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Systematic salt tolerance-related physiological mechanisms of wild soybean and their role in the photosynthetic activity and Na^+ distribution of grafted soybean plants

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Abstract

Systematic salt tolerance-related physiological mechanisms in roots and shoots of halophyte Dongying wild soybean have not yet been thoroughly studied. In this study, photosynthesis, modulated 820-nm reflection, chlorophyll *a* fluorescence, and Na^+ distribution in cultivated (G_{mc}) and wild (G_{sw}) soybean leaves of grafted soybean plants were investigated after NaCl treatment. Results showed that the decreases in photosynthetic rate, performance index, active P_{700} content, and plastocyanin reduction were significantly greater in the G_{sw} leaves than those in the G_{mc} leaves. The observed increases in the Na^+ concentration in the G_{sw} leaves were likely responsible for the severe decrease in the photosynthetic activity of grafted plants. We suggest that Na^+ accumulation in G_{sw} roots, which prevents the transport of Na^+ from the roots to the shoots, effectively maintains the concentration of Na^+ at a comparatively low level in the leaves to prevent the destruction of the photosynthetic apparatus by salt.

Keywords: grafting; ion distribution; photosynthetic activity; salt resistance; wild soybean.

Introduction

Soybean, as a major source of plant proteins and oils, is a crop of significant importance (Miransari 2016, Liu *et al.* 2020). However, its production can dramatically decrease when grown in salinized soil, so it has been listed among crops with moderate salt tolerance. Thus, developing new salt-tolerant soybean cultivars is significantly meaningful for making use of marginal land and ensuring food security. The traditional breeding of cultivated soybean prioritizes high yields to meet human needs, leading to several gene losses that are essential for accommodating the environment (Qi *et al.* 2014). Therefore, it is necessary to introduce salt-tolerance genes from exotic soybean

Highlights

- Increases in Na^+ concentration in G_{sw} leaves are responsible for the decrease of P_N
- Na^+ accumulation in G_{sw} roots prevents its transport from roots to shoots
- Maintaining a low Na^+ concentration in G_{sw} leaves protects the photosynthetic apparatus

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Abbreviations: ABS/RC – absorption flux per RC; CE – carboxylation efficiency; C_i – intercellular CO_2 concentration; G_{mc} – cultivated soybean; g_s – stomatal conductance; G_{sw} – Dongying wild soybean; PI_{ABS} – performance index; P_N – net photosynthetic rate; RC – reaction center; V_{ox} – the maximal slopes of the rates of P_{700} and plastocyanin oxidation; V_{red} – the maximal slopes of the rates of P_{700} and plastocyanin reduction; V_t – relative variable fluorescence at the time t; φ_{Po} – maximum quantum yield for primary photochemistry; Ψ_{Eo} – the probability that an electron moves further than Q_A^-.

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germplasm resources through traditional or transgenic breeding methods.

The progenitor of cultivated soybean is wild soybean (*Glycine soja* Sieb. et Zucc.), which has greater genetic diversity than that of cultivated soybean (Chen and Nelson 2004). Moreover, it has been proven that reproduction can occur between wild and cultivated soybeans (Lam *et al*. 2010). Hence, wild soybean is considered a vital germplasm resource to promote the resistance of cultivated soybean to abiotic and biotic stresses. Many studies have already been performed to test the mechanism of salt tolerance in wild soybean at the genome (Qi *et al*. 2014), transcriptome (Tuyen *et al*. 2010), proteome (Lee *et al*. 2009), and metabolome levels (Yang *et al*. 2017). Qi *et al*. (2014) identified an ion-transporter gene, *GmCHX1*, that maintains a low Na^+/K^+ ratio under salt stress conditions in wild soybean. Ji *et al*. (2010) found that overexpression of a glutathione S-transferase gene, *GsGST*, in wild soybean can enhance transgenic tobacco tolerance to drought and salt. Moreover, many studies have made great efforts to demonstrate the physiological mechanisms by which wild soybean accommodates saline stress, such as changes in photosynthetic activity, induction of antioxidant activity, synthesis of osmolytes, and accumulation of Na^+ in roots. Based on our previous studies, the halophyte Dongying wild soybean (*Glycine soja* Sieb. et Zucc. ZYD 03262) can maintain photosynthetic activity at a higher level under salt stress conditions than cultivated soybean due to a mechanism that maintains the Na^+ concentration in shoots at a low level through the accumulation of this ion in the roots (Chen *et al*. 2013, Xue *et al*. 2014). However, the systematic salt tolerance-related physiological mechanisms in the roots and shoots have not yet been thoroughly studied.

