**Effects of Salt Stress on Photosynthesis and Fluorescence Characteristics of Endangered Plant *Barringtonia racemosa***

Fang Liang1, Xiaohui Tan2, Caihong Bai1, Qiuwei Huang2, Ping Li3, Xu Deng1, Ju Hu1\*

1 College of Biology and Pharmacy, Yulin Normal University, Yulin 530700, China

2 Guangxi Subtropical Crops Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530001, China

3 Guangxi South Subtropical Agricultural Science Research Insitute, Guangxi Academy of Agricultural Sciences, Chongzuo 532415, China

\***Corresponding author**:

Ju Hu. Tel. + 86 151 9810 8651. Fax: 151 9810 8651

E-mail: huju918@yahoo.com

## Abstract

*Barringtonia racemosa* is one of endangered plant species, and salt stress plays a role in the demise of endangered plant species. In order to investigate the effects of salt stress on photosynthesis and chlorophyll fluorescence of *B. racemosa*, the photosynthetic and chlorophyll fluorescence parameter ofleaves under eight different salt concentration treatments were determined. The results showed that the net photosynthetic rate (*P*n) of the treated leaves were extremely significantly different from the control (*P*<0.01), and reached the peak at 6‰ salt concentration; transpiration rate (Tr) and stomatal conductance (Gs) were not significantly different from control; intercellular carbon dioxide concentration (Ci) were significantly different between 6‰ and 12‰ treatments compare with control (*P*<0.05). Meanwhile, there were no significant difference between treatments and control in initial fluorescence (Fo), maximum fluorescence (Fm), and maximum PSII quantum yield (Fv/Fm) with the salt concentration increasing; non-photochemical quenching coefficient (qN) was significantly different from that of control at the concentration of 4‰ to 14‰ (*P*<0.05); the apparent electron transport rate (ETR) was significantly different from that of control at the concentration of 8‰ to 14‰ (*P*<0.05). It indicated that the salt stress of 0 to 14‰ had little effect on the photosynthesis and chlorophyll fluorescence characteristics, and the photosynthesis ability of the leaves was the strongest when the salt concentration was 6‰, but it did not damage the PSII reaction center of the leaves when the salt stress of less than 14‰, and the leaves were in normal physiological state.

**Key words:** endangered plants, salt stress, photosynthetic characteristics, chlorophyll fluorescence, *Barringtonia racemosa*

## 1 Introduction

Currently, soil salinization was increasing year by year in our country (Wang *et al*., 2017). The study revealed that the salinized soil contained high salt content and excessive mineral components, which would cause the soil solution concentration to be too high, increase the rhizosphere osmotic pressure, reduce the ability of plants to absorb water and nutrients, and inhibit the growth of plant tissues and organs differentiation, and leaves damage (Lie and Xue, 2017). High salt, water logging and low oxygen were the characteristics of the mangrove habitat (Liu *et al*., 2017). Thus, cultivation of ornamental tree species that could withstand flood disasters in flood-prone areas, such as along the river and coastal, and this was of great significance for the construction and restoration of its landscape (Liu *et al*., 2018b).

It was found that salt stress had a significant effect on the chlorophyll fluorescence index of *Cordyline fruticosa*, *Hibiscus rosa-sinensis*, *Ixora chinensis*, and *Duranta repens* seedling leaves by She *et al*. (2018). Among them, *C. fruticosa* had the strongest salt-resistance ability, but *H. rosa-sinensis* had the weakest. For *Robinia pseudoacacia*, the chlorophyll fluorescence activity of Yuyin ‘1’ was better than that of Yuyin‘3-I’ under salt stress, that is, the salt tolerance of Yuyin ‘1’ was greater than that of Yuyin ‘3-I’ (Ren *et al*., 2018). The photosynthetic rate and chlorophyll synthesis of *Solanum melongena* were significantly affected when the NaCl concentration was greater than 15 mmol/L (Zhao *et al*., 2018). Scholars of Niu *et al*. (2018) obtained the salt tolerance of different varieties by studying the growth and chlorophyll fluorescence characteristics of six *Vitis vinifera* rootstock varieties under salt stress. Thus, it can be seen that the study of photosynthetic characteristics and chlorophyll fluorescence characteristics can reflect to some extent the tolerance of plants under salt stress.

