Biological Characteristics of Drought Resistance in Coastal Tung Tree (Postprint)

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Date: 2020-09-15T00:00:00+00:00

Abstract

The soils of the Nansha Islands are predominantly composed of coral sand. Due to environmental conditions such as intense light, poor soil moisture, and nutrient deficiency, few plants can grow normally on the islands. To rapidly restore vegetation on the Nansha Islands, it is necessary to screen for plants with strong drought resistance and introduce them. Guettarda speciosa is a typical tropical coastal plant that plays an important role in windbreak and sand fixation, as well as in vegetation ecological restoration on islands and coastal zones. This study utilized Guettarda speciosa under natural conditions on Yongxing Island in the Xisha Islands as research material to investigate its morphological and anatomical characteristics, antioxidant capacity, stress-resistant substance content, and nutrient element content. The results demonstrated that: as drought severity increased, structural parameters such as the ratio of palisade tissue to spongy tissue and leaf thickness increased, which could effectively reduce water loss within the plant and indicated strong water-saving capacity; the activities of superoxide dismutase and catalase in the plant increased rapidly, and proline content also continuously increased, proving that Guettarda speciosa possesses a strong regulatory capacity to resist drought; its root nutrient content was low but leaf nutrient content was high, and the total chlorophyll content decreased slowly, indicating that Guettarda speciosa can efficiently utilize nutrients and suffers less damage from drought stress. Overall, Guettarda speciosa exhibits strong drought resistance and can grow normally in environments with low soil moisture, making it suitable for introduction to the Nansha Islands.

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Full Text

Biological Characteristics of Drought Resistance in *Guettarda speciosa*

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Abstract: The soil of the Spratly Islands is primarily composed of coral sand. Due to environmental conditions such as intense sunlight, poor soil moisture, and nutrient deficiency, few plants can grow normally on these islands. To rapidly restore vegetation on the Spratly Islands, it is necessary to select and introduce drought-resistant plants. *Guettarda speciosa* is a typical tropical coastal plant that plays an important role in windbreak and sand fixation on islands and coastal zones, as well as in vegetation ecological restoration. This study investigated the morphological and anatomical characteristics, antioxidant capacity, stress-resistant substance content, and nutrient element content of *G. speciosa* under the natural environment of Yongxing Island in the Xisha Islands. The results showed that as drought intensity increased, structural features such as the ratio of palisade tissue to spongy tissue and leaf thickness increased, effectively reducing water loss within the plant and demonstrating strong water-saving capacity. The activities of superoxide dismutase and catalase in the plant increased rapidly, and proline content continued to rise, proving that *G. speciosa* possesses strong regulatory capacity to resist drought. Although nutrient content in the roots was low, it was high in the leaves, and the rate of total chlorophyll content reduction was slow, indicating that *G. speciosa* can efficiently utilize nutrients and suffers minimal damage from drought stress. Overall, *G. speciosa* demonstrates strong drought resistance, can grow normally in environments with limited soil moisture, and can be introduced to the Spratly Islands.

Key words: *Guettarda speciosa*, drought resistance, morphological and anatomical characteristics, stress-resistant substances, nutritional elements

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Introduction

The Spratly Islands are located at the southernmost tip of China and hold important strategic significance (Liu et al., 2015). The islands have a tropical monsoon climate, characterized by year-round heat and perpetual summer. The annual average temperature is 27–28°C, with annual rainfall of 1,500–2,200 mm.

The rainy season extends from June to November, while the dry season lasts from December to May of the following year. The island soil is primarily coral sand, which has poor water retention and lacks true soil structure and fertility. The islands experience strong sea winds, high salinity (including salt spray in the air), high soil pH, intense sunlight, strong evaporation, and particularly severe drought during the dry season. Under these habitat conditions, only drought- and salt-tolerant plant species can successfully colonize and grow.

Guettarda speciosa, belonging to the Rubiaceae family, is an evergreen small tree, 3–5 m tall, occasionally reaching 8 m (Fig. 1 [Figure 1: see original paper]). It has black, smooth bark; robust branchlets in opposite arrangement with conspicuous lenticels and shedding pubescence. Leaves are opposite, thinly papery, broadly obovate or broadly elliptic, with acute, obtuse or rounded apices and gradually tapering bases. Cymes are often borne in leaf axils of fallen leaves, with short, widely spreading, dichotomous branches densely covered with pubescence; the calyx tube is cup-shaped, 2–2.5 mm long, with a tubular calyx limb that is truncate; the corolla is white, 3.5–4 cm long when fully open, with a narrow tube and 7–8 lobes at the apex, the lobes are obovate, approximately 1 cm long, with acute apices; filaments are extremely short. The drupe is hairy when young, flattened globose, 2–3 cm in diameter, with a fibrous mesocarp; seeds are small and curved. The flowering period is April to July. It is native to Hainan and Taiwan, distributed along tropical coasts, and grows on the edges of thickets in coastal sandy areas. This species is one of the littoral tidal zone tree species, commonly growing along tropical coasts, particularly densely on the eastern and western coasts of the Malay Peninsula (Flora of China, 1990). *G. speciosa* has an attractive tree form and is an excellent greening plant (Ren et al., 2017). Its wood is of good quality, with strong wind resistance, appropriate tree height, and as a native species, it can serve as an excellent coastal windbreak forest.