In this research, we performed a grafting experiment to comparatively study the photosynthetic activity and ion distribution of grafted plants after treatment with different NaCl concentrations to explore the function of roots in the salt tolerance-related physiological mechanisms of wild soybean. This research can provide a thorough exploration of salt tolerance physiological mechanisms in wild soybean and may assist in the breeding of cultivated soybean.

Materials and methods

Plant materials and treatments: Dongying wild soybean (G_{sw}) seeds were obtained from the estuary of the Yellow River in Kenli County, Shandong Province, China. The wild soybean seeds were immersed in concentrated sulfuric acid for 3 min to remove the seed coat. The cultivated soybean [*Glycine max* (L.) Merr. Shanning 11] (G_{mc}) seeds purchased from the market were cultivated in Shandong Province, China, on a large scale. Then, both wild and cultivated seeds were put on vermiculite for germination. When the unifoliolate leaves began to expand (10 d after sowing), plug grafting was conducted following the methods described by Pan *et al*. (2011) with some modifications. The unexpanded compound leaves of G_{mc} were removed, and a hole was made at an angle of 30 degrees along the stem by using bamboo sticks. On the other hand, the scion of G_{sw} was whittled into a wedge shape at an angle of 30 degrees less than 1 cm below the cotyledons and was then inserted into the hole of the G_{mc} rootstock (Fig. 1*A*). Then, the grafted plants were maintained indoors at ambient temperature (20–28°C) in the dark with good ventilation. After 2 d, the successfully grafted seedlings were transplanted to pots with a diameter of 25 cm and a height of 40 cm that contained quartz sand without other soluble minerals. Three plants were arranged in one pot. The grafted plants were grown in a greenhouse in which the temperature was controlled at 20–28°C (night/day) and the PPFD was controlled at 0–300 μmol m^{-2} s^{-1}. To protect the plants from the influence of nutrient or drought stress, ½ Hoagland solution, which contained 5 mM KNO_3, 5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2 mM $MgSO_4 \cdot 7H_2O$, 1 mM KH_2PO_4, 0.1 mM EDTA-Fe, 47 μM H_3BO_3, 1 μM $MnCl_2 \cdot 4H_2O$, 1 μM $ZnSO_4 \cdot 7H_2O$, 0.25 μM $CuSO_4 \cdot 5H_2O$, and 0.01 μM H_2MoO_4, was supplied to the plants every day. A new growth point developed between the cotyledons of G_{mc}, so the grafted plant had both G_{sw} and G_{mc} shoots (Fig. 1*B*).

In addition, NaCl treatments were initiated at the moment of full expansion of the second compound leaf, and ½ Hoagland solution alone was used as the control. NaCl was added to the Hoagland solution to generate solutions with final NaCl concentrations of 50 and 100 mM. The NaCl concentrations in the solutions were increased by 50 mM NaCl daily until the final concentrations (50, 100 mM) were achieved. To maintain the NaCl concentrations during the treatment, the treatment solutions were used to flush the pots, and ½ Hoagland solution was used for the control. The amount of flushing solution used was 2 times the total water content of the quartz sand in

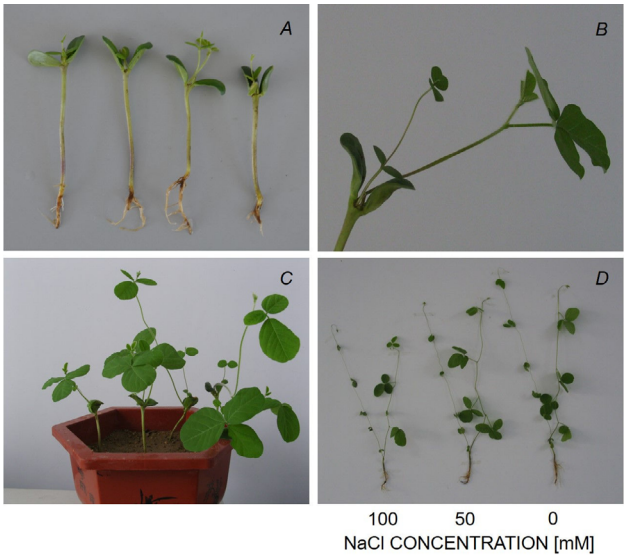

Fig. 1. Growth of the grafted soybean plants on different days. (*A*) The growth of grafted soybean plants after grafting. (*B*) The grafting sites. (*C*) The growth of the grafted soybean plants after transplantation. (*D*) The growth of the grafted soybean plants after treatment with 0, 50, and 100 mM NaCl for 12 d.

each pot. The treatments continued for 12 d at the final concentrations. Then, four pots for each treatment were used for measurement (Fig. 1*C*,*D*).

