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ORIGINAL PAPER

Salt tolerance of *Hibiscus hamabo* seedlings: a candidate halophyte for reclamation areas

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Abstract In order to evaluate the salinity tolerance of *Hibiscus hamabo* Siebold & Zuccarini (Malvaceae), a candidate halophyte for reclamation areas, we analyze the effects of NaCl concentration, ranging from 0 to 500 mM, on the morphological, photosynthetic and chlorophyll fluorescent traits of this species. The optimal concentration for the germination of *H. hamabo* was 25 mM NaCl, and the optimal concentration for the survival and growth of *H. hamabo* ranged from 5 to 10 mM NaCl. Growth traits of *H. hamabo* at 25 mM, including the plant height, canopy diameter, number of leaves and width of the largest leaf, showed no statistical differences from the control. Net photosynthetic rate, stomatal conduction, light utilization efficiency, water utilization efficiency, maximal photosynthetic rate, light saturation point and chlorophyll content were the highest at 7.5 mM NaCl. F_v/F_m and F_v/F_0 at 5 and 7.5 mM were significantly higher than the others, while F_0 was significantly lower. F_m and F_v at NaCl concentrations ranging from 2.5 to 10 mM were significantly higher than the others. Pearson correlation analysis showed that the chlorophyll content, maximal photosynthetic rate and light saturation point were significantly positively correlated with the number of leaves, while F_0 was significantly negatively correlated with the width of the largest leaf. Light compensation point was significantly negatively correlated with plant height, leaf number, width of the largest leaf and canopy diameter, and might be a good indicator for the salt tolerance of *H. hamabo*.

Keywords *Hibiscus hamabo* · Salt stress · Photosynthesis · Chlorophyll fluorescence · Chlorophyll content · Growth

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Introduction

In recent years, the utilization of coastal and ocean space for human activities has increased due to economic and population growth (Hsu and Chang 2000). To compensate for this increased use, land is being reclaimed from the sea for new industry and agriculture. Sodic (alkaline) soils are widespread in reclamation areas and need to be ameliorated (Qadir et al. 2007). Afforestation using halophytes (salt-tolerant plants) (Ashraf 2004) has proven to be effective in re-vegetating higher salinity landscapes (Khamzina et al. 2009) and as an important tool for land reclamation and soil amelioration (Ritter 2007; Mishra and Sharma 2010). Studies evaluating the salt tolerance of halophytes can provide useful information for the afforestation project of reclamation areas.

Salinity poses several problems for plant growth and development (Goldstein et al. 1996; Rawat and Banerjee 1998; Parida and Das 2005; Nedjimi 2009). Salinity may reduce plant growth through water deficit, ion toxicity, ion imbalance or a combination of these factors (Shannon et al. 1998). Increasing salinity is accompanied by a significant reduction in biomass, plant height, number of leaves per plant, root length, root/shoot ratio and so on (Mohammad et al. 1998; Meloni et al. 2001; Yu et al. 2002; Parida and Das 2005). Some studies have suggested that low saline concentration may have a stimulating effect on plant growth (Rawat and Banerjee 1998; Parida and Das 2005). Rawat and Banerjee (1998) found that 40 mM of salt concentration stimulated growth and biomass production of *Eucalyptus camaldulensis* and *Dalbergia sissoo*. Different plant species had different growth responses to the given salinity regime, and significant variations in the growth and salt tolerance among different species of woody plants have been reported (Bell et al. 1994; Rawat and Banerjee 1998; Nasim et al. 2009). Plants respond to salinity stress at physiological, biochemical, molecular and morphological levels (for review, see Parida and Das 2005). Physiological criteria should be able to supply more direct and reliable information than agronomic characters (Ashraf 2004). Photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl (Praxedes et al. 2010). The combination of chlorophyll fluorescence measurements together with net gas exchange parameters provides a good means of evaluating the photosynthetic performance in stressed plants (Jiménez et al. 1997) and to gain insight into the behavior of the photosynthetic machinery under stress (Maxwell and Johnson 2000). However, for different plant species, the response magnitude and the optimal physiological criteria would be different. Therefore, it is necessary to evaluate the salt tolerance of a plant species and test the physiological responses before it is widely used in afforestation, vegetation restoration or soil amelioration.

