

Photosynthetic gas exchange and chlorophyll *a* fluorescence of three wild soybean species in response to NaCl treatments

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Abstract

Responses of photosynthetic gas exchange and chlorophyll (Chl) *a* fluorescence of three wild soybeans, *Glycine soja*, *G. tomentella*, and *G. tabacina* occurring in different habitats of Taiwan, to four NaCl treatments, 0S, LS, MS, and HS (*i.e.* 0, 17, 51, and 85 mM NaCl) were compared. In *G. soja* following exposure to NaCl treatment for one month, the photon saturated photosynthetic rate (P_N), the ratio of variable to maximum fluorescence (F_v/F_m), the quantum yield of photosystem 2 (Φ_{PS2}), and the electron transport rate (ETR) decreased dramatically. These reductions increased with increasing concentration of NaCl treatment. Plants of MS and HS treatments did not survive after extending the treatment to two months. Reductions in P_N , Φ_{PS2} , and ETR (but not in F_v/F_m) were found in *G. tabacina* after two months of exposure to MS and HS treatments, but the reduction was not as severe as that in *G. soja*. In *G. tomentella*, significant reductions in P_N and g_s were found only in HS plants after two months of treatment, but no significant differences in F_v/F_m , Φ_{PS2} , and ETR were found among plants of the four treatments. Thus the three wild soybeans in Taiwan have differentiated in their photosynthetic susceptibility to salinity, *G. tomentella* being the least susceptible, *G. soja* the most sensitive, and *G. tabacina* the intermediate. Different mechanisms are attributed to the inhibition effect of salinity on photosynthesis of the three species.

Additional key words: electron transport rate; *Glycine* species; irradiance; net photosynthetic rate; quantum yield of photosystem 2; salinity; species differences; stomatal conductance.

Introduction

There are three wild soybean species in Taiwan, *Glycine soja* Sieb & Zucc., *G. tomentella* Hayata, and *G. tabacina* (Labill.) Benth. Differences in morphological and physiological responses to water availability among these three species have been reported and the species differences were consistent with the water availability of their habitats (Kao and Tsai 1998). Plant responses to salt and water stress have much in common (Munns 2002), however, drought and salt tolerance are not necessarily linked (Nagy and Galiba 1995). In addition, among the three species, *G. tomentella* is the only species that grows along seaside (Huang and Ohashi 1993). Thus, variation might exist among these three wild soybeans in their sensitivity to salinity, which could be different from their susceptibility to low water availability. No information

on their difference in salt sensitivity is available and therefore we studied this question.

In contrast to halophytes, glycophytes subjected to saline conditions often show reduction in photosynthetic rates (Walker *et al.* 1981, Seemann and Critchley 1985, Bongi and Loreto 1989, Brugnoli and Björkman 1992). The decreases in photosynthetic rate may result from the closure of stomata induced by osmotic stress, or from salt damaging of photosynthetic apparatus (reduced efficiency of the electron transport chain and damage to the light-harvesting complex) or other metabolic processes. Measurements of photosynthetic gas exchange provide information on the salinity effect on the net CO₂ assimilation (P_N) and stomatal conductance (g_s). Application of the chlorophyll (Chl) fluorescence technique provides a rapid

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Abbreviations: c_a – ambient CO₂ concentration; c_i – intercellular CO₂ concentration; Chl – chlorophyll; ETR – electron transport rate; F_0' – minimal fluorescence in light-adapted state; F_m – maximum fluorescence in dark-adapted state; F_m' – maximum fluorescence in light state; F_i – steady-state fluorescence in the light-adapted state; F_v – variable fluorescence in dark-adapted state; F_v' – variable fluorescence in light state; g_s – stomatal conductance; HS – 85 mM NaCl; LS – 17 mM NaCl; MS – 51 mM NaCl; P_N – photon saturated net photosynthetic rate; PFD – photon flux density; 0S – 0 mM NaCl; Φ_{PS2} – quantum yield of PS2.