Morphological traits: The number of leaves and the lengths of the roots, rootstock, and shoots were measured when the experiment was complete. The measurements were conducted on five different plants for each treatment.

Photosynthetic parameters: According to Xue *et al.* (2014), the photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and carboxylation efficiency (CE) were determined by a *CIRAS-2* portable photosynthetic system (*PP Systems*, USA), which controls the PPFD at 1,200 μmol m^{-2} s^{-1}, CO_2 concentration at 360 μmol mol^{-1}, and temperature in the leaf chamber at 25°C.

Chlorophyll (Chl) *a* fluorescence and modulated 820-nm reflection transients were measured by using a multifunctional plant efficiency analyzer (*MPEA*, *Hansatech*, UK). The grafted plants were illuminated with the natural light of 800 μmol(photon) m^{-2} s^{-1} for 1 h. Then, leaf dark-adaptation clips were used on the G_{sw} and G_{mc} leaves before measurement for 30 min. Then, the leaves were illuminated in an orderly manner by a red light for 1 s [627 nm; 5,000 μmol(photon) m^{-2} s^{-1}], far red light for 10 s [735 nm, 200 μmol(photon) m^{-2} s^{-1}], and red light for 2 s [627 nm; 5,000 μmol(photon) m^{-2} s^{-1}]. The modulated 820-nm reflection and Chl *a* fluorescence were noted at the same time throughout illumination. The Chl *a* fluorescence transients were calculated based on the JIP-test proposed by Strasser *et al.* (2004). Based on the modulated 820-nm reflection (MR) signal, the relative value of the maximal difference of 820-nm reflection throughout the final 2 s of red light illumination was taken as the MR/MR_0 ratio (Oukarroum *et al*. 2013). MR represents the value of the maximal difference in the 820-nm reflection in the final 2 s of red light illumination, and MR_0 represents the value of the 820-nm reflection at 0.7 ms (the first reliable MR measurement). Then, the maximal slopes of the rates of P_{700} and PC oxidation (V_{ox}) and the subsequent re-reduction (V_{red}) were calculated from the kinetics of the light-induced changes in MR/MR_0 (Salvatori *et al*. 2014).

Na^+ concentrations: The dried grafted soybean plants, which were divided into three parts of roots, shoots, and leaves, were ground to a fine powder and digested in 6 ml of nitric acid and hydrogen peroxide (2:1, v/v). Then, the Na^+ concentrations were measured by an inductively coupled plasma mass spectrometer (*Agilent 7700X*, *Agilent Technologies*, USA).

Statistical analysis: One-way analysis of variance (*ANOVA*) was used to assess all the data for significant differences ($P<0.05$) by *SPSS 22.0* statistical software. The *SigmaPlot 12* was used to construct graphs.

Results

Growth of the plants: The root length, rootstock length, and leaf number of the grafted soybean plants did not change in response to NaCl treatment, but the shoot length of G_{mc} and G_{sw} decreased by 22.8 and 31.5%, respectively, after 100 mM NaCl treatment (Table 1).

Photosynthesis: In this study, the P_N in both the G_{sw} and G_{mc} leaves of the grafted soybean plants decreased as the concentrations of NaCl in the nutrient solution increased (Fig. 2*A*). The decrease in the G_{sw} leaves was greater than that in the G_{mc} leaves. The P_N in the G_{sw} and G_{mc} leaves decreased by 38.0 and 20.0% after 100 mM NaCl treatment, respectively (Fig. 2*A*). However, the g_s in both types of leaves decreased after the NaCl treatment (Fig. 2*B*), the C_i in G_{sw} leaves increased significantly after the 100 mM NaCl treatment, and that in the G_{mc} leaves decreased when the concentration of NaCl increased (Fig. 2*C*). Moreover, no obvious change in CE in the G_{mc} leaves was observed after NaCl treatment, but the CE in the G_{sw} leaves decreased significantly after 100 mM NaCl treatment (Fig. 2*D*).

Chl *a* fluorescence: The shapes of the Chl *a* fluorescence transients in both the G_{mc} and G_{sw} leaves of the grafted soybean plants were altered by the NaCl treatments (data not shown). Moreover, the G_{sw} leaves showed considerable differences compared to the G_{mc} leaves, which is demonstrated by the relatively variable fluorescence intensity (Fig. 3). There were more distinct G_{sw} leaves in the 50 and 100 mM NaCl treatment groups than in the control group. Moreover, the parameters derived from the transients according to the JIP-test are shown in Table 2. The φ_{Po}, Ψ_{Eo}, and PI_{ABS} of the G_{sw} leaves decreased significantly, while the ABS/RC of the G_{sw} leaves increased significantly with increasing NaCl concentration, and these

Table 1. Changes in the number of leaves and the lengths of roots, rootstocks, and shoots of the grafted soybean plants after treatment with 0, 50, and 100 mM NaCl for 12 d. The data are means ± SD ($n = 5$). *The different lowercase letters* presented in each column show significant differences at $P<0.05$. G_{mc} – cultivated soybean; G_{sw} – Dongying wild soybean.

