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Responses of gas exchange, chlorophyll synthesis and ROS-scavenging systems to salinity stress in two ramie (*Boehmeria nivea* L.) cultivars

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Abstract

Ramie (*Boehmeria nivea* L.) is an important crop that serves as fine fiber material, high protein feedstuff, and valuable herbal medicine in China. However, increasing salinity in soil limits the productivity. We investigated in a greenhouse experiment responses to salinity in two ramie cultivars, Chuanzhu-12 (salt-tolerant cultivar, ST) and Xiangzhu-2 (salt-sensitive cultivar, SS), to elucidate the salt tolerance mechanism of this species. Salinity stress substantially reduced both chlorophyll and carotenoid contents. In addition, net photosynthesis, transpiration rate, stomatal conductance, intercellular CO_2 concentration, and the ratio of intercellular CO_2 to ambient CO_2 were affected, less in ST. Nevertheless, salinity stress markedly improved water use efficiency and intrinsic water use efficiency in both species. Moreover, relative water contents, soluble proteins, and catalase activity were substantially impaired, while proline accumulation and superoxide dismutase activity were enhanced substantially, more in ST. Furthermore, noteworthy increase in peroxidase activity and decrease in malondialdehyde content was recorded in ST, whereas, in SS, these attributes changed conversely. Overall, the cultivar ST exhibited salt tolerance due to its higher photosynthetic capacity, chlorophyll content, antioxidative enzyme activity, and nonenzymatic antioxidants, as well as reduced lipid peroxidation and maintenance of the tissue water content. This revealed the salt tolerance mechanism of ramie plants for adaptation to salt affected soil.

*Additional key words***:** abiotic stress; photosynthesis; pigments; lipid peroxidation; antioxidant enzymes.

Introduction

Soil salinity is one of the major abiotic stresses that limit agricultural production worldwide (Läuchli and Grattan 2007). Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang *et al*. 2003). The response to salinity stress differs noticeably among different crop species or cultivars of same species due to their inherent differences in salt tolerance. The effects of salt stress are more evident in sensitive species or genotypes (Munns *et al.* 2006). It also depends on a salt concentration and growth stage. Some species and plants are particularly susceptible to salinity during the seedling and the early vegetative growth stage as compared to other growth stages (Läuchli and Grattan 2007).

Salinity stress reduces the growth and production of crops by affecting a variety of physiological and biochemical processes, including photosynthesis, protein synthesis, and lipid metabolism (Khan *et al*. 2009, Hu *et al*. 2012). Growth inhibition observed in many plants exposed to salinity stress is often associated with a decrease in their photosynthetic capacity (Jiang *et al*. 2006). The salinity-induced decrease in photosynthesis may involve stomatal and nonstomatal limitations and inhibition of photochemical processes (Hichem *et al*. 2009, Huang *et al*. 2011). A substantial inhibition of photosynthesis under salt conditions seems to be associated with degradation of chlorophyll (Chl) (Meng *et al*. 2011). Salt stress also induces modifications in plant gene expression. This may lead to the accumulation or

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Abbreviations: Car – carotenoids; CAT – catalase; Chl *a* – chlorophyll; C_i – intercellular CO_2; C_i/C_a – intercellular CO_2 to ambient CO_2 concentration ratio; E – transpiration rate; g_s – stomatal conductance; MDA – malondialdehyde; P_N – net photosynthesis; POD – peroxidase**;** RWC – relative water content; ROS – reactive oxygen species; SOD – superoxide dismutase; SS – salt-sensitive cultivar; ST – salt-tolerant cultivar; WUE – water-use efficiency**;** WUE_i – intrinsic water-use efficiency.
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depletion of certain metabolites, resulting in an imbalance in the cellular proteins, which may increase, decrease, appear or disappear after a salt treatment (Kong-Ngern *et al*. 2005). An important consequence of salinity stress in plants is the excessive generation of reactive oxygen species (ROS) including $O_2^{\cdot -}$, $\cdot OH$, H_2O_2, and $^1O_2$. ROS, being highly reactive and toxic, can seriously disrupt normal metabolism through oxidative damage to proteins, lipids, carbohydrates, DNA, and ultimately to cellular structures (Apel and Hirt 2004). Malondialdehyde (MDA) production is taken as an index of ROS-induced oxidative damage (Teisseire and Guy 2000). Nonetheless, plants have developed complex enzymatic and nonenzymatic antioxidant systems. These defense systems work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS (Gill and Tuteja 2010). Salt-tolerant cultivars generally have the better antioxidative system when compared to the sensitive cultivars (Rout and Shaw 2001).

