



## Research article

## Effects of concentrations of sodium chloride on photosynthesis, antioxidative enzymes, growth and fiber yield of hybrid ramie

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## ABSTRACT

Ramie (*Boehmeria nivea* L.) is one of the oldest and most important fiber crops in China due to the comfortable textile of its fine fiber. Increased ramie fiber demand brings ramie cultivation to salt-affected regions. The aim of this research was to determine morphological, physiological and biochemical responses of ramie by subjecting plants to varying concentrations of NaCl (0, 2, 4, 6 and 8 g NaCl/kg dry soil) at vigorous growth stage for 10 and 20 days. Results indicated that salinity stress substantially inhibited the growth of hybrid ramie plants and led to remarkable decline in fiber yield. However, when grown at 2 g NaCl/kg growth and fiber yield were similar to non-saline control. In addition, chlorophyll fluorescence and gas exchange parameters were correlated with growth and yield response. Salt treatments promoted a subsequent decrease in maximum quantum efficiency of PSII photochemistry (Fv/Fm), quantum efficiency of open PSII reaction centers (Fv'/Fm') and quantum yield of PSII ( $\phi_{PSII}$ ) while non-photochemical quenching (NPQ) changed conversely. Photochemical quenching (qP) and electron transport rate of PSII (ETR) increased at 2 and 4 g NaCl/kg then decreased at 6 and 8 g NaCl/kg. Substantial decline in the PSII activity at high salinity was associated with the loss of chlorophyll contents. Moreover, marked decrease in net photosynthetic rate (A), transpiration rate (E), stomatal conductance ( $g_s$ ) was also recorded. Nonetheless, intercellular CO<sub>2</sub> (Ci) decreased at low salt stress, subsequently increased at high salt stress while water use efficiency (WUE) and instantaneous water use efficiency (WUEi) altered in opposite direction. Substantial decrease of photosynthesis at high salinity was due to non-stomatal factors. Furthermore, salinity stress led to decrease of proteins and accumulation of proline and malondialdehyde (MDA), as well as enhanced activities of superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POD, EC 1.11.1.6), whereas, catalase (CAT, EC 1.11.1.7) enhanced at low salinity, decreased at high salinity. Nonetheless, these changes were closely related with the severity and duration of the salinity stress and their interaction. The results suggested a certain tolerance to salinity stress for hybrid ramie. This meets the essential condition for utilization in salinity-prone environments.

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**Abbreviations:** A, net photosynthesis; CAT, catalase; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a + b, total chlorophyll; Ci, intercellular CO<sub>2</sub>; E, transpiration rate; ETR, electron transport rate of PSII; Fv/Fm, maximum quantum efficiency of PSII photochemistry; Fv'/Fm', quantum efficiency of open PSII reaction centers;  $g_s$ , stomatal conductance; MDA, malondialdehyde; NPQ, non-photochemical quenching; POD, peroxidase; PSII, photosystem II;  $\phi_{PSII}$ , quantum yield of PSII; qP, photochemical quenching; RWC, relative water content; SOD, superoxide dismutase; WUE, water use efficiency; WUEi, intrinsic water use efficiency.

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## 1. Introduction

Salinity is one of the most important abiotic stresses widely distributed in both irrigated and non-irrigated areas of the world (Cha-Um and Kirdmanee, 2009). The annual global income losses due to salinization of agricultural land could reach US\$11.4 billion in irrigated land and US\$1.2 billion in non-irrigated areas (Ghassemi et al., 1995). Salinity stress limits growth and productivity of crop species by disrupting physiological processes (Sudhir and Murthy, 2004). Photosynthesis is among the primary processes to be affected most by salinity (Munns et al., 2006). Salinity influences photosynthetic capacity and its effects vary with the salt concentration, duration of stress and with the cultivar used.

Photosynthetic ability of some plant species reduced in the presence of salinity (Parida et al., 2003; Stepien and Johnson, 2009), while that of other species reduced only at high stress and long term stress (Nie et al., 2011; Koyro et al., 2013). Both stomatal and non-stomatal limitations contribute to the reduction in photosynthesis (Stepien and Klobus, 2006). Under saline conditions, substantial reduction in photosynthesis has been associated with decrease in total chlorophyll contents and distortion in chlorophyll ultra structures (Meng et al., 2011). In photosynthesis, photosystem II (PSII) is particularly more susceptible to salinity stress. Measurement of chlorophyll fluorescence has been used as a mean to evaluate the integrity of PSII upon exposure to stress (Shabala, 2002). The analyses of fluorescence quenching provide information on the fundamental processes of energy absorption, utilization, and dissipation, and electron transport in PSII (Schreiber et al., 1986). This makes it possible to evaluate the plant's photosynthetic performance and the extent of its tolerance to salt stress (Maxwell and Johnson, 2000). However, salinity influences the activity of PS II and its effects also vary with the salinity levels and with the species used. Under salt stress, inhibition of PSII activity has been reported in some species e.g. wheat (Kanwal et al., 2011) and castor bean (Li et al., 2010), while some other species e.g. eggplant (Shaheen et al., 2013) exhibited no effect on PSII indicating non-significant effect of salinity stress on photosynthetic activity.