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and convenient method for obtaining non-destructive estimates of photosynthetic performance. Chl fluorescence measurements are often applied in the investigations of salt stress damage (Smillie and Nott 1982, Ball and Anderson 1986, Jimenez *et al.* 1997, Kao and Tsai 1999). In particular, the maximum quantum yield of photosystem 2 (PS2), *i.e.* F_v/F_m , and the efficiency of excitation capture by open PS2 (quantum yield) provide important information on photosynthetic activity. To differentiate the effect of salt treatment on the aspects of

Materials and methods

Plants and treatments: *Glycine soja* seeds were collected from Shihmen (25°02'N, 120°30'E) in northern Taiwan, *G. tomentella* from Hengchun (22°00'N, 120°44'E) in southern Taiwan, and *G. tabacina* from Penghu island (23°34'N, 119°33'E). Seeds were germinated in a Petri dish. After germination, seedlings were transferred to 2 000 cm³ plastic pots, with one seedling per pot, filled with a mixture of vermiculite : soil of 1 : 1 by volume. Plants were grown in a glasshouse of the Academia Sinica, Taipei in natural daylight, watered every day, and fertilised using inorganic fertiliser (N : P : K of 20 : 20 : 20) once every 2 weeks. The salinity treatment began when plants were 4 weeks old. The pots were immersed in a modified Hoagland solution (Haines and Dunn 1976), containing 0 (0S), 17 (LS), 51 (MS), or 85 (HS) mM NaCl, for two hours every day, with four replicates per species for each salinity treatment. Final NaCl treatment concentrations were reached gradually by increment of 17 mM every two days. Then, plants were subjected to the final treatment concentration for two months.

Measurements: Potted plants, one and two months after the NaCl treatment started, were brought into the laboratory for the measurements of photosynthetic gas exchange and Chl *a* fluorescence. The F_v/F_m values were determined on leaves being dark-adapted for 30 min. To measure the fluorescence-PFD response, terminal leaflets were held in a leaf-clip holder (2030-B, Walz, Effeltrich, Germany). The PFD on leaves, provided by a halogen lamp (2050-H, Walz, Effeltrich, Germany), was adjusted from darkness to 1 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in steps of 50–200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The halogen lamp was equipped with a

Results

Photosynthetic gas exchange: P_N of 0S treated plants were similar among the three species after one month of treatment (Fig. 1A). However, the corresponding g_s were significantly different among the three species, *G. soja* showing the highest, *G. tomentella* the intermediate, and *G. tabacina* the lowest g_s (Fig. 1B) ($p < 0.05$). As a result, *G. soja* had the highest ratio of intercellular to ambient

photosynthesis, we measured both gas exchange and Chl *a* fluorescence of the three wild soybean species subjected to different NaCl treatments.

The main objective of this study was to assess the effects of salinity on photosynthesis of three wild soybean species in Taiwan. We tested the hypothesis that, in addition to their differences in response to drought (Kao and Tsai 1998), the three wild soybeans in Taiwan are also different in their photosynthetic activity under salinity.

heat-reflecting filter to reduce heat generated by the lamp. PFD on the leaf was monitored with a micro-quantum sensor installed on the leaf-clip holder next to the spot where fluorescence was measured. After the leaf was exposed to the desired PFD for 10 min, the Chl *a* fluorescence of PS2 was measured using a portable, pulse amplitude modulated fluorometer (*Mini-PAM*, Walz, Effeltrich, Germany). The quantum yield of PS2 [$\text{yield} = (F_m' - F_t)/F_m'$] and the apparent electron transport rate ($\text{ETR} = \text{yield} \times \text{PFD} \times 0.5 \times 0.84$) were computed from the Chl fluorescence, where F_m' is the maximal fluorescence and F_t is the steady-state fluorescence in the light-adapted state. During the fluorescence measurement, room temperature was maintained at 25 °C while leaf temperature was not controlled.