Currently, research has been conducted on coastal zone-adapted plants and their biological characteristics (Wang and Ren, 2017). For example, studies have investigated the drought resistance biological characteristics of tropical coastal plants such as *Pisonia grandis* (Wang et al., 2017), *Scaevola sericea* (Xu et al., 2018), and *Calophyllum inophyllum* (Zhang et al., 2019), analyzing changes in morphological structure and physiological-biochemical characteristics of these plants under drought stress and proposing relatively comprehensive methods for evaluating plant stress resistance and adaptability. *Guettarda speciosa* is an important component of the vegetation community on the Xisha Islands, yet domestic research on its drought resistance biological characteristics and its ability to adapt to tropical coral sand environments is lacking, as are scientific data for its protection, development, and utilization. This study used wild *G. speciosa* from Yongxing Island as experimental material, subjecting it to light, moderate, and severe drought stress through controlled experiments. We measured and analyzed the ecological and biological characteristics, including morphological and anatomical features, antioxidant capacity, stress-resistant substance content, and nutrient element content under different conditions, aiming to provide fundamental data and references for plant introduction in vegetation construction and restoration on the Spratly Islands.

Materials and Methods

1.1 Materials and Study Site Overview

Guettarda speciosa materials were collected from Yongxing Island in the Xisha Islands. Yongxing Island has a tropical oceanic monsoon climate, with an average annual precipitation of 1,382 mm, concentrated mainly from May to September, showing distinct wet and dry season differences. The annual average temperature is 26.3°C, with high annual solar radiation and long sunshine hours, high temperatures year-round, and strong evaporation. The soil substrate is phosphatic limestone soil and coastal saline soil formed from coral and shell debris under long-term bird guano deposition (Li et al., 2004). Yongxing Island originally had good vegetation with over 300 plant species. In recent years, due to human activity disturbance, the area of natural vegetation has significantly decreased. Common wild plant species include *Scaevola sericea*, *Argusia argentea*, *Guettarda speciosa*, *Pisonia grandis*, *Cordia subcordata*, *Ipomoea pescaprae*, *Phyla nodiflora*, *Wedelia biflora*, among others. On Yongxing Island and Dong Island, which have better vegetation in the Xisha Islands, *G. speciosa* communities are widely distributed, either as monodominant stands or mixed with *Cordia subcordata*, *Pisonia grandis*, and other species.

In April 2017, robust *G. speciosa* plants approximately 80 cm tall were selected from their native habitat on Yongxing Island, Xisha, and transported to the experimental greenhouse at the South China Botanical Garden, Chinese Academy of Sciences in Guangzhou. They were cultivated in 30 cm × 25 cm pots with a substrate mixture of coral sand:red soil:coconut coir:peat soil:organic fertilizer at a ratio of 8:1:0.5:0.3:0.2. Twenty-four *G. speciosa* plants with relatively uniform growth were selected for the simulated drought control experiment.

Before the experiment began, the 24 potted *G. speciosa* seedlings were randomly divided into four groups of six pots each. All plants were maintained under identical conditions with watering for seven days to keep soil water content between 45%–55%. Three groups were subjected to natural drought. Drought experiments commenced when soil water content reached the ranges of 35%–45% (light drought), 25%–35% (moderate drought), and 15%–25% (severe drought), and the drought duration was calculated for each group. On days 0, 1, 6, 9, 13, and 18, healthy mature leaves and branches were randomly collected from each group and brought back to the laboratory for measurement of various indicators and data analysis. During the drought experiment, soil water content in each group was measured daily using a moisture meter, and 50–200 mL of water was added based on the moisture data to maintain soil water content within the required experimental ranges. Additionally, plant positions were randomly swapped regularly within each group to ensure consistent growth conditions except for water availability, thereby minimizing experimental error.