Ramie (*Boehmeria nivea* L.), or 'Chinagrass', is one of the oldest fibre crops. It has been traditionally used as a refreshing material for summer clothes in China. In addition, ramie possesses various functions; it is widely used as feedstuff for its 25% of protein in the stems and leaves (Lee *et al*. 2009), as a common herbal medicine for anti-inflammation, anti-bacteria, diuresis, hemostasis, liver protection, and prevention of miscarriages for many years (Lin *et al*. 1997, Huang *et al.* 2006, Tian *et al*. 2011). It is also a potential material for functional food and medicine (Lee *et al*. 2009). However, ramie seedlings are very sensitive to soil salinity (Huang *et al*. 2014). Increasing salinity in the soil seriously limits ramie growth and development. The best effective approach of fighting against salinity is development of the tolerant crop varieties. Hence, it is important to identify the genetic resources with high tolerance, and to understand the mechanisms of salinity tolerance in plants (Sabir *et al*. 2011). The present study evaluated in two contrasting varieties of ramie the effect of salinity stress on gas exhange, pigment contents, water status, proline synthesis, lipid membrane peroxidation, and antioxidative systems in order to better understand the basis of the salt resistance.

Materials and methods

Experimental design: This experiment was carried out during the summer 2012 in a greenhouse at College of Agronomy and Biotechnology, Southwest University, Chongqing, China (latitudes 29°49′32″N, longitudes 106°26′02″E, and altitude 220 m). Two ramie (*Boehmeria nivea* L.) cultivars, Chuanzhu-12 (ST, salt-tolerant cultivar) and Xiangzhu-2 (SS, salt-sensitive cultivar), obtained from Dazhou Institute of Agricultural Sciences, Sichuan, China, were used. The seedlings were planted during the previous autumn as rhizomes; regrowth started early in March after a dormancy of 4 months. For salt stress treatment, sodium chloride [8 g(NaCl) kg^{-1}(dry soil)] was mixed with the dry soil in plastic pots (28 cm in diameter, 24 cm in depth, 10 kg of dry soil) two months before sowing, while the controls were grown without NaCl addition. Irrigation was done daily to yield a field capacity to promote the incorporation of NaCl into the soil. The soil contained 5.20 g(organic matter) kg^{-1}, 0.50 g(total nitrogen) kg^{-1}, 1.28 g(total phosphorus) kg^{-1}, 3.25 g(total potassium) kg^{-1}, 150.53 mg(alkali-hydro nitrogen) kg^{-1}, 23.03 mg(available phosphorus) kg^{-1}, 55.21 mg(readily-available potassium) kg^{-1}, pH 6.66, and 0.038 g(Na^+) kg^{-1}. On 13 April 2012, 10 cm tall young plants from the nursery beds in the fields were transplanted into pots, one seedling per pot. After transplanting, watering was performed daily to maintain the soil at the field capacity. The pots were arranged in a completely randomized design (CRD) and five replications of each experimental unit with 10 pots per treatment. The greenhouse temperature, relative humidity, and photosynthetically active radiation varied in a range of 18.5–34.0°C, 40.5–72.9%, and 217.9–667.0 μmol m^{-2} s^{-1}, respectively, during the entire experimental period.

Plants: Gas-exchange parameters were measured at the seedling stage (after 30 d from the seedling transplantation, DAT). The plants were sampled (6–7th leaf from the top of plants) after 30 DAT to assess the Chl content, the relative water content (RWC), protein, proline, MDA contents, and the activity of superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.6), and catalase (CAT, EC 1.11.1.7). After washing, the leaves were frozen in liquid nitrogen and stored at –80 °C until biochemical analysis.

Gas-exchange measurements: Net photosynthesis (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 (C_i) were evaluated using a portable gas exchange system (*LI-6400*, *LI-COR*, Lincoln, USA), which was equipped with a leaf chamber (*LI-6400-40 LCF*). The gas exchange analysis was made during 8:40–11:40 h. The 6–7th leaf from the top of ramie plants were measured following the methods of Liu (2010). Sixteen leaves were selected for each treatment with the following adjustments: molar flow of air per unit of leaf area was 499.57 mmol mol^{-1} m^{-2} s^{-1}, water vapour pressure in the leaf chamber was 3.72 mPa, PAR at leaf surface was up to 1,000 μmol m^{-2} s^{-1}, temperature of leaf ranged from 33.25 to 35.02°C, ambient temperature was 34.79–36.08°C, ambient CO_2 concentration was 390.87 mol mol^{-1}, and relative humidity (RH) was 54.63%. Water-use efficiency (WUE) was calculated as the ratio of P_N to E. Intrinsic water-use efficiency (WUE_i) was calculated as the ratio of P_N and g_s. The C_i/C_a ratio was calculated as the ratio between intercellular CO_2 and ambient CO_2 concentration.