NaCl concentration [mM]	Root length [cm]	Rootstock length [cm]	Shoot length [cm]		Number of leaves	
			G_{mc}	G_{sw}	G_{mc}	G_{sw}
0	6.20 ± 1.30^a	4.40 ± 0.89^a	45.33 ± 5.50^a	65.66 ± 6.02^a	4.60 ± 0.55^a	4.60 ± 0.55^a
50	5.40 ± 0.55^a	4.00 ± 0.71^a	38.00 ± 5.29^b	44.00 ± 7.94^b	4.00 ± 0.70^a	4.00 ± 0.70^a
100	6.00 ± 0.82^a	4.50 ± 0.58^a	35.00 ± 3.60^b	45.00 ± 10.58^b	4.00 ± 0.22^a	4.25 ± 0.96^a

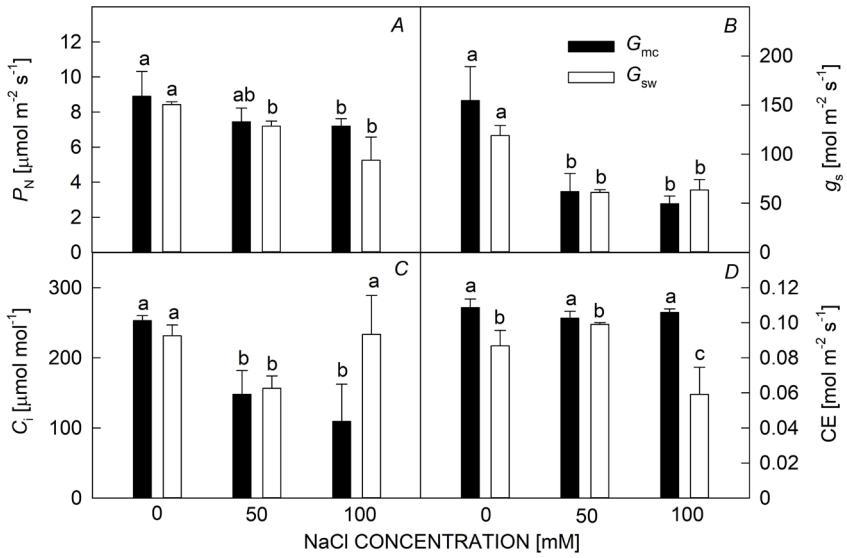

Fig. 2. Changes in the net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), intercellular CO_2 concentration (C_i) (*C*), and carboxylation efficiency (CE) (*D*) in the G_{sw} and G_{mc} leaves of grafted soybean plants after treatment with 0, 50, and 100 mM NaCl for 12 d. The values are means ± SD (n = 4). *Different lowercase letters* above the bars show significant differences at P<0.05.

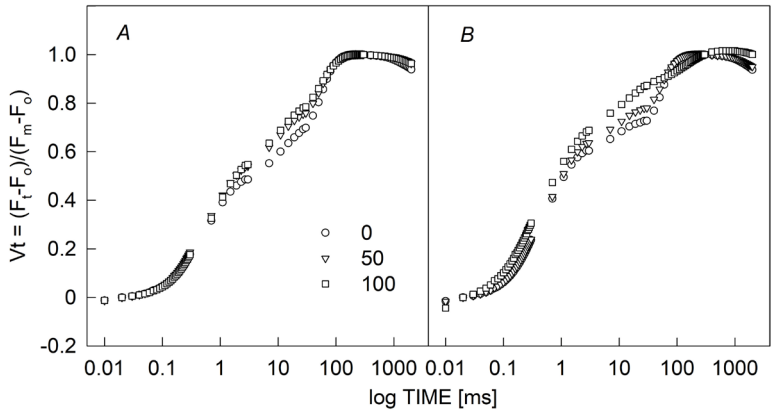

Fig. 3. Changes in the relative variable fluorescence at the time t (V_t) in the G_{mc} (*A*) and G_{sw} (*B*) leaves of the grafted soybean plants under PPFD of 800 μmol m^{-2} s^{-1} for 1 h after treatment with 0, 50, and 100 mM NaCl for 12 d. Ten measurements were taken for each treatment, after which the average result was calculated. G_{mc} – cultivated soybean; G_{sw} – Dongying wild soybean.

parameters did not change in the G_{mc} leaves, indicating that the electron transport of PSII in the G_{sw} leaves was more seriously destroyed than that in the G_{mc} leaves.