Hibiscus hamabo Siebold & Zuccarini (Malvaceae) is an endangered deciduous tree or shrub (Wu et al. 1994). It is a dominant species on islands and can reach 1–5 m in height. It is naturally distributed in coastal sands near sea level in China (Dinghai Islands in Zhejiang Province), Japan (Bonin and Ryukyu Islands) and Korea, and is cultivated in India and the Pacific islands (Wu et al. 1994). It is halophytic and lives well in habitats with NaCl concentrations ranging from 1.1 to 1.5 % (Yang et al. 2008). It is considered one of the best afforestation tree species used in reclamation areas and shelterbelt tree species along the coast (Yang et al. 2008). Although there are several studies on the photosynthetic responses of *H. hamabo* to salinity (Zhou et al. 2009; Yang et al. 2008), little has been clarified regarding whole-plant responses, such as morphological, growth and photosynthetic fluorescent responses. To our knowledge, there are few studies focusing on the growth traits of *H. hamabo*, and the salt tolerance mechanism is still unknown. This may be one of the main reasons limiting the application of *H. hamabo* in the development of afforestation and shelterbelts (Yang et al. 2008).

Here, we analyze the effects of NaCl concentration, ranging from 0 to 500 mM, which is the salinity of seawater (Guja et al. 2010), on the morphological, photosynthetic and chlorophyll fluorescent traits of *H. hamabo* to evaluate the salinity tolerance of this species. The aims of the project were the following: (1) to determine the suitable salt concentration for seed germination and seedling growth of *H. hamabo*, (2) establish the salt tolerance range of seedlings, and (3) determine whether photosynthetic responses or chlorophyll fluorescence parameters can be good criteria for evaluating salt tolerance.

Materials and methods

Plant material

Hibiscus hamabo seeds were provided by Zhejiang Yuhuan Lűye Agriculture Technology Co. Ltd in January 2010 and stored in a low-humidity storage cabinet (HZM-600, Beijing Biofuture Institute of Bioscience & Biotechnology Development, Beijing, China) until use.

Seed germination and seedlings survival

In March 2010, seeds were selected and pre-treated for 14 min with 98 % H_2SO_4, rinsed with distilled water five times, sterilized with 3 % $NaClO_3$ for 10 min and rinsed with sterilized distilled water three times. Pre-treated *H. hamabo* seeds were placed in Petri dishes (11 cm in diameter) containing silica sand and 10 mL of 1/4 Hoagland's nutrient solution with different NaCl concentrations (0, 2.5, 5, 7.5, 10, 25, 50, 100, 200 or 500 mM). Thirty seeds were used in every dish, and three replicates were used for each treatment. The Petri dishes were placed in a climate-controlled incubator at 28 °C (day)/20 °C (night) for 20 days with a light (12 h) and dark (12 h) cycle. Nutrients were replaced every day to maintain a stable salt concentration. Germination was recorded when the root length was 2-mm long. The date and rate of germination were measured daily for 20 days: germination rate (%) = (number of germinated seeds/total number of seeds) × 100 %. The mean and median germination days were calculated. After germination, 2-cm tall seedlings were transplanted into pots filled with sand and were sprayed with 50 ml of 1/4 Hoagland's nutrient solution containing the same NaCl concentration (0, 2.5, 5, 7.5, 10, 25 or 50 mM) every day. Seedling survival rate was measured 35 days after sowing.

Morphological traits

Four months after transplanting, the morphological traits of *H. hamabo* seedlings, including the height, width of the largest leaf, number of leaves per plant and the canopy diameter, were measured.

Photosynthetic traits

In situ measurements of photosynthesis were made on fully expanded, mature sun leaves at the same position on the main stems using a portable photosynthesis system (GFS-3000, Waltz, Effeltrich Germany) on clear days. Net photosynthetic rate (P_n), photosynthetically active radiation (PAR), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (C_i) were recorded. Light utilization efficiency (LUE) was calculated as P_n/PAR (Long et al. 1993), water utilization efficiency (WUE) was calculated as P_n/E (Hamid et al. 1990) and the apparent carboxylation efficiency (CE) was calculated as P_n/C_i (Flexas et al. 2001). Three leaves per plant were chosen, and six consecutive measurements were performed. For each measurement, three randomly selected *H. hamabo* plants were sampled.

To construct the light response curves, photosynthesis measurements were made between 9:30 and 11:00 a.m. on fully expanded leaves from each plant with a leaf temperature of 25 °C, a CO_2 concentration of 380 ppm and a relative humidity of 70 %. Light was provided by a photosynthetically active radiation lamp. Prior to measurements, leaves were allowed to acclimate under a PAR of 1,000 μmol m^{-2} s^{-1} to avoid photo-inhibition. Once stable, leaves were exposed to a series of PAR values: 2,000, 1,500, 1,200, 1,000, 800, 600, 400, 200, 100, 50, 20 and 0 μmol m^{-2} s^{-1}.