Photosynthetic gas exchange of trifoliolate leaf was measured using a steady-state open gas exchange system (Kao and Tsai 1998). The system consisted of an infrared carbon dioxide analyser (*LI-6262*, Licor, Lincoln, NE, USA) and a leaf chamber (*Pacsys 9900*, DDG, La Jolla, CA, USA) with integral temperature and humidity sensor. P_N and g_s were determined by enclosing the leaf in a cuvette under a photon flux of 1 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, coming from a fibre illuminator (*FL-440*, Walz, Germany). Measurement conditions within the cuvette were: leaf temperature 30 °C, leaf-to-air water vapour pressure difference 1.0–1.2 kPa, and ambient CO₂ concentration $360 \pm 5 \text{ cm}^3 \text{ m}^{-3}$.

All statistical tests were performed using the computer software *SYSTAT* (*Statistical Solution Limited*, Ireland). Means were compared by the least significant difference test. Significant levels are reported as $p < 0.05$.

CO₂ concentration (c_i/c_a), *G. tomentella* the intermediate, and *G. tabacina* the lowest one (Table 1). Increment of NaCl concentration had different effect on photosynthetic gas exchange of these three species. P_N and g_s of *G. soja* were significantly reduced by NaCl treatments and the reduction increased with increasing of NaCl concentration (Fig. 1A,B). The reduction was greater in P_N than in g_s ,

and as a consequence, c_i/c_a of *G. soja* increased with increasing NaCl concentration (Table 1). In contrast, P_N and g_s of *G. tomentella* and *G. tabacina* were not significantly affected after one month of continuous salinity

treatment. Consequently, no significant difference was found in c_i/c_a of either species among the four NaCl treatments (Table 1).

Table 1. Effect of NaCl (0, 17, 51, and 85 mM) treatments for one and two months on c_i/c_a and F_v/F_m (mean \pm SE, $n = 4$) of the most recent, fully expanded trifoliolate leaf of wild soybean species. Means within each row followed by different superscripts were significantly different ($p < 0.05$) using Fisher's LSD test.

	Duration [month]	Species	NaCl [mM]			
			0	17	51	85
c_i/c_a	1	<i>G. soja</i>	0.66 \pm 0.02 ^a	0.70 \pm 0.01 ^{ab}	0.72 \pm 0.02 ^{bc}	0.76 \pm 0.01 ^c
		<i>G. tomentella</i>	0.63 \pm 0.02 ^a	0.63 \pm 0.01 ^a	0.64 \pm 0.01 ^a	0.63 \pm 0.02 ^a
		<i>G. tabacina</i>	0.60 \pm 0.00 ^a	0.60 \pm 0.02 ^a	0.62 \pm 0.01 ^a	0.61 \pm 0.01 ^a
	2	<i>G. soja</i>	0.67 \pm 0.02 ^a	0.72 \pm 0.01 ^a	–	–
		<i>G. tomentella</i>	0.63 \pm 0.01 ^a	0.57 \pm 0.01 ^b	0.58 \pm 0.01 ^b	0.58 \pm 0.01 ^b
		<i>G. tabacina</i>	0.60 \pm 0.02 ^a	0.58 \pm 0.02 ^a	0.57 \pm 0.04 ^a	0.57 \pm 0.02 ^a
F_v/F_m	1	<i>G. soja</i>	0.80 \pm 0.01 ^a	0.79 \pm 0.01 ^a	0.75 \pm 0.02 ^b	0.51 \pm 0.13 ^c
		<i>G. tomentella</i>	0.79 \pm 0.01 ^a	0.82 \pm 0.01 ^a	0.79 \pm 0.01 ^a	0.81 \pm 0.01 ^a
		<i>G. tabacina</i>	0.82 \pm 0.00 ^a	0.81 \pm 0.01 ^a	0.81 \pm 0.01 ^a	0.83 \pm 0.00 ^a
	2	<i>G. soja</i>	0.80 \pm 0.02 ^a	0.75 \pm 0.02 ^b	–	–
		<i>G. tomentella</i>	0.80 \pm 0.01 ^a	0.80 \pm 0.01 ^a	0.81 \pm 0.01 ^a	0.81 \pm 0.01 ^a
		<i>G. tabacina</i>	0.82 \pm 0.01 ^a	0.83 \pm 0.01 ^a	0.82 \pm 0.01 ^a	0.81 \pm 0.01 ^a