1.2.1 Morphological and Anatomical Characteristics

Morphological and anatomical characteristics were analyzed through leaf structure dissection. Leaf sections were prepared using conventional methods (Liang et al., 2007), and leaf thickness, upper and lower epidermis thickness, and palisade and spongy tissue thickness were observed under a microscope.

1.2.2 Antioxidant Enzyme Activity and Stress-Resistant Substance Content Determination

Superoxide dismutase (SOD) activity was measured using the nitroblue tetrazolium photoreduction method (Li et al., 2000). Catalase (CAT) activity was determined using the ultraviolet absorption method (Zou, 2000). Peroxidase (POD) activity was measured using the guaiacol method (Amalo et al., 1994). Proline (Pro) content was determined using the acidic ninhydrin staining method (Li et al., 2000). Malondialdehyde (MDA) content was measured according to the method of Zhang (1992).

1.2.3 Leaf Chlorophyll Content and Root, Stem, and Leaf Nutrient Element Determination

Leaf chlorophyll content was determined using the acetone extraction colorimetric method (Gao, 2006). Root, stem, and leaf samples collected from the field were oven-dried at 65°C, then ground and sieved. Nitrogen content was measured using the indophenol blue colorimetric method, phosphorus content using the molybdenum-antimony anti-colorimetric method, and potassium content using flame photometry (Cornelissen et al., 2003).

1.2.4 Data Analysis

Experimental data were processed using Excel 2018 software. One-way analysis of variance (ANOVA) was used to analyze the effects of stress treatments on antioxidant enzyme activities, osmotic adjustment substances (proline), malondialdehyde, and other substances. Duncan' s test was used to compare differences among different drought stress levels, and graphs were generated using GraphPad Prism 8.0.2.

Results

2.1 Morphological and Anatomical Characteristics

The morphological and anatomical characteristics of *G. speciosa* are shown in Table 1 and Fig. 2 [Figure 2: see original paper]. As shown in Table 1, the ratio of palisade tissue to spongy tissue increased with drought intensity, ranging from 0.555-0.595 at soil water content of 25%-55%, and significantly increasing to 0.718 at 15%-25% water content. Fig. 2 shows that spongy tissue cells in the mesophyll were loosely arranged with large intercellular spaces and large vacuoles. Under normal water conditions, leaf thickness, upper epidermis thickness, and palisade tissue thickness were 201.658 m, 25.379 m, and 56.388 m, respectively. As drought intensity increased, these values increased to 263.362 m, 40.430 m, and 78.884 m, respectively. In contrast, lower epidermis thickness, spongy tissue thickness, and palisade tissue width showed decreasing trends, reducing to 56.7% (from 32.494 m to 18.422 m), 84.3% (from 115.040 m to 96.969 m), and 38.5% (from 20.120 m to 7.741 m) of normal water condition values, respectively.

Table 1 Morphological and anatomical characteristics of *Guettarda speciosa* under drought stress

Index	正常 CK	Low drought	Medium drought	High drought
Leaf thickness (m)	201.658$±26.207$b$\|220.092±20.478ab\|246.724±6.856ab\|263.362±28.642a\|\|Epicuticlethickness(\mu m)\|2$			

Note: Different letters indicate significant differences between groups (P<0.05). The same below.

Fig. 1 Whole plant of *Guettarda speciosa*

Fig. 2 Anatomical structure of *Guettarda speciosa* leaves

2.2 Antioxidant Enzyme Activity and Stress-Resistant Substance Content

The antioxidant enzyme activities and stress-resistant substance contents in *G. speciosa* leaves are shown in Fig. 3 [Figure 3: see original paper]. Under normal water conditions, SOD activity, CAT activity, POD activity, MDA content, and PRO content were 125.531 $U \cdot g^{-1}$, 105.148 $U \cdot g^{-1}$, 25.161 $U \cdot g^{-1}$, 116.584 $nmol \cdot g^{-1}$, and 26.975 $nmol \cdot g^{-1}$, respectively. As drought stress intensified, SOD, CAT, and POD activities all increased, while MDA and PRO contents also increased, reaching 152.257 $U \cdot g^{-1}$, 168.099 $U \cdot g^{-1}$, 32.252 $U \cdot g^{-1}$, 199.251 $nmol \cdot g^{-1}$, and 43.354 $nmol \cdot g^{-1}$, respectively, at 15%-25% water content. Following the onset of drought stress, by day 6, CAT activity, MDA content, and PRO content showed significant increases. As drought intensity increased further, by day 18, CAT activity and PRO content had continuously increased to 159.9% and 160.7% of normal water condition values, respectively, while MDA content showed a decreasing trend. Throughout the drought stress process, SOD and POD activities continued to increase but with smaller magnitudes, reaching only

121.3% and 128.2% of normal water condition values, respectively. During the drought stress period, MDA content, PRO content, and SOD, CAT, and POD activities showed no significant differences from the control under light drought stress, but significant differences emerged under severe drought stress.