Modulated 820-nm reflection transients: The decrease in the MR/MR_0 ratio in both the G_{sw} and G_{mc} leaves on the grafted soybean plants was not altered by NaCl treatment, while the increase in the MR/MR_0 ratio in the G_{sw} leaves slowed under 100 NaCl treatment (Fig. 4). Correspondingly, there were no significant variations in the V_{ox} in either the G_{sw} or G_{mc} leaves on the grafted soybean plants, while the V_{red} in the G_{sw} leaves decreased more markedly than that in the G_{mc} leaves with increasing NaCl concentration in the nutrient solution (Fig. 5).

Na^+ distribution in the plants: The NaCl treatments had a marked effect on the Na^+ distributions in the roots, stems, and leaves of the grafted soybean plants. As shown in Table 3, the Na^+ concentrations in the grafted soybean plants increased obviously due to the increase in NaCl concentration in the nutrient solution. The Na^+ concentrations in the roots were 23.28 g kg^{-1} and 32.67 g kg^{-1} after 50 and 100 mM NaCl treatment for 12 d. The grafted soybean plants with both wild and cultivated soybean shoots had the same cultivated roots. Thus, the Na^+ concentrations in the roots were uniform in the G_{sw} and G_{mc} shoots after NaCl treatment. However, the Na^+ concentration in the G_{sw} stems and leaves was approximately 22.8 and 17.2% higher, respectively, than that in G_{mc} stems and leaves after 100 mM NaCl treatment (Table 3).

Discussion

Photosynthesis provides energy for the growth of plants and is very sensitive to salt stress (He *et al.* 2016). In general, a reduction in photosynthesis in leaves may occur due to stomatal or nonstomatal limitations, which are explained by osmotic stress and ionic toxicity in plants under increased salinity conditions (Kao *et al.* 2003, Sharkey *et al.* 2007). Observations by He *et al.* (2016) showed that the P_N was different among different soybean lines in the anthesis period and that stomatal limitation could occur due to reduced P_N under salt stress conditions. Tsai *et al.* (2019) pointed out that there is an obvious variation in the effect of salt on photosynthetic

Table 2. Chlorophyll *a* fluorescence parameters in the G_{sw} and G_{mc} leaves of grafted soybean plants under PPFD of 800 μmol m^{-2} s^{-1} for 1 h after treatment with 0, 50, and 100 mM NaCl for 12 d. Ten measurements were taken for each treatment, after which the average result was calculated. *Different lowercase letters* presented in the column show significant differences at $P<0.05$ between different treatments. φ_{Po} – maximum quantum yield for primary photochemistry; Ψ_{Eo} – probability that an electron moves further than Q_A^-; PI_{ABS} – performance index; ABS/RC – absorption flux per RC; G_{mc} – cultivated soybean; G_{sw} – Dongying wild soybean.

NaCl treatment [mM]		φ_{Po}	Ψ_{Eo}	ABS/RC	PI_{ABS}
G_{mc}	0	0.78 ± 0.02[a]	0.54 ± 0.05[a]	1.92 ± 0.32[c]	2.27 ± 0.77[a]
	50	0.77 ± 0.01[a]	0.50 ± 0.01[a]	1.92 ± 0.14[c]	1.80 ± 0.04[a]
	100	0.78 ± 0.01[a]	0.46 ± 0.02[a]	1.72 ± 0.32[c]	1.76 ± 0.16[a]
G_{sw}	0	0.74 ± 0.02[a]	0.49 ± 0.06[a]	2.13 ± 0.24[c]	1.78 ± 0.80[a]
	50	0.70 ± 0.03[b]	0.32 ± 0.01[b]	2.38 ± 0.17[b]	0.39 ± 0.16[b]
	100	0.50 ± 0.06[c]	0.30 ± 0.01[b]	3.07 ± 0.23[a]	0.19 ± 0.14[b]