Photosynthetic pigments: Chl *a*, *b*, and Chl (*a*+*b*) were analyzed according to the methods of Arnon (1949).

Carotenoids (Car) were measured according to Lichtenthaler and Wellburn (1983). Samples containing 100 mg of fresh leaf matter were extracted at dark place for 48 h with 10 mL of 95.5 % acetone and absolute ethylalcohol (1:1, v/v) ratio until the sample changed to white (Huang *et al.* 2014). Pigment concentrations were measured using an ultravoilet (UV)-visible spectrophotometer (*UC-5500PC*, *Shanghai Yuanxi Co., Ltd*. Shanghai, China). The absorbance was recorded at 470, 645, 652, and 663 nm.

RWC, free proline, and MDA: The RWC was determined using the methods of Yamasaki and Dillenburg (1999) by recording the fresh mass (FM); then the leaf samples were immersed into the distilled water for 12 h for turgid mass (TM). Leaves were then drying at 70ºC for 48 h to determinate the dry mass (DM). The RWC was calculated as: RWC = [(FM – DM)/(TM – DM)] × 100.

Proline concentration was measured following the methods of Bates *et al*. (1973), using a standard curve and calculating on a FM basis. Fresh leaf samples of 0.5 g were ground in 5 mL of 3% sulphosalicylic acid and the mixture was centrifuged at 4,000 × *g* for 10 min; 2 mL of the filtrate was mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid in a test tube. The mixture was incubated in water bath for 40 min at 97–99°C and then cooled at room temperature. The mixture was extracted with 4mL of toluene and the absorbance was measured at 520 nm.

The MDA concentration was measured as described by De Vos *et al*. (1991). Fresh leaves (0.5 g) were homogenized in 5 mL of 5% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 4,000 × *g* for 10 min at 25°C and 3 mL of 2-thiobarbituric acid in 20% trichloroacetic acid was added to 2 mL aliquot of the supernatant. The mixture was incubated in boiled water (97–99°C) for 10 min, then cooled rapidly in an ice bath. After centrifugation at 3,000 × *g* for 10 min, the absorbance was recorded at 450, 532, and 600 nm. MDA content in the aqueous phase was calculated based on the following formula: $C\ (\mu mol\ L^{-1}) = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$.

Soluble protein and the activities of antioxidative enzymes: The soluble protein concentration was determined according to the method of Bradford (1976), using bovine serum albumin (BSA) as a standard. Frozen leaf samples (500 mg) were ground in liquid nitrogen with a mortar and pestle and homogenized in 5 mL of 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA-Na_2 and 2% (w/v) polyvinylpyrrolidine-40 (PVP-40). The homogenate was centrifuged at 12,000 × *g* for 20 min at 4°C. The supernatant was collected and used for antioxidant enzyme activity analysis. One mL of Bradford solution was added to 0.1 mL of the crude extract and absorbance was recorded at 595 nm for estimation of the total protein content. The protein concentration was calculated from a BSA standard curve.

The POD activity was determined spectrophotometrically as described by Upadhyaya *et al*. (1985). The reaction mixture (3.9 mL) contained 50 mM phosphate buffer (pH 7.0), 28 µL of guaiacol, 100 µL of the enzyme extract, and 19 µL of H_2O_2. The POD activity was measured following the increase of absorbance at 420 nm due to guaiacol oxidation. The absorbance was recorded per 30 s for at least 2 min. One unit of POD activity was defined as $\mu mol\ min^{-1}\ mg^{-1}$(protein) using the exctinction coefficient of 26.6 mM cm^{-1}. Both the SOD and CAT activities were measured by ready kits provided by Nanjing Jiancheng Bioengineering Institute, China. Assay for SOD and CAT were carried out using the protocol mentioned with the detection kit. One unit of SOD activity was defined as the amount of enzyme required for 1 mg of tissue proteins in 1 mL of a reaction mixture SOD inhibition rates to 50% inhibition of SOD as monitored at 550 nm by spectrophotometer (*UC-5500PC, Shanghai Yuanxi Co., Ltd*. Shanghai, China) and calculated per mg of proteins. One unit of CAT activity was defined in $\mu mol(H_2O_2)\ s^{-1}\ mg^{-1}$(protein) at 405 nm. Both SOD and CAT activity were expressed as enzyme units per mg of protein [$U\ mg^{-1}$(protein)].

Statistical analysis: Data were analyzed by one-way analysis of variance (*ANOVA*) test using *SPSS16.0* (*SPSS Inc*., Chicago, IL, USA) for *Windows*. Means were compared using *Newman-Keul*s test at 5% level of significance. Linear regression equations between physiological and biochemical parameters are also reported.