Salinity stress leads to the overproduction of reactive oxygen species (ROS) such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radical ( $HO^\cdot$ ). Excessive ROS in plants are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress (Gill and Tuteja, 2010). Malondialdehyde (MDA) is generally taken as a tool to assess the severity of the oxidative stress and the degree of plant sensitivity towards ROS induced oxidation (Perez-Lopez et al., 2009). Nonetheless, Plants possess efficient systems for scavenging ROS that protect them from destructive oxidative reactions, in which SOD, POD and CAT are most important anti-oxidative enzymes. SOD converts superoxide radicals ( $O_2^-$ ) into hydrogen peroxide ( $H_2O_2$ ), which is further scavenged by POD using various substrates as electron donors in the extracellular space and cytosol, and mainly by CAT in peroxisomes (Tarchoune et al., 2010; Yildiztugay et al., 2011). Proline at high concentrations may protect plants from environmental stress through contribution to cellular osmotic adjustment, detoxification of ROS, protection of membrane integrity, and stabilization of enzymes/proteins (Ashraf, 2009). Higher antioxidant enzyme activities and higher proline contents are associated with higher salt tolerance in most plants (Cha-Um and Kirdmanee, 2009; Ashraf and Foolad, 2007).

Ramie (*Boehmeria nivea* L.), or 'China grass', is a perennial herbaceous plant of the Urticaceae family. It is one of major foreign exchange earning crops in China due to the high quality textile of its fine fiber (Huang et al., 2013). Its fabrics are strong, smooth and durable. It is extremely absorbent and comfortable to wear, especially in warm weather (Cengiz and Babalik, 2009). Currently, the practical utilization ranges from clothes, bed clothes and decorations. Increased fiber demand can be achieved by increasing cultivated land, increasing fiber productivity. This brings ramie cultivation to salt-affected regions. Ramie hybrid varieties have higher yield potential compared to conventional varieties (Huang et al., 2013). However, little is known of the salt tolerance mechanism of ramie plants as per our knowledge. Therefore, the specific objective of this study was undertaken to explore hybrid ramie tolerance to salinity stress, based on changes in chlorophyll fluorescence, gas exchange traits, pigments content, soluble protein, proline, MDA, enzymatic antioxidants, growth and yield.

## 2. Materials and methods

### 2.1. Plant materials and growth conditions

The study was conducted during summer 2011 in a rain-protected net house at Department of Agronomy and Biotechnology, Southwest University, Chongqing, China (latitudes 29° 49' 32" N, longitudes 106° 26' 02" E and altitude 220 m). The hybrid seedlings (Chuan Zhu 11, a two-line hybrid ramie variety developed by good male sterile line C9451 and restorer line R79-20) of ramie (*Boehmeria nivea* L.) were provided by Dazhu Ramie Research Station, Sichuan, China. On June 18, 2011 40-day-old nursery seedlings from the nursery beds in the fields were transplanted into plastic pots (24 cm in diameter, 18 cm in depth), one seedling per pot. The pots were filled with sandy loam soil. Selected physical and chemical properties of the soil are presented in Table 1. Total weight of each pot was 7 kg after filling with dry soil. The pots were arranged in a completely randomized design (CRD) and three replications of each experimental unit with 20 pots per treatment. Salt treatment was started 40 days after transplanting when plants were at the start of vigorous growth stage. The experiment consisted of five treatments involving varying salinity levels in soil. These five salinity levels were created by adding 0, 2, 4, 6 and 8 g NaCl/kg dry soil ( $g\ kg^{-1}$ ).

### 2.2. Salt treatments

Hybrid ramie plants were subjected to salt stress by adding sodium chloride (NaCl) solutions, with the calculated amount of NaCl dissolved in water. To minimize salt shock, NaCl concentration was raised stepwise in aliquots of  $2\ g\ kg^{-1}$  every day until the final salinity levels were achieved. The plants were irrigated with appropriate volume of fresh water to maintain soil water at field capacity and avoid drought stress till harvest. Moisture was regularly monitored by TRIME-EZ/-IT (IMKO Micromodultechnik GmbH, Germany). The net house temperature and relative humidity varied 22.8–38.1 °C and 45.8–81.6%, respectively for the entire growth period.

### 2.3. Observations

Gas exchange parameters and chlorophyll fluorescence measurements were determined at the 19th and 20th day after salt treatment, respectively. Ramie plants were sampled (6–7th leaf from top) at the 10th and 20th day after salt treatment to assess the photosynthetic pigments, soluble protein, relative water content (RWC), malondialdehyde (MDA) and proline. After washing, leaves were frozen in liquid  $N_2$  and stored at  $-80\ ^\circ C$  until biochemical analysis. The remaining plants were harvested on September 10, 2011, i.e. 84 days after transplanting for assessing the growth and yield-related traits.

**Table 1**  
The basic nutrients of experimental soil in hybrid ramie.

No.	Item	Value
1	pH	6.66
2	Organic matter ( $g\ kg^{-1}$ )	5.20
3	Total nitrogen ( $g\ kg^{-1}$ )	0.50
4	Total phosphorus ( $g\ kg^{-1}$ )	1.28
5	Total potassium ( $g\ kg^{-1}$ )	3.25
6	Alkali-hydro nitrogen concentration ( $mg\ kg^{-1}$ )	150.53
7	Available phosphorus ( $mg\ kg^{-1}$ )	23.03
8	Readily-available potassium ( $mg\ kg^{-1}$ )	55.21
9	$Na^+$ ( $g\ kg^{-1}$ )	0.038

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