*Barringtonia racemosa* is a semi-mangrove plant, as evergreen small tree or medium large tree with thick shades leaves, graceful posture, and long flowering period characteristics (Lin, 1998). It is native to tropical and subtropical regions in Africa, Asia and Oceania, naturally distributed in Hainan, Taiwan, Yunnan, Guangxi and Guangdong in China, and was listed as an endangered plant in 2017 (Nong and Li, 2006; Qin *et al*., 2017). The research found that *B. racemosa* had a strong carbon storage capacity compared to *Cyclobalanopsis glauca* when used as a landscape tree (Tsay *et al*., 2015). Moreover, our preliminary study found that *B. racemosa* showed a good resistance under 10 to 14‰ salinity stress (Liang *et al*., 2019). Thus, it is recommended that more applications should be made in mangrove construction and wetland landscape ecological restoration.

At present, the research of *B. racemosa* is mostly concentrated on its ecological protection, economic and medicinal value (Gowri *et al*., 2009; Musman, 2010; Sulaiman and Ooi, 2014; Zhong *et al*., 2018). The research on its application of garden landscape is little, however, it has not been reported that the research on photosynthesis and chlorophyll fluorescence characteristics under adversity of *B. racemosa*. Thus, in this study, the tidal wetland system environment was simulated to deeply study the growth and development of *B. racemose* seedlings in response to salt stress from the aspects of photosynthetic characteristics and chlorophyll fluorescence parameters. The aim is to explore the salt-tolerant characteristics of *B. racemosa*, and the application would provide a theoretical basis and has high value for further research on the resistance mechanism of plants to salt stress.

## 2 Materials and methods

**Plant materials** **-** The seeds for culturing of seedlings were collected from the natural forest of *B. racemose* in Danzhou, Hainan, China (N19°31′, E109°35′), and sown in nursery shed at subtropical crops research institute, Guangxi Academy of Agricultural Sciences, Nanning, China (N22°53′, E108°70′) (Fig. 1). The sand beds were used for sowing, then seedlings in height of 10 to 15 cm were moved to nutrition bags with orchard soil and coconut bran (1:1, mass ratio) for growth. Finally, the 2-year-old seedlings were used for treatment of salt stress and determination of photosynthesis and fluorescence characteristics.

**Fig. 1** Two-year-old seedlings of *B. racemosa*

**Treatment of salt stress** **-** This test was performed at July to August of 2018 in the greenhouse at subtropical crops research institute, Nanning. Automatic tide simulation control device equipped with an aqua storage tank, a plant cultivation tank, a timer, a water pump, a filtering system, a fill light system and a shading system were used for salt stress treating, which could automatically control the pumping time by timer to reach the set test water level and control the treating time.

To simulate half-day tides, a cycle of 12 h was used for flooding, and each half-day tide seedlings were submerged for 3 h under a high level. 0‰ (Control), 2‰, 4‰, 6‰, 8‰, 10‰, 12‰, 14‰ (water to salt was m: m) were used for treatment, each treatment was performed in 6 plants, and the biological testing repeated three times.

**Determination of photosynthetic characteristics** **-**At 9:00 to11:00 Am in mid-August 2018, three plants with well and consistent growth status selected randomly from different treatments were used for net photosynthetic rate (*P*n), intercellular carbon dioxide concentration (Ci), stomatal conductance (Gs) and transpiration rate (Tr) determination by portable photosynthetic analyzer (TPS-2, PP Systems, USA). Each treatment was selected randomly of three leaves, and each leaf was measured three times.

**Measure of fluorescence parameters -** After salt stress treating, CF Imager chlorophyll fluorescence imaging system (Technologica, Ltd., UK) was used to measure the fluorescence parameters of *B. racemose* leaves. Among which, minimal fluorescence (Fo), maximum fluorescence (Fm), electron transport rate (ETR), maximum PS II quantum yield (Fv/Fm) and non-photochemical quenching coefficient (qN) and the PSⅡ reaction center photochemical quenching coefficient (qP) under steady state were measured. Then, a comprehensively analysis for photosynthetic capacity of leaves after salt stress was carried out.

**Statistics and Analysis** **-** All data were analysed using SPSS Statistics 19.0 (SPSS Inc., Chicago, USA) software for statistical analysis, one-way ANOVA and least significant difference test (Duncan, *P* < 0.05). Excel 2017 and Origin 9.0 (OriginLab Inc., Massachusetts, USA) were used for data tabulation.

## 3 Results

**Effects of salt stress on photosynthetic characteristics of *B. racemosa* seedlings -** As shown in Fig. 2-A, the *P*n value was increased and then decreased, but all were higher than that of control with the increasing of salt concentration, indicating that 2‰ to 14‰ salt concentration had a good promoting effect on *P*n. In addition, *P*n value was significantly different from control (*P*<0.01) showed a single-peak change trend, and reaching the peak when the salt concentration was 6‰, suggesting that proper salt concentration could increase the *P*n of *B. racemosa*, and reached the highest at 6‰ salt concentration.