Results

Gas exchange: The gas-exchange attributes of ramie seedlings were noticeably affected by salinity. Both cultivars showed parallel impairment in P_N, E, g_s, C_i, and the C_i/C_a ratio, while WUE and WUE_i changed in the opposite direction under salinity stress (Table 1). In ST, P_N, E, g_s, and C_i decreased by 25.0%, 41.3%, 41.1%, and 8.4%, respectively, compared with the control. In SS, however, P_N, E, g_s, and C_i were reduced by 43.0%, 54.2%, 55.9%, and 7.7%, respectively, in comparison with the control. The considerably higher reduction in P_N, E, and g_s was observed in SS than that in ST (Table 1).

Photosynthetic pigments: Upon exposure to salinity, photosynthetic pigment concentration of ramie cultivars declined in comparison to their controls (Table 2). Salinity led to substantial decrease in Chl *a*, *b*, Chl (*a*+*b*),

Table 1. Gas-exchange traits and water-use efficiency of seedlings in two ramie cultivars grown under salinity stress. P_N – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; WUE – water-use efficiency; WUE_i – intrinsic water-use efficiency; C_i – intercellular CO_2 concentration; C_i/C_a –intercellular CO_2 to ambient CO_2 concentration. ST – salt-tolerant cultivar Chuanzhu-12; SS – salt-sensitive cultivar Xiangzhu-2. Values in the table are mean ± SE (n = 5). Values followed by *the different letter* within columns differ significantly according to *Newman–Keuls*' test (p<0.05).

Treatment	P_N [μmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	g_s [μmol m^{-2} s^{-1}]	WUE [μmol $mmol^{-1}$]	WUE_i [μmol $mmol^{-1}$]	C_i [μmol mol^{-1}]	C_i/C_a
ST control	15.76 ± 0.51^a	5.81 ± 0.38^b	0.292 ± 0.014^a	2.68 ± 0.03^b	54.91 ± 2.35^c	277.80 ± 3.73^a	0.722 ± 0.011^a
ST salt	11.80 ± 0.77^b	3.41 ± 0.14^c	0.172 ± 0.004^b	2.97 ± 0.04^a	70.08 ± 2.68^b	254.58 ± 4.19^b	0.651 ± 0.019^{bc}
SS control	17.14 ± 0.68^a	7.31 ± 0.32^a	0.290 ± 0.009^a	2.36 ± 0.02^c	59.81 ± 2.16^c	259.19 ± 3.25^b	0.687 ±0.010^{ab}
SS salt	9.77 ± 0.48^c	3.35 ± 0.13^c	0.128 ± 0.006^c	2.90 ± 0.06^a	78.26 ± 2.60^a	239.34 ± 3.85^c	0.634 ± 0.009^c

Table 2. Chlorophyll (Chl) *a*, Chl *b*, total Chl (*a*+*b*), Chl *a*/*b* ratio, carotenoids (Car), and Chl (*a*+*b*)/ Car) ratio of seedlings in two ramie cultivars grown under salinity stress. FM – fresh mass. ST – salt-tolerant cultivar Chuanzhu-12; SS – salt-sensitive cultivar Xiangzhu-2. Values in the table are mean ± SE (n = 5). Values followed by *the different letter* within columns differ significantly according to *Newman–Keuls*' test (p<0.05).

Treatment	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Chl (*a*+*b*) [mg g^{-1}(FM)]	Chl *a*/*b*	Car [mg g^{-1}(FM)]	Chl (*a*+*b*)/Car
ST control	2.16 ± 0.04^a	0.906 ± 0.016^b	3.35 ± 0.06^a	2.39 ± 0.03^{bc}	8.48 ± 0.12^a	0.395 ± 0.009^a
ST salt	1.77 ± 0.02^b	0.720 ± 0.011^c	2.70 ± 0.05^b	2.45 ± 0.02^b	6.78 ± 0.13^b	0.403 ± 0.008^a
SS control	2.21 ± 0.03^a	0.958 ± 0.024^a	3.45 ± 0.09^a	2.31 ± 0.04^c	8.61 ± 0.20^a	0.407 ± 0.008^a
SS salt	1.35 ± 0.01^c	0.517 ± 0.008^d	1.97 ± 0.01^c	2.61 ± 0.04^a	4.63 ± 0.08^c	0.426 ± 0.010^a

and Car by 18.1%, 20.5%, 19.4%, and 21.0% in ST, respectively, whereas, a comparatively larger reduction by 38.9%, 46.0%, 42.9%, and 46.3% were found in SS, respectively. The Chl *a*/*b* ratio was not affected by salt treatments in ST, in contrast to the significant increase in SS. There was no significant difference in the Chl/Car ratio neither due to salinity nor between the varieties (Table 2).