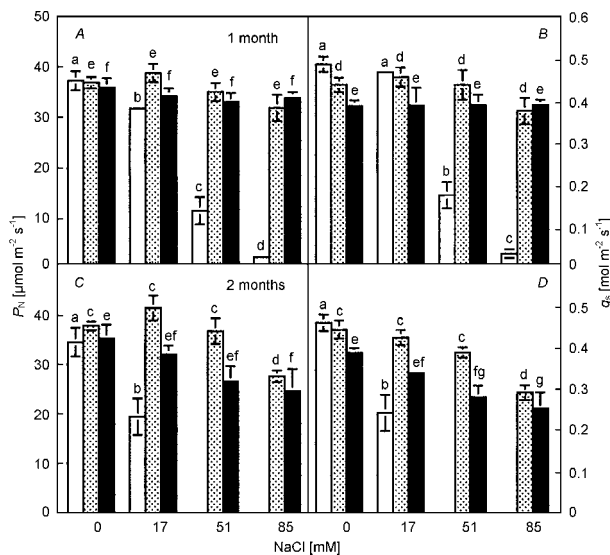


Fig. 1. Effect of four NaCl treatments on photon saturated photosynthetic rate (P_N) and stomatal conductance (g_s) of three wild soybeans, *G. soja* (open bars), *G. tomentella* (bars with dots), and *G. tabacina* (solid bars). Measurements were taken one (A, B) and two months (C, D) after the beginning of treatment. Vertical bars represent 1 S.E. of treatment ($n = 4$). Values with different letters are significantly different ($p < 0.05$) among NaCl treatments.

MS and HS plants of *G. soja* did not survive after two months of treatment, hence no P_N was measured in plants of these two treatments. Though LS treated *G. soja* did survive after two months of salinity treatment, their P_N

and g_s were significantly lower than those of 0S treated plants (Fig. 1C,D). *G. tomentella* and *G. tabacina* plants showed similar pattern of changes in P_N and g_s in response to NaCl treatments after two months of the treatment: 0S, LS, and MS plants had similar P_N and g_s , only the HS plants showed significantly lower g_s in comparison to the other treatments. For *G. tabacina*, no significant difference was found in c_i/c_a among the four treatments (Table 1). In contrast, LS, MS, and HS treated *G. tomentella* had significantly lower c_i/c_a than the 0S treated one.

Chl *a* fluorescence: NaCl had no effect on F_v/F_m of both *G. tomentella* and *G. tabacina* after one or two months of treatments (Table 1). In contrast, MS and HS treatments resulted in significant reduction of F_v/F_m of *G. soja* after one month of treatment. Significant reduction in F_v/F_m was also found in LS treated *G. soja* after two months of the treatment.

Similar to the result from F_v/F_m measurement, MS and HS treatments resulted in significant reduction in the quantum yield (Φ_{PS2}) of *G. soja* after one month of treatment (Fig. 2). Significant reduction in Φ_{PS2} was also measured in LS treated *G. soja* after two months of salinity treatment. No significant effect of NaCl treatment on Φ_{PS2} was found in *G. tabacina* after one month of treatment, however, extending treatment time resulted in significant reduction in Φ_{PS2} in MS and HS treated *G. tabacina*. In contrast, no significant effect of NaCl on Φ_{PS2} was found in *G. tomentella* throughout the treatment period.