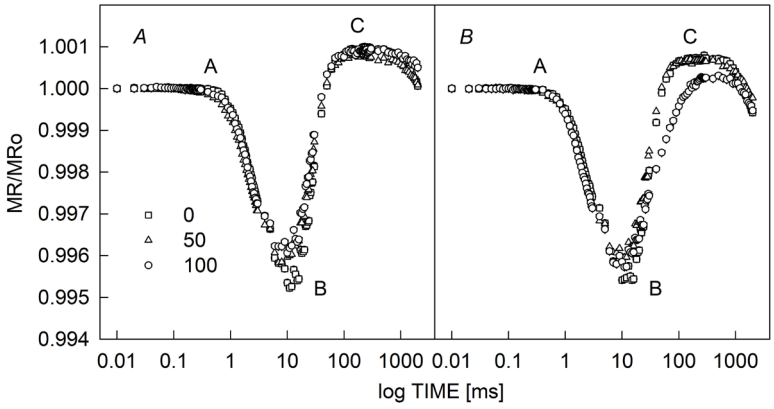

Fig. 4. Changes in MR/MR_0 ratio in the G_{mc} (*A*) and G_{sw} (*B*) leaves of grafted soybean plants under PPFD of 800 μmol m^{-2} s^{-1} for 1 h after receiving treatment with 0, 50, and 100 mM NaCl for 12 d. Ten measurements were taken for each treatment, after which the average result was calculated. G_{mc} – cultivated soybean; G_{sw} – Dongying wild soybean.

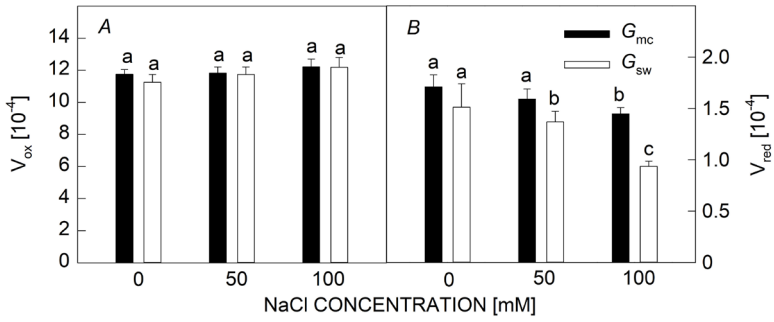

Fig. 5. Changes in the maximal slopes of the rates of P_{700} and PC oxidation (V_{ox}) (*A*) and the maximal slopes of the rates of P_{700} and PC re-reduction (V_{red}) (*B*) in the G_{mc} and G_{sw} leaves of grafted soybean plants under PPFD of 800 μmol m^{-2} s^{-1} for 1 h after receiving treatment with 0, 50, and 100 mM NaCl for 12 d. Ten measurements were taken for each treatment, after which the average result was calculated. *Different lowercase letters* above the bars show significant differences at $P<0.05$. G_{mc} – cultivated soybean; G_{sw} – Dongying wild soybean.

Table 3. Na^+ distribution in the grafted soybean plants in roots, stems, and leaves after treatment with 0, 50, and 100 mM NaCl for 12 d. The mean ± SE values of three replicates are shown. *Different lowercase letters* presented in a column represent significant differences at $P<0.05$ between different treatments. G_{mc} – cultivated soybean; G_{sw} – Dongying wild soybean.

NaCl treatment [mM]	Na^+ concentration in roots [g kg^{-1}]	Na^+ concentration in stem [g kg^{-1}]		Na^+ concentration in leaves [g kg^{-1}]	
		G_{mc}	G_{sw}	G_{mc}	G_{sw}
0	2.43 ± 0.18[c]	0.97 ± 0.06[c]	1.44 ± 0.12[c]	0.42 ± 0.04[c]	1.01 ± 0.14[c]
50	23.28 ± 1.97[b]	7.77 ± 0.60[b]	14.12 ± 0.11[b]	6.45 ± 0.16[b]	12.51 ± 0.47[b]
100	32.67 ± 1.53[a]	23.53 ± 1.06[a]	42.12 ± 4.63[a]	13.60 ± 1.56[a]	16.43 ± 0.52[a]

efficiency in eight rice varieties. These findings are consistent with those of Mohamed *et al.* (2020) who reported that photosynthetic gas exchange and stomatal anatomical traits had a significant effect on the tolerance of rapeseed cultivars to salinity. We have demonstrated that wild soybean G_{sw} could maintain photosynthetic

activity at a higher level under salt stress conditions than that of G_{mc} (Xue *et al*. 2014). The significant reduction in photosynthetic capacity is consistent with the significantly reduced growth under salt conditions (Phang *et al*. 2008), and compared with the decrease in root growth, the decrease in shoot growth is much more obvious (Yadav *et al* 2019).