Soluble protein, RWC, proline, and MDA: As the result of the salinity treatment, both soluble protein contents and RWC declined, while proline accumulation enhanced in both ramie cultivars (Table 3). Salinity stress led to a notable decrease in RWC (13.8%) and protein content (42.0%) in ST. However, greater reductions in RWC (23.8%) and protein content (59.4%) were recorded in SS (Table 3). The biosynthesis of proline was activated under salinity stress in ramie leaves. Leaf free proline accumulated more in ST (34.1% increase) than that of SS (13.2%). Higher proline content was also observed in ST compared to SS both under salinity and control conditions (Table 3). Nonetheless, the trends of MDA content in both ramie cultivars were completely disparate when exposed to salinity. MDA content rose by 32.6% in SS, whereas it dropped by 19.0% in ST (Table 3).

Antioxidative enzymes: Salt stress resulted in significant modulation of antioxidant defense in leaves of ramie seedlings (Table 3). Higher activities of SOD, POD, and CAT were found in ST in comparison to SS (Table 3). The SOD activity was enhanced by 29.9% and 28.4% in ST and SS, respectively. However, the CAT activity was reduced by 16.7% and 27.4% in ST and SS, respectively. Interestingly, the trends in the activity of POD differed evidently between both ramie cultivars. The POD activity increased by 32.8% in ST, whereas it declined by 29.9% in SS.

Table 3. Relative water content (RWC), soluble protein, malonialdehyde (MDA), and leaf proline content, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities of seedlings in two ramie cultivars grown under salinity stress. FM – fresh mass. ST – salt-tolerant cultivar Chuanzhu-12; SS – salt-sensitive cultivar Xiangzhu-2. Values in the table are mean ± SE (n = 5). Values followed by *the different letter* within columns differ significantly according to *Newman–Keuls*' test (p<0.05).

Treatment	RWC [%]	Soluble protein [mg g^{-1}(FM)]	MDA [μmol g^{-1}(FM)]	Proline [μg g^{-1}]	SOD [U mg^{-1}(protein)]	POD [U mg^{-1}(protein)]	CAT [U mg^{-1}(protein)]
ST control	88.57 ± 1.80^a	11.80 ± 0.50^a	20.92 ± 1.11^b	109.72 ± 2.05^b	17.53 ± 0.62^c	83.86 ± 1.65^b	0.688 ± 0.020^b
ST salt	76.36 ± 1.61^b	6.84 ± 0.20^b	15.78 ± 0.82^c	147.15 ± 3.20^a	22.77 ± 0.75^b	111.33 ± 2.79^a	0.573 ± 0.023^c
SS control	89.29 ± 1.69^a	7.42 ± 0.17^b	19.75 ± 1.33^b	85.37 ± 1.85^d	21.29 ± 0.81^b	78.11 ± 1.91^b	0.870 ± 0.026^a
SS salt	68.08 ± 1.45^c	3.01 ± 0.09^c	24.39 ± 1.09^a	96.63 ± 1.44^c	27.33 ± 1.22^a	54.78 ± 1.64^c	0.632 ± 0.018^{bc}

Relationships between physiological and biochemical parameters: The P_N was linearly and positively correlated with g_s and C_i (Fig. 1*A*,*B*), whereas, there was a negative and linear relationship of P_N with MDA content (Fig. 1*C*). Significant, positive correlation was recorded between P_N and Chl *a*, Chl *b*, Chl (*a*+*b*), and Car (Fig. 1*D*-*G*). Although g_s was linearly and positively correlated with C_i and *E* (Fig. 2*A,B*), it was linearly and negatively correlated with WUE_i (Fig. 2*C*). Similar relationship was observed between *E* and WUE (Fig. 3). MDA was linearly and negatively correlated with the activity of SOD, POD, and CAT as well as the content of Car and leaf proline (Fig. 4*A*-*E*).

Discussion

The present study was conducted to elucidate the mechanism of salt tolerance in two contrasting ramie cultivars. Both cultivars responded differently to NaCl stress as we observed by changes in water relations, gas exchange, photosynthetic pigments, soluble protein contents, MDA and proline accumulation, and activities of antioxidative enzymes.