Fig. 3 Effects of drought stress on leaf MDA and Pro contents and the activities of SOD, POD and CAT enzymes

2.3 Root, Stem, and Leaf Nutrient Content and Leaf Chlorophyll Content

The nutrient contents in roots, stems, and leaves of *G. speciosa* are shown in Table 2 . As shown in Table 2, nitrogen, phosphorus, and potassium contents in roots were relatively low but showed an increasing trend with drought intensity. Total nitrogen, total phosphorus, and total potassium contents reached maximum values of 11.83, 1.089, and 9.804 $g \cdot kg^{-1}$, respectively, with N/P ratio, K/P ratio, and K/N ratio ranging from 7.907-12.575, 0.096-0.135, and 0.940-1.375, respectively. Nitrogen, phosphorus, and potassium contents in stems were higher than in roots, with maximum total nitrogen, total phosphorus, and total potassium contents reaching 13.942, 1.986, and 10.998 $g \cdot kg^{-1}$, respectively, and N/P ratio, K/P ratio, and K/N ratio ranging from 5.818-10.073, 0.126-0.187, and 1.068-1.378, respectively. Nitrogen, phosphorus, and potassium contents in leaves were significantly higher than in roots and stems, with maximum total nitrogen, total phosphorus, and total potassium contents reaching 15.631, 3.007, and 24.477 $g \cdot kg^{-1}$, respectively, and N/P ratio, K/P ratio, and K/N ratio ranging from 4.846-10.460, 0.056-0.181, and 0.583-0.876, respectively.

The leaf chlorophyll contents of *G. speciosa* are shown in Table 3 . As shown in Table 3, under normal water conditions, leaf chlorophyll a, chlorophyll b, and total chlorophyll contents were 1.668, 0.709, and 2.317 $mg \cdot g^{-1}$, respectively. As drought stress progressed, leaf chlorophyll a, chlorophyll b, and total chlorophyll contents all decreased, reducing to 1.467, 0.526, and 1.831 $mg \cdot g^{-1}$, respectively. The chlorophyll a/b ratio ranged from 2.389-2.862, showing a trend of initial increase followed by a slight decrease.

Table 2 Nutrient contents in *Guettarda speciosa*

Treatment group	Plant tissue	Total nitrogen content (g · kg^{-1})	Total phosphorus content (g · kg^{-1})	Total kalium content (g · kg^{-1})	N/P ratio	P/K ratio	N/K ratio
正常 CK	根部营养物 Root nutrient content	-	-	-	-	-	-
Low drought	根部营养物 Root nutrient content	-	-	-	-	-	-
Medium drought	根部营养物 Root nutrient content	-	-	-	-	-	-
High drought	根部营养物 Root nutrient content	-	-	-	-	-	-

Treatment group	Plant tissue	Total nitrogen content (g · kg^{-1})	Total phosphorus content (g · kg^{-1})	Total kalium content (g · kg^{-1})	N/P ratio	P/K ratio	N/K ratio
正常 CK	茎部营养物 Stem nutrient content	-	-	-	-	-	-
Low drought	茎部营养物 Stem nutrient content	-	-	-	-	-	-
Medium drought	茎部营养物 Stem nutrient content	-	-	-	-	-	-
High drought	茎部营养物 Stem nutrient content	-	-	-	-	-	-

Treatment group	Plant tissue	Total nitrogen content (g · kg^{-1})	Total phosphorus content (g · kg^{-1})	Total kalium content (g · kg^{-1})	N/P ratio	P/K ratio	N/K ratio
正常 CK	叶部营养物 Leaf nutrient content	-	-	-	-	-	-
Low drought	叶部营养物 Leaf nutrient content	-	-	-	-	-	-
Medium drought	叶部营养物 Leaf nutrient content	-	-	-	-	-	-
High drought	叶部营养物 Leaf nutrient content	-	-	-	-	-	-

Table 3 Contents of chlorophyll in the leaf of *Guettarda speciosa*

Index	正常 CK	Low drought	Medium drought	High drought
Chlorophyll a content (mg·g^{-1})	1.668±0.023a	1.639±0.064a	1.553±0.072a	1.467±0.135a
Chlorophyll b content (mg·g^{-1})	0.709±0.102a	0.640±0.081a	0.546±0.058a	0.526±0.059a
Chlorophyll a + b content (mg·g^{-1})	2.317±0.174a	2.169±0.194ab	1.931±0.149ab	1.831±0.202b
Chlorophyll a/b ratio	2.389±0.123	2.5[illegible]	[illegible]	[illegible]

Discussion and Conclusion

The soil of the Spratly Islands is primarily coral sand, which has poor water retention and lacks true soil structure and fertility, resulting in barren soil with low nutrient content. The islands experience strong sea winds, high salinity, high soil pH, intense sunlight, strong evaporation, and particularly severe drought during the dry season. Therefore, studying the drought resistance biological characteristics of *G. speciosa*, a tropical coastal plant, can provide fundamental data and references for plant introduction in vegetation construction and restoration on the Spratly Islands.