Entire photosynthetic light response curves were fitted in Origin 8.0 using the non-rectangular hyperbola function (McAlpine et al. 2008).

$$P_n = \frac{\alpha I + P_{max} - \sqrt{(\alpha I + P_{max})^2 - 4\theta \alpha I P_{max}}}{2\theta} - R_d$$

where P_n is the net photosynthetic rate, α is the quantum yield (the initial slope of the light response curve), I is the incident PAR, R_d is leaf dark respiration rate, P_{max} is the maximum leaf light-saturated photosynthetic rate and θ is the curvature parameter (Ögren 1993). Light compensation point (LCP) and light saturation point (LSP) were obtained using the equation above (Prado and Moraes 1997).

Chlorophyll content

The relative chlorophyll content was measured using a chlorophyll content meter (CCM-200 plus, Opti-Science Inc., Hudson, NH, USA). For each measurement, three leaves per plant and three randomly selected plants per measurement were sampled.

Chlorophyll fluorescence traits

Chlorophyll fluorescence parameters were measured between 8:00 and 11:00 a.m. with a portable chlorophyll fluorometer (OS30P, Opti-Science Inc., Hudson, NH, USA). Measurements were performed on the third undamaged adult leaf from the plant top after 30 min of dark adaptation using light exclusion clips. For each measurement, three leaves per plant and three randomly selected plants per measurement were sampled. The data of three measurements of three leaves were averaged and used as the mean of each plant. The variable-to-maximum fluorescence ratio (F_v/F_m), which has been used to express the maximum PSII photochemical efficiency, was calculated.

Data analysis

One-way ANOVA was used to analyze the effects of NaCl concentration on the growth of *H. hamabo*. Post hoc pairwise comparisons of means were made to examine the difference between treatments using the Fisher protected least significant difference (LSD) test at the 0.05 level of confidence. Because the data of survival rate measured on the 20th day did not meet the requirements for ANOVA, non-parametric test was used to examine the differences among the treatments. All statistical analyses were performed in SPSS 16.0 for Windows. All the figures were created in Sigma Plot 11.0.

Results

Effect of NaCl concentration on seed germination and seedling survival

On the 7th day after sowing, NaCl concentrations of 25 and 50 mM had significantly increased the germination rate, while the 100 and 200 mM concentrations had significantly decreased the germination rate (Fig. 1). On the 20th day

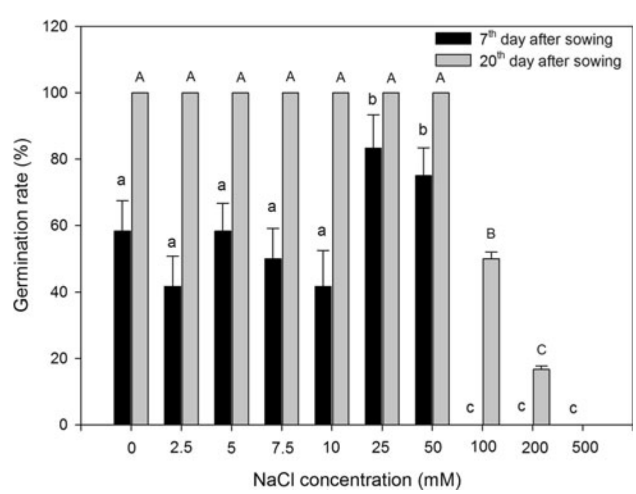

Fig. 1 Effect of NaCl concentration on the seed germination rate of *Hibiscus hamabo* at the 7th and 20th days after sowing. Different *lower* and *uppercase letters* indicate the significant difference of the germination rate at the 7th days and 20th days, respectively, after sowing among different treatments