Salinity stress can detrimentally affect photosynthesis (Cha-um and Kirdmanee 2009). It may lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, resulting in lower photosynthesis (Gossett *et al*. 1994, Mundree *et al.* 2002). In the present study, P_N, *E*, g_s, and C_i of both ramie cultivars were markedly reduced, while in contrast, WUE and WUE_i

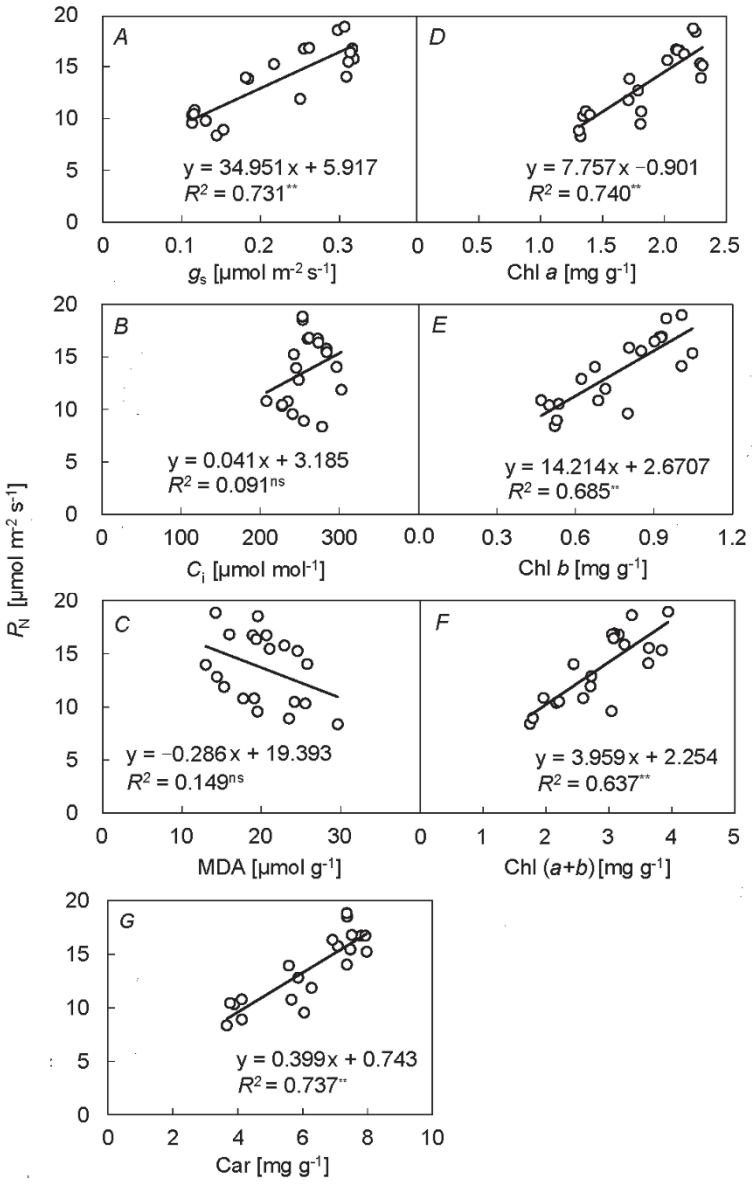

Fig. 1. Relationship between the net photosynthetic rate (P_N) and stomatal conductance (g_s), intercellular CO_2 (C_i), malondialdehyde content (MDA), chlorophyll (Chl) *a*, Chl *b*, total Chl (*a*+*b*), and carotenoids (Car) of ramie seedlings grown under salinity stress (n = 20): * – significant at p<0.05; ** – significant at p<0.01; ns – not significant.

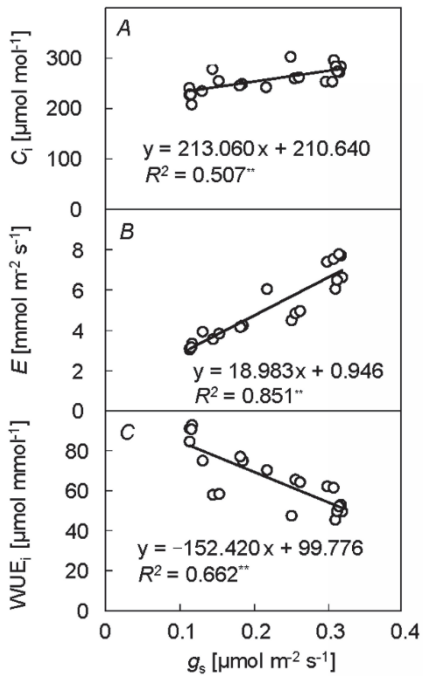

Fig. 2. Relationship between stomatal conductance (g_s) and intercellular CO_2 (C_i), transpiration rate (E), intrinsic water-use efficiency (WUE$_i$) of ramie seedlings grown under salinity stress (n = 20): * – significant at p<0.05; ** – significant at p<0.01; ns – not significant.