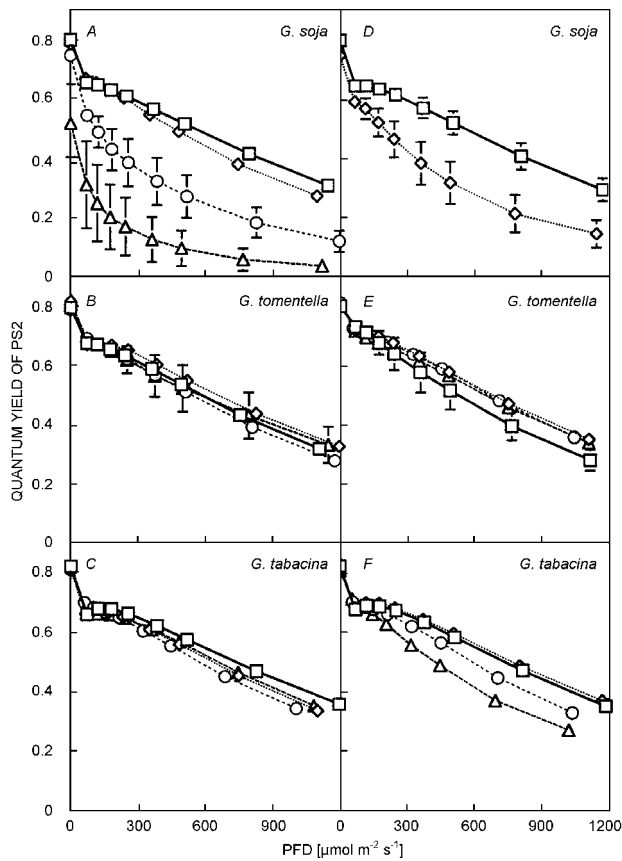


Fig. 2. The photon response curve of quantum yield of photosystem 2 of three wild soybean species (*G. soja*, *G. tomentella*, and *G. tabacina*), after one (A, B, C) and two (D, E, F) months of 0 (square), 17 (diamond), 51 (circle), and 85 (triangle) mM NaCl treatments. Vertical bars represent 1 S.E. of treatment ($n = 4$).

MS and HS treatments resulted in significant reduction in the apparent electron transport rate (ETR) of *G. soja* after one month of treatment (Fig. 3). Significant reduction in ETR was also found in LS plants of *G. soja* after two months of the treatment. No significant effect of

Discussion

P_N and g_s in general declined when the wild soybean plants were exposed to NaCl (Fig. 1). However, the degree of reduction differed among the three species, with *G. soja* being the most sensitive to NaCl treatment and *G. tomentella* the least affected. This is consistent with the field observation that among the three species, *G. tomentella* is the only species ever being found growing along the seaside.

The inhibition of P_N by NaCl may be a consequence of salinity induction of stomatal closure, caused by the reduction in osmotic potential, and/or non-stomatal inhibition of photosynthesis, caused by direct effects of NaCl on photosynthetic apparatus independent of stomatal