From the perspective of morphological and anatomical characteristics, as drought intensity increased, leaf thickness, the ratio of palisade tissue to spongy tissue, palisade tissue thickness, and upper epidermis thickness of *G. speciosa* increased, while spongy tissue thickness, lower epidermis thickness, and palisade tissue width showed decreasing trends. This indicates that *G. speciosa* has excellent adaptability to water-stressed environmental conditions, can reduce mechanical damage during plant wilting from water deficiency (Guo et al., 2009) and decrease plant transpiration to adapt to water shortage (Li and Bao, 2005), and can store water to cope with environmental changes involving water deficit. This demonstrates that *G. speciosa* has good water control capacity and strong drought resistance.

Regarding the effects of different drought stress levels on antioxidant enzyme activities and stress-resistant substances in *G. speciosa*, the results showed that as drought stress occurred and intensified, malondialdehyde (MDA) content increased, showing an initial increase followed by a decreasing trend throughout the drought process, with relatively small magnitude. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) all increased with intensifying drought stress. Compared with the magnitude of CAT activity increase, POD activity showed a smaller increase, but SOD, POD, and CAT activities had not yet reached their maximum values. This indicates that *G. speciosa*, when subjected to drought stress, experiences disruption of the dynamic balance between reactive oxygen species production and scavenging in plant tissues (Zhao et al., 2009), leading to accumulation of large amounts of reactive oxygen and causing membrane lipid peroxidation (Fridovich, 1975), which results in plant damage or senescence. However, the activities of endogenous reactive oxygen scavengers in the plant were enhanced (Karim et al., 2004), effectively scavenging reactive oxygen species such as O_2^- and H_2O_2 (Guo et al., 2016), thereby protecting the membrane system from damage (Wan et al., 1997). This demonstrates that *G. speciosa* experiences minimal damage, has high membrane permeability, can protect the membrane system by scavenging reactive oxygen through antioxidant enzymes to prevent tissue damage or senescence, possesses strong drought resistance capacity, and can withstand severe drought stress environments. The study showed that compared with normal water conditions, PRO content in *G. speciosa* continuously increased with intensifying drought stress, which can help reduce cellular osmotic potential (Wu et al., 2017) and maintain cell turgor pressure, further indicating that *G. speciosa* has the capacity to adapt to drought-stressed environments and possesses good drought resistance (Zhang, 2006).

Regarding nutrient elements in roots, stems, and leaves and leaf chlorophyll content of *G. speciosa*, the N/P ratios in all three parts were less than 14, with the N/P ratios in descending order: leaves > stems > roots. *Guettarda speciosa* showed increased absorption and utilization of potassium. As drought intensity increased, total leaf chlorophyll content of *G. speciosa* continuously decreased, and both chlorophyll a and chlorophyll b also decreased progressively with drought stress. The chlorophyll a/b ratio showed a trend of initial increase followed by a slight decrease. This indicates that nitrogen is the primary limiting factor for *G. speciosa* growth (Zhang et al., 2014), but *G. speciosa* can absorb potassium to increase net photosynthetic rate (Lü et al., 2012) to maintain normal growth. However, chlorophyll was damaged by drought stress, affecting photosynthetic rate (Mou et al., 2014), though the degree of damage was not severe, and changes in the chlorophyll a/b ratio did not affect the reduction in plant photosynthesis (Li, 1990). This demonstrates that *G. speciosa* has adaptability to drought environments, can grow well in arid and barren conditions, and possesses strong stress resistance.

Therefore, considering water and nutrient stress factors, *G. speciosa* can serve as a pioneer species for community construction on tropical coral sand islands and can be introduced and planted on tropical coral sand islands such as the Spratly Islands for vegetation construction and ecological restoration. However, this study only measured and analyzed the drought resistance physiological characteristics of *G. speciosa*. To comprehensively understand the adaptability of *G. speciosa* on tropical coral sand islands, further experimental research on different substrate ratios, nutrients, environmental temperature, and other factors is needed for verification.

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Note: Figure translations are in progress. See original paper for figures.