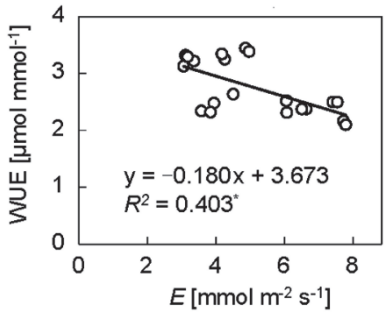

Fig. 3. Relationship between transpiration rate (E) and water-use efficiency (WUE) of ramie seedlings grown under salinity stress (n = 20): * – significant at p<0.05; ** – significant at p<0.01; ns – not significant.

improved with salinity. Lesser reduction in P_N, E, g_s, and C_i was observed in ST cultivar, Chuanzhu 12, as compared to SS, Xiangzhu 2 (Table 1, Figs. 1*A*,*B*, 2*A*). This could be attributed to salt-induced stomatal closure that prevented the transpirational water loss from the cell of plants, resulting in a low E (Table 1, Fig. 2*B*). This in turn led to tissue water maintenance and an improvement in WUE and WUE$_i$ under salt condition (Table 1, Figs. 2*C*, 3). In comparison to SS cultivar, the ST cultivar demonstrated salt tolerance by maintaining higher P_N due to lesser reduction and higher g_s and C_i under saline conditions (Table 1, Figs. 1*A*, 2*A*). Our findings are in accordance with the results which has been reported in cucumber (Huang *et al*. 2011), winter radish (Munir *et al*. 2013), and cotton (Shaheen *et al*. 2012) cultivars.

In addition to stomatal factors, pigment contents showed a positive relationship with the photosynthetic rate. Salinity stress, however, variously affects the biosynthesis of these pigments (Yadav *et al*. 2011). Decreased or unchanged Chl and Car during salt stress have been reported in many species, depending on the duration and severity of salt treatment (Panda and Khan 2009, Li *et al*. 2010, Sadder *et al*. 2013). In our study, the decline in Chl *a*, Chl *b*, Chl (*a*+*b*), and Car was observed in leaves of ramie seedlings. The loss of Chl is accompanied by inactivation of photochemical reactions, especially those mediated by PSII in plants exposed to salt stress (Sharma and Hall 1992). The reduction of photosynthesis under salt stress was associated also with the reduction of photosynthetic pigment contents (Fig. 1*D*-*G*). Nonetheless, the smaller decrease in the Chl content might contribute to the greater P_N in ST than in SS (Table 2).

Salt stress caused the sharp decrease in soluble proteins, while leaf proline changed conversely in both ramie cultivars. Lesser reduction in soluble proteins and higher accumulation of proline enabled the ST cultivar to maintain tissue water potential. This could help in osmoregulation and maintenance of the cell turgor under stress conditions (Rhodes and Samaras 1994), which in turn prevented water loss from the cell and led to the maintenance of comparatively higher RWC in ST (Table 3).

When exposed to NaCl stress, ROS are continuously produced in plants, which causes oxidative stress to plants (Gill and Tuteja 2010). The content of MDA is usually used as a tool to assess the severity of the oxidative damage and the degree of plant sensitivity (Pérez-López *et al*. 2009). The amount of MDA in the leaves was strongly variable in two ramie cultivars (Table 3), which indicated SS as more sensitive to salinity, along with a higher degree of lipid peroxidation than that in ST. Salt sensitive species or genotypes differ from the more tolerant ones by inability to scavenge excessive ROS in leaves. This is closely linked to nonenzymatic antioxidants and the activity of antioxidant enzymes.

As nonenzymatic antioxidants, Car are responsible for quenching of singlet oxygen ($^1O_2$) (Knox and Dodge 1985). When exposed to salinity stress, ST cultivar showed lesser reduction and higher content of Car than SS cultivar (Table 2), suggesting that the concentration of Car in the variety might determine its relative tolerance. This could be aparent from the significantly negative and linear relationships between Car and MDA content (Fig. 4*D*). Similar results were also reported in corn cultivars subjected to salinity stress (Hichem *et al*. 2009).