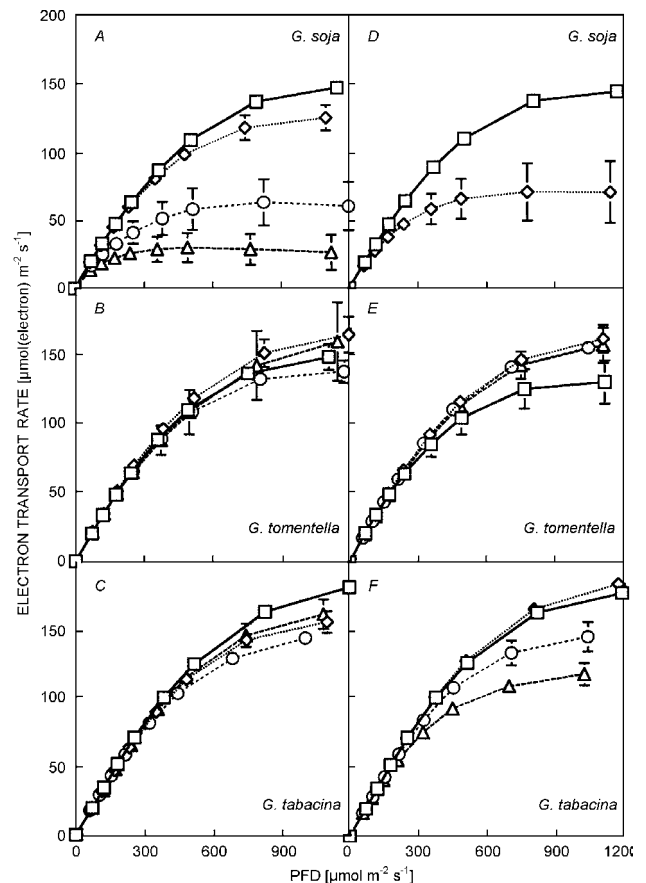


Fig. 3. The photon response curve of apparent electron transport rate of three wild soybean species (*G. soja*, *G. tomentella*, and *G. tabacina*) after one (A, B, C), and two (D, E, F) months of 0 (square), 17 (diamond), 51 (circle), and 85 (triangle) mM NaCl treatments. Vertical bars represent 1 S.E. of treatment ($n = 4$).

NaCl treatment on ETR was found in *G. tabacina* after one month of treatment. However, extending the treatment time to two months did result in significant reduction in ETR in MS and HS treated *G. tabacina* (Fig. 3F). In contrast, no effect of NaCl treatment on ETR was found in *G. tomentella* throughout the treatment period.

closure. Analysis of gas exchange measurement and Chl *a* fluorescence revealed that the reduction of P_N in *G. tomentella* was probably not caused by the ion toxicity but mainly due to the salinity modification of g_s (a lesser decline in P_N than in g_s and concomitantly a lower c_i/c_a by NaCl treatment, but no significant changes in F_v/F_m and Φ_{PS2}) (Figs. 1 and 2, Table 1). Similar result was found in *Phaseolus vulgaris* (Seemann and Critchley 1985, Brugnoli and Lauteri 1991) and spinach leaves (Downton *et al.* 1985) in response to salinity. In contrast, the significantly higher reduction in P_N than in g_s , hence an increase in c_i/c_a (Table 1), and significant reduction in F_v/F_m and Φ_{PS2} in NaCl treated *G. soja* suggest that non-

stomatal inhibition of photosynthesis, caused by direct effects of NaCl on photosynthetic apparatus independent of stomatal closure, might be the major factor responsible for the drastic reduction in photosynthetic rate in NaCl treated *G. soja*. Differences in both P_N and F_v/F_m are often used as characteristics of cultivar or species differences (for soybean cultivars see Jiang and Xu 2001). Non-stomatal inhibition of photosynthesis by salinity was also reported for several plant species (Seemann and Critchley 1985, Ball and Anderson 1986). This inhibition of photosynthetic activity may attribute to a reduced efficiency of ribulose-1,5-bisphosphate (RuBP) carboxylase, to a reduction in RuBP regeneration capacity, or to the sensitivity of PS2 to NaCl (Ball and Anderson 1986). Parallel reduction in P_N and g_s was found among *G. tabacina* that received salinity treatments. Consequently no significant difference was detected in c_i/c_a among the four treatments (Table 1). Hence the reduction of CO₂ assimilation by salinity treatment in *G. tabacina*

was caused by both stomatal and non-stomatal factors to a similar degree.

In comparison to their susceptibility to low water availability, *G. soja* was the most susceptible to low water availability, *G. tabacina* the least sensitive, and *G. tomentella* the intermediate species (Kao and Tsai 1998). Our results showed a different ranking among the three wild soybeans in their sensitivity to NaCl treatment. Thus, similar to the finding in wheat cultivars (Nagy and Galiba 1995), drought and salt tolerance are not necessarily linked among these three species of wild soybean.

In conclusion, the degree of inhibition effect of salinity on photosynthesis differs among the three species, *G. soja* being the most sensitive, *G. tabacina* the intermediate, and *G. tomentella* the least sensitive to NaCl treatment. The reduction of P_N by salinity in these species was caused by stomatal and non-stomatal factors to a different degree.

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