However, in this study, we generated grafted plants that had both G_{sw} and G_{mc} shoots (Fig. 1*B*) using grafting technology to explore the role of roots in the mechanism of salt resistance in wild soybean. Sugiyama *et al*. (2007) proved that roots play a role in the variation of the cadmium concentration in seeds within soybean varieties by using grafting experiments. However, the photosynthetic activity of G_{sw} was more severely damaged than that of G_{mc} in the grafted soybean plants after NaCl treatment, which was proven by the finding that the decrease in P_N in the G_{sw} leaves was significantly greater than that in the G_{mc} leaves (Fig. 2*A*). The shoot length of G_{mc} and G_{sw} decreased by 22.8 and 31.5%, respectively, after 100 mM NaCl treatment (Table 1). The g_s within the G_{sw} and G_{mc} leaves decreased significantly because of the increase in the NaCl concentration in the nutrient solution (Fig. 2*B*). However, the decreased C_i and unchanged CE in the G_{mc} leaves indicate that stomatal limitation can contribute to the decrease in P_N, and the increased C_i and decreased CE in the G_{sw} leaves indicate that the activities of Rubisco, the main photosynthetic enzyme catalyzing the absorption of CO_2 from the atmosphere into plants in the Calvin cycle, may be inhibited by salt treatment (Fig. 2*C*,*D*). It is well established that salinity exposure leads to decreased Rubisco activity in the majority of plants (Hameed *et al*. 2021). Moreover, the decreases in CO_2 assimilation through the Calvin cycle lead to a decrease in photochemical electron sinks under salt stress conditions because compared to the photochemical reactions of photosynthesis, CO_2 assimilation reactions to salinity are considered to be more sensitive (Stepien and Johnson 2009, Hameed *et al*. 2021). Therefore, photosynthetic electron transport is inhibited by the increase in a major electron acceptor in PSI (Stepien and Johnson 2009). In this study, Chl *a* fluorescence and a modulated 820-nm reflection technique were used to gain insights into the change in electron transport. The observation that the shape of the Chl *a* fluorescence transient of the G_{sw} leaves was changed to a significantly greater degree by the NaCl treatments than that of the G_{mc} leaves indicates that the electron transport of PSII in the G_{sw} leaves was damaged much more severely than that in the G_{mc} leaves. Many studies have shown that disruptions in electron transport are affected by salt stress, and salinity can amplify this disruption, affecting the heterogeneity and activity of the photosynthetic apparatus (Mehta *et al*. 2011, Duarte *et al*. 2013). Tiwari *et al*. (1997) showed that an increase in salinity causes a progressive decrease in the activities of PSI and PSII in four rice varieties. Yan *et al*. (2020) proved that the interaction of PSI and PSII is among the mechanisms of salt adaptation in wild soybean and depends on the stability of PSI and the quick reaction of the PSII acceptor to defend against salt-induced oxidative stress in the photosynthetic apparatus. Therefore, the data in this study revealed that the electron transfer of PSII in the G_{sw} leaves was blocked, which was proven by the significant decrease in PI_{ABS} in the G_{sw} leaves. PI_{ABS} characterizes the three major structural and functional properties of PSII (ABS/RC, φ_{Po}, Ψ_{Eo}) and is obviously correlated with the energy-storing ability and photosynthetic apparatus activity (Strasser *et al*. 2004). The drop in Ψ_{Eo} suggested that the probability that an electron resides on Q_A reflects that the accumulation of Q_A^- caused damage to the electron transport of PSII. The lack of significant difference in φ_{Po} in the G_{sw} leaves indicates that NaCl treatment did not affect the main photochemical responses. On the other hand, the decrease in the MR/MR_0 ratio with the increasing concentration of the oxidized states of P_{700}^+ and PC^+ as well as the increase in the MR/MR_0 ratio are indicative of P_{700}^+ and PC^+ re-reduction. It has been proven that the oxidized states of P_{700}^+ and PC^+ are significantly influenced by more severe stress and that the re-reduction of P_{700}^+ and PC^+ is more likely to be affected by stress (Schansker *et al*. 2003). Under saline conditions, PSI seems less likely to be affected by stress than PSII and is likely to impart salt tolerance through the increase in cyclic electron flow to produce ATP around PSI, helping to avoid the overaccumulation of Na in chloroplasts (He *et al*. 2015, Niewiadomska and Wiciarz 2015). This is consistent with our results showing that the decrease in the MR/MR_0 ratio in both the G_{sw} and G_{mc} leaves was not changed by NaCl treatment, which is also supported by the value of V_{ox} (Fig. 5*A*). The increase in the MR/MR_0 ratio in the G_{sw} leaves slowed under the 100 mM NaCl treatment conditions, and the V_{red} in the G_{sw} leaves decreased more markedly than that in the G_{mc} leaves.