Proline is also considered as effective scavenger of ·OH (Smirnoff 1989). In fact, proline can now be added to the elite list of nonenzymatic antioxidants, which plants

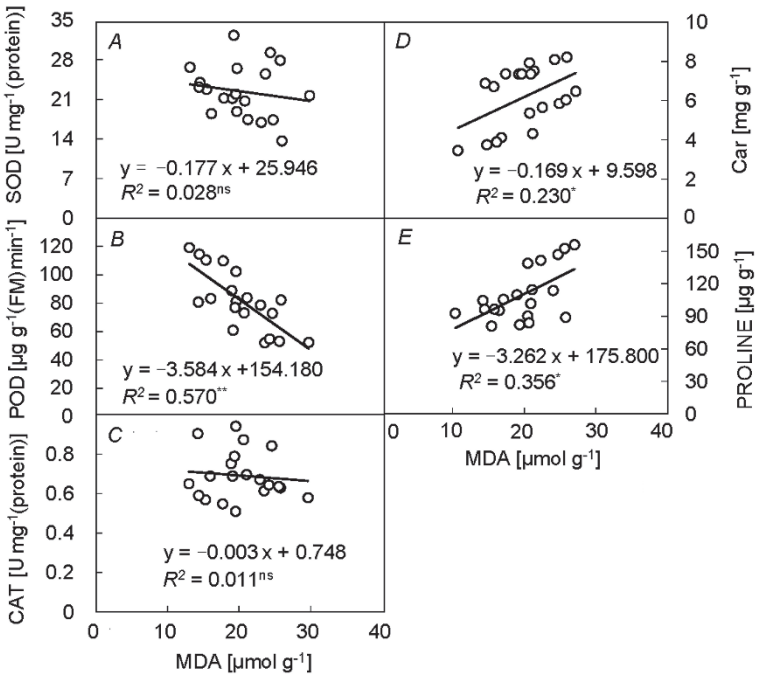

Fig. 4. Relationship between malondialdehyde content (MDA) and superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), carotenoids (Car), and proline of ramie seedlings grown under salinity stress (n = 20). * – significant at p<0.05; ** – significant at p<0.01; ns – not significant.

need to counteract the inhibitory effects of ROS (Chen and Dickman 2005).

In this study, a negative correlation between proline and MDA was detected in ST (Table 3), which meant that oxidative stress tolerance was enhanced and MDA decreased. In contrast, the increase in proline was accompanied by the increase in MDA in SS (Table 3), likely as a result of a high demand for detoxification of ROS and protection of membrane integrity. Nonetheless, the proline content was markedly higher in ST than that in SS. This suggested the accumulation of proline under salinity stress was correlated with salt tolerance (Fig. 4*E*).

Salt tolerant cultivars have generally the enhanced or higher constitutive antioxidant enzyme activity under salt stress when compared with the sensitive cultivars (Rout and Shaw 2001). Upon salinity stress, the two ramie cultivars exhibited different trends in the activity of SOD, CAT, and POD. In ST, although CAT activity decreased, SOD and POD activity increased compared with the control (Table 3). These antioxidant enzymes cooperated with each other to scavenge ROS in plant cells. The ROS was well scavenged by the scavenging system, and damage to membranes was controlled, leading to the increase of tolerance to oxidative stress, thus MDA contents declined. In contrast, in SS, SOD activity increased, while POD and CAT activity decreased as compared with the control. This was accompanied by the accumulation of MDA (Table 3). It seemed that the activity of POD and CAT was not sufficient for the complete scavenging of H_2O_2, thus, H_2O_2 content elevated. Increased H_2O_2 severely destroyed cell membrane stability and caused lipid peroxidation and accumulation of MDA (Table 3). The increase in MDA in SS could be ascribed to the low activity of POD and CAT under salinity stress. A high concentration of H_2O_2 in plant cells directly inhibits CO_2 fixation, resulting in lower photosynthetic capacity (Yamazaki *et al*. 2003); it was represented by the negative relationship between P_N and MDA (Fig.1*C*). The damage of oxidative stress was a critical factor for considerably decreased P_N in SS (Table 1, Fig. 1*D*). Nonetheless, higher activities of SOD, POD, and CAT and lower MDA content were recorded in ST than in SS (Table 3); it suggests that salt-tolerant ramie varieties might be better protected against ROS by increasing the activity of antioxidant enzymes under salt stress. Thus, it indicated that salt tolerance was related to a higher activity of antioxidant enzymes (Fig. 5*A*-*C*). Our results were analogous to those previously found in cotton cultivars (Meloni *et al.* 2003), mustard cultivars (Khan *et al*. 2009), and perennial ryegrass cultivars (Hu *et al*. 2012).

Conclusion: Salinity caused detrimental effects by modulating the content of nonenzymatic antioxidants, the activity of antioxidant enzymes, and gas exchange parameters in ramie seedlings. Salt-tolerant ramie cultivar Chuanzhu-12 performed better under salinity condition than the salt-sensitive cultivar Xiangzhu-2 due to the higher photosynthetic capacity, chlorophyll contents, and maintenance of tissue water contents, as well as more efficient enzymatic and nonenzymatic antioxidant defense systems. This revealed the salt tolerance mechanism of ramie plants for adaptation to salt affected soil.

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