**Fig. 2** Effects of salt stress on photosynthetic characteristics of mature leaves of *B. racemosa* seedlings. Vertical bars represent standard deviations (SDs) of the mean (n=3). The different lowercase letters indicate that the mean values are significantly different among the treatments at *P*<0.05 according to Duncan’s test (the same as below).

Similarly, as shown in Fig. 2-B, 2-C, and 2-D, Tr and Gs were no significant difference from control among each treatment, indicating that all the treatment of salt concentrations were had little effect on the Tr and Gs. However, Ci showed a reverse trend of *P*n by overall downward with increasing salt concentration, and showed a significantly different from control at 6‰ and 12‰ salt concentration (*P*<0.05), indicating that the photosynthesis of plants was related to non-stomatal factors at this time.

**Effects of salt stress on chlorophyll fluorescence characteristics of *B. racemosa* seedlings -** As shown in Fig. 3-A, except for 4‰ salt concentration, the FO value of all the other treatments were no significant difference from the control with the increasing of salt concentration. Fm value was no significant difference from the control as well (Fig. 3-B), indicating that there was no photoinhibition in the PSⅡ reaction center.

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**Fig. 3** Effects of salt stress on Fo and Fm in leaves of *B. racemosa* seedlings

As shown in Fig. 4-A, the Fv/Fm value does not change significantly with the increasing of salt concentration, and there was no significant difference between the treatments and the control. This showed that the light energy utilization efficiency was not significantly affected, and could be seen that different levels of salt stress less than 14‰ did not damage the PSⅡ reaction center of *B. racemose*. For Fig. 4-B, the ETR value showed a downward trend, and significantly different from the control at 8‰ to 14‰ salt concentration (P<0.05) with the salt concentration increasing, suggesting that the rate of electron transfer became lower at this salt concentration, which would pose a certain stress to *B. racemose*.



**Fig.4** Effects of salt stress on Fv/Fm and ETR in leaves of *B. racemosa* seedlings

It could be seen from Fig. 5-A that the qN value was significantly different from 2‰ salt concentration and the control (*P<0.05*) by an obvious upward trend with the salt concentration increasing (4‰ to14‰). This was indicating that it may adapt to the influence of salt stress through the energy dissipation mechanism to protecting the photosynthetic apparatus of the plant from damage in *B. racemose* at this time. In Fig. 5-B, there was no significant effect on the qP value from all the salt concentrations. It could be seen that qP value was more stable than qN value under different treatments.

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**Fig. 5** Effects of salt stress on qN and qP in leaves of *B. racemosa* seedlings

## 4 Discussion

Research showed that the *P*n value could directly reflect the functions of the photosynthetic system of plants, and was a reflection of the energy conversion process between plants and the external environment (Shao *et al*., 2013), meanwhile, the *Pn* of plants was the accumulation rate of plant organic matter, which directly reflects the functions of the photosynthetic system (Gong *et al*., 2017). Stomata was the gateway for water vapor and CO2 to enter and exit, and plant leaves exchanged gas with the outside through the stomata (Zhang *et al*., 2017), environmental stress would cause plant stomata to close, and CO2 entry into the leaves was blocked, resulting in a decreasing of *Pn*. In addition, the decrease in photosynthetic capacity of leaf mesophyll cells would cause a decreasing of *Pn* value, however, the Ci value does not change or increase when the *Pn* value decrease (Farquhar and Sharkey, 1982). In this study, Tr, Gs and Ci decreased to varying degrees when *Pn* increased under different treatments of *B. racemosa*. However, Ci showed a downward trend when *Pn* increased at 2‰ to 6‰ salt concentration, speculating that the reason for this change may be that salt stress did not destroy the chloroplast structure of *B. racemosa* leaves, and the photosynthetic capacity of mesophyll cells was increased. Therefore, the non-stomatal factors were the main reasons for the *Pn* increasing of *B. racemosa* leaves at this stage. Moreover, *Pn* decreased at 8‰ to 14‰ salt concentration accompanied by Ci decreasing, indicating that the decreasing in photosynthesis at this time may be caused by salt concentration increasing, and caused stress to *B. racemosa* leaves. Thereby, the entry of CO2 and water vapor were blocked, and the gas exchange was also affected, causing the stomata to close. Thus, the stomata factor was the main limiting factor of photosynthesis in *B. racemose* under this condition. This phenomenon was similar to net photosynthetic rate changes under sand and dust stress of *Cydonia oblonga* by Wang *et al*. (2018).

