity (Wang et al., 2017). The divergent trends between CAT and POD activities align with previous findings (Liang et al., 2020; Aires et al., 2021), possibly because POD participates not only in H_2O_2 detoxification but also in lactate and ethanol detoxification (Su et al., 2021), leading to differential responses to shading. Aires et al. (2021) suggested that plants selectively activate either POD or CAT under different light intensities to prevent membrane lipid peroxidation, generally preferring the more efficient CAT (Hasanuzzaman et al., 2018). The suppression of CAT activity under shading in this study suggests that elevated POD activity may be an important pathway for protecting cell membranes from H_2O_2 damage in mangroves.

Studies have shown that the ascorbate-dependent H_2O_2 scavenging pathway typically increases under low-light stress (Shou et al., 2000; Chen et al., 2013). Ascorbate peroxidase and GR, the primary enzymes in this pathway, are mainly localized in chloroplasts (Shou et al., 2000). Under low-light stress, expression of antioxidant enzyme genes in chloroplasts is activated, increasing enzyme content to avoid potential oxidative damage from insufficient light (Wang et al., 2020; Aires et al., 2021). In this study, APX and GR showed different trends:

APX generally increased initially then decreased with declining light intensity, while GR showed a decreasing trend, suggesting that excessive H_2O_2 generation in chloroplasts under shading exceeded the scavenging capacity, damaging the enzyme system and reducing stress resistance. Wang et al. (2020) also reported that when light intensity exceeds certain thresholds, antioxidant enzyme gene expression declines, potentially causing membrane lipid peroxidation damage.

Antioxidant enzymes are primary agents for alleviating oxidative damage from environmental stress, with the antioxidant system being rapidly activated under stress (Deng et al., 2012; Wang et al., 2017; Sano et al., 2020). However, under chronic photosynthetic radiation deficiency, SOD, POD, and APX activities decrease, reducing ROS scavenging capacity, obstructing metabolism, and ultimately inhibiting growth (He et al., 2021; Zhang et al., 2021; Zhao et al., 2022). In this study, overall trends in antioxidant enzyme activities under shading varied among species, reflecting different physiological-ecological strategies for light adaptation. *Bruguiera gymnorrhiza*, *A. ilicifolius*, and *A. aureum* maintained SOD and APX activities at 10% light intensity comparable to the control, indicating normal antioxidant system function under severe shading. In contrast, *S. apetala*, *K. candel*, *A. corniculatum*, *H. littoralis*, and *H. tiliaceus* showed overall declining antioxidant enzyme activities, suggesting their antioxidant systems could not adapt appropriately, with impaired function and balance, leading to poor growth status. Tan et al. (2020) found that *S. apetala* survival and height growth decreased significantly under shading, with biomass reduction in *S. apetala*, *K. candel*, and *H. tiliaceus*, while *A. ilicifolius* biomass and height growth increased significantly. Combined with our results, soluble protein content and antioxidant enzyme activities can characterize plant growth status to some extent. We infer that *B. gymnorrhiza*, *A. ilicifolius*, and *A. aureum* are physiologically adaptable to different light intensities and suitable as understory species for mangrove community transformation, whereas *S. apetala*, *K. candel*, *A. corniculatum*, *H. littoralis*, and *H. tiliaceus* are better suited as mid- or upper-layer species or for planting in low-canopy-density forests.

4 Conclusion

This study demonstrates that *B. gymnorrhiza*, *A. ilicifolius*, and *A. aureum* exhibit adaptability in soluble protein content to different light intensities, making them candidate species for understory cultivation in mangrove regeneration. In contrast, *S. apetala*, *K. candel*, *A. corniculatum*, *H. littoralis*, and *H. tiliaceus* showed stress responses in soluble protein content under shading, indicating their suitability for low-canopy-density understory or mid-upper layer positions. Regarding SOD, POD, and APX activities, when light intensity decreased below 30%, the capacity of *S. apetala*, *K. candel*, *A. corniculatum*, *H. littoralis*, and *H. tiliaceus* to alleviate ROS toxicity through antioxidant enzymes declined, with plants showing stress damage, confirming their unsuitability for high-canopy-density understory planting. Therefore, scientific selection of native mangrove species for stand improvement in high-canopy-density *S. apetala* communities requires careful consideration of differential adaptive responses in growth status and physiological-ecological characteristics under varying light conditions to enrich biodiversity and enhance ecosystem service functions.

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