The Na^+ concentration in the leaves is the main factor that determines salt resistance in various plants (He *et al*. 2016, Umezawa *et al*. 2000). Moreover, soybean is more likely to be affected by high Na^+ concentrations (Le *et al*. 2021). High tissue Na^+ contents have significantly greater negative influences, including a reduction in the Chl content, inhibition of electron transport, and disruption of the enzymatic process of the Calvin cycle (Hameed *et al*. 2021). As discussed by Liu *et al*. (2017), the wild soybean W8 performs better in terms of the growth of seedlings, photosynthetic properties, and physiological indexes because the Na^+ and Cl^- contents are lower and the K^+ content is higher in W8, and there is a higher K^+/Na^+ ratio in its leaves and roots than either the semi-wild *G. gracilis* SW18 or the salt-sensitive *G. max* Melrose plants. Our previous study also showed that a lower Na^+ concentration allows the maintenance of higher photosynthetic activity in G_{sw} leaves under salt stress conditions (Xue *et al*. 2014). Na^+ is absorbed by the root and allocated to different organs and cells *via* a transpiration stream under salt stress conditions. 'Tissue tolerance' in plants helps in the development of tolerance mechanisms by plants to cope with stress conditions (Flowers *et al*. 2015). According to this study, the grafted soybean plants had the same cultivated roots, and the Na^+ concentrations in the roots were uniform for the G_{sw} and G_{mc} shoots after

NaCl treatment (Table 3). However, the Na^+ concentration in the G_{sw} stems and leaves was higher than that in the G_{mc} stems and leaves. Therefore, the differences in the Na^+ concentrations in both the G_{sw} and G_{mc} leaves of the grafted soybean plants are likely to result in their different photosynthetic responses to NaCl treatments. This was further supported by the fact that a significant relationship existed between the P_N and Na^+ concentration in the leaves of the grafted soybean plants ($R = -0.90$, $P<0.01$), and compared with that in the G_{mc} leaves, the greater decrease in P_N in the G_{sw} leaves was mainly attributed to the higher concentrations of Na^+ in the G_{sw} leaves.

From the discussion above, we suggest that the concentrations of Na^+ in the G_{sw} leaves of the grafted soybean were too high to affect the growth conditions and regular metabolism of the G_{sw} leaves, including photosynthesis. According to our previous research, a higher Na^+ concentration was observed in the G_{sw} leaves because of differences in the roots (Xue *et al*. 2014). The G_{sw} shoots were grafted with G_{mc}, and the grafted soybean plants had only cultivated roots. The difference in the roots led to the different levels of Na^+ accumulation in the G_{sw} leaves. The higher the Na^+ concentration in the G_{sw} leaves was, the more the photosynthetic apparatus was damaged, which was demonstrated by the decrease in the photosynthetic activity in the leaves. That is, excessive Na^+ concentrations entering the G_{sw} leaves could cause serious damage to the photosynthetic apparatus. Under salinity, G_{sw} accumulated higher Na^+ concentrations in the roots and exhibited fine regulation of Na^+ transport from the roots to the leaves, maintaining a lower Na^+ concentration in the leaves. The results suggested that the roots of G_{sw} play an obvious role in salt resistance. Tissue tolerance refers to its function when there is a relatively high internal Na^+ concentration. Furthermore, G_{sw} showed strong regulation of Na^+ transport from the roots to the leaves, and in G_{sw}, there was less accumulation of Na^+ in the leaves than in the roots. By sequestering Na^+ in the roots, the accumulation of Na^+ in leaves was regulated to prevent ion toxicity. In addition, Na^+ in the xylem flow of soybean can be reabsorbed or intercepted by parenchyma cells of the xylem in the process of transport to leaves and can be transported horizontally to the phloem and then to the roots to prevent Na^+ transport to leaves (Durand and Lacan 1994). Kotula *et al.* (2019) reported that overaccumulation of Na^+ in the mesophyll cells of salt-sensitive chickpea is consistent with chloroplast structural destruction by comparing the salinity tolerance of the two contrasting chickpea genotypes. Salinity tolerance in salt-tolerant plants is related to the ability to exclude Na^+ from leaflets, particularly from photosynthetically active mesophyll cells.

Conclusions: Based on the grafting experiment, we demonstrated, again, that the roots of G_{sw} are of great significance to salt resistance. The absence of G_{sw} roots led to more Na^+ entering the leaves, destroying the photosynthetic apparatus. Therefore, we suggest that G_{sw} can effectively accumulate Na^+ to keep the Na^+ concentration in the leaves at a lower level, which protects the photosynthetic apparatus from salt damage. We believe that a kind of Na^+ transporter gene is involved in salt resistance in G_{sw} root systems, and more molecular biological studies on the role of Na^+ transport in G_{sw} root systems in salt resistance are needed to elucidate this mechanism.

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