The chlorophyll fluorescence parameter could reflect the photosynthetic efficiency and tolerance to adversity stress of plants (Liu *et al*., 2018a), was one of the indicators that reflected he adaptability to adverse environments with more rapid, sensitive and profoundly in terms of photosynthetic physiology of plant leaves (Efeoglu *et al*., 2009). Among the many chlorophyll fluorescence parameters, Fv/Fm was an indicator of plant tolerance to poor environments, and it would decrease when the PSⅡ effectiveness affected under any adversity, and indicating that the plant was photoinhibited (Li *et al*., 2000; Xiong *et al*., 2017). Under different salt stress, seedlings of *B. racemose* needed to maintain a certain amount of photosynthetic carbon acquisition, but also to avoid excessive transpiration causing water loss. Thus, seedlings required to achieve balance by regulating stomatal conductance. In this study, all the treatments were no significant effect on the Fv/Fm value of leaves, indicating that different degrees of salt stress less than 14‰ did not damage the PSⅡ reaction center of *B. racemosa* leaves. At this time, the chloroplast energy obtained was fully used for photosynthesis of *B. racemosa* leaves, and the leaves were in a normal physiological state.

Previous studies had shown that PSⅡ was one of the more sensitive and fragile parts of plants under adversity in photosynthesis, and Fo expressed that the PSⅡ reaction center of the leaf was completely at the fluorescence level when it was open, but the PSⅡ reaction center had been destroyed or reversible inactivation when Fo increased (Peterson *et al*., 1988; Xu *et al*., 1992). However, Fm value was the fluorescence output of the PSⅡ reaction center under completely closed conditions, and it could be used as one of the characteristics of photoinhibition when it decreased (Ma and Wu, 2018). In addition, a study showed that the maximum light energy conversion efficiency of plants was not the same as the maximum photosynthetic rate (Shen *et al*., 2015). Also, the ETR value could be used to reflect the transfer rate of photosynthetic energy (Chen *et al*., 2010). In this study, the values of Fo, Fm, Fv/Fm have been close to the control level with the salt concentration increasing of *B. racemosa*, showing that it could effectively maintain the original light energy conversion efficiency of PSⅡ by enhancing the electron transfer ability of PSⅡ under salt stress, and at the same time enhance the ability to use light energy. In terms of ETR, it showed a significant downward trend at the 8‰ to 14‰ salt concentration, indicating that this salt concentration range had a certain stress on *B. racemosa*, but at this timeinitiated a self-protection mechanism, meanwhile, the qN and qP values both showed an upward trend to effectively activate the non-radiative heat dissipation mechanism, and dissipated the excess light energy absorbed by PSⅡ in the form of heat, and protected the photosynthetic apparatus of plants in time, also to preventing excessive decline of *P*n (Gilmore and Yamamoto, 1991; Guo *et al*., 2009; Gao, 2011). This may be one of the reasons why the *P*n value of *B. racemosa* was always higher than the control when the salt concentration was high. This phenomenon was similar to *Cerasus humilis* through heat dissipation in qN response to drought stress to achieve different degrees of self-protection (Duan *et al*., 2018).

At the same time, the qP value increased slowly with the increase of salt concentration, which mean that the open ratio of the PSⅡ reaction center and the e-involved in the fixation of CO2 were increasing, and the photosynthetic electron transfer capacity was increasing, so, the plant carbon response was not hindered. The photosynthetic rate was increased, which helps plants cope with high salt stress. *P*n value reached the maximum rate when treated with 6‰ salt concentration, accompanied by a significant decreasing in Ci value at 6‰, but the *P*n and Ci values showed reverse changes, and non-stomatal factors were activated at this time. The *P*n and Ci values showed a consistent decreasing after the treatment concentration increasing, however, *P*n was always higher than that of the control. This may be due tostrong salt tolerance and certain adaptability to salt stress environments of *B. racemosa*, but the specific adaptations mechanism needs to be further studied and perfected.

## 5 Conclusion

The results of this study showed that 0‰ to 14‰ salt concentration had little effect on the photosynthetic and chlorophyll fluorescence characteristics of *B. racemosa* seedlings. The photosynthetic capacity was the strongest when it submerged at 6‰ salt concentration of *B. racemosa* leaves. However, it was not damaged of PSⅡ reaction center of *B. racemosa* leaves under different salt concentrations less than 14‰, and all the leaves were in normal physiological state.

## Author contribution statement

Experimental design, XT and XD; experimentation and result analysis, FL; experimental verification, QH and PL; paper writing, JH; Revision, CB.

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