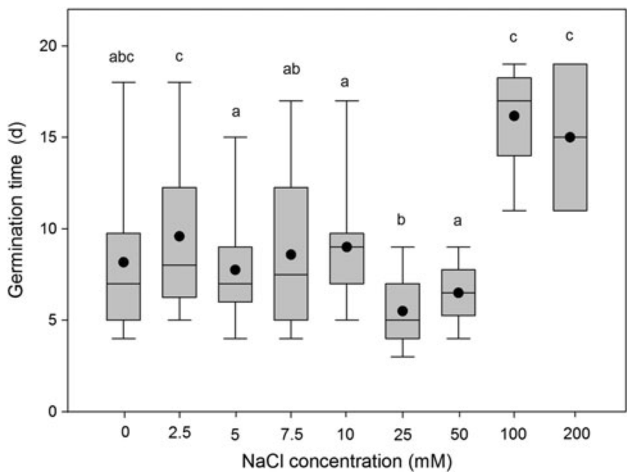

Fig. 2 Effect of NaCl concentration on the seeds germination time of *Hibiscus hamabo*. The *lower* and *upper boxes* show the lower quartile and upper quartile, respectively. The *dashes* and *filled dots* in the *boxes* indicate the median value and mean value, respectively. *Whiskers* represent the minimal and maximal value. Different *lowercase letters* indicate the significant difference among different treatments

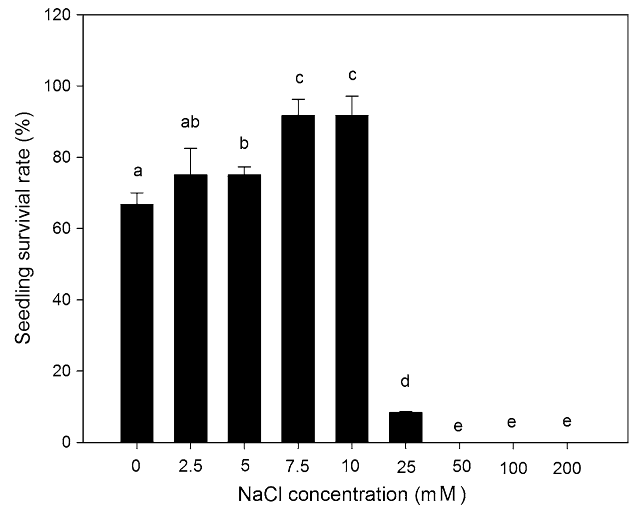

Fig. 3 Effect of NaCl concentration on the seedling survival rate of *Hibiscus hamabo*. Different *lowercase letters* indicate the significant difference among different treatments

after sowing, all seeds germinated under NaCl concentrations ranging from 0 to 50 mM, whereas the 100 and 200 mM NaCl concentrations significantly decreased the seed germination rate and the 500 mM NaCl concentrations completely inhibited seed germination (Fig. 1). The germination time at 25 mM NaCl was significantly higher than the other treatments, while the germination times at 100 and 200 mM NaCl were significantly lower (Fig. 2). Thirty-five days after sowing, the seedling survival rate at the 7.5 and 10 mM NaCl concentrations was significantly higher than at 0, 2.5 and 5 mM NaCl, but NaCl concentrations of 50, 100 and 200 mM completely inhibited the survival of seedlings (Fig. 3).

Effect of NaCl concentration on morphological traits

Plant height, width of the largest leaf, number of leaves per plant and canopy diameter at 5.0, 7.5 and 10 mM NaCl were significantly higher than those at the other concentrations (Fig. 4).

Effect of NaCl concentration on photosynthetic traits

The photosynthetic curve was a typical bimodal diurnal variation pattern (Fig. 5). The two peaks of P_n for *H. hamabo* leaves at NaCl concentrations ranging from 0 to 7.5 mM were at 10:00–12:00 a.m. and 14:00–16:00 p.m., while they shifted to 8:00–10:00 a.m. and 12:00–14:00 p.m. at a concentration of 10 mM. The bimodal pattern at 25 mM was weak. P_n, LUE, g_s and WUE were highest at 7.5 mM NaCl, while CE was the highest at 2.5 mM NaCl (Fig. 6).

Effect of NaCl concentration on the photosynthetic response to light

Photosynthetic light response curves showed that P_n increased with the increase of PAR until light saturation (Fig. 7). *H. hamabo* had significantly higher LSP, P_{max} and lowest LCP at 7.5 mM NaCl (Table 1).

Effect of NaCl concentration on chlorophyll content

The relative chlorophyll content increased with increasing NaCl concentration, peaked at 7.5 mM NaCl, and decreased at higher concentrations (Fig. 8).

Effect of NaCl concentration on fluorescence traits

Values of F_v/F_m and F_v/F_0 at the concentrations of 5 and 7.5 mM were significantly higher than at other concentrations, while the value of F_0 was significantly lower (Table 2). F_m and F_v were significantly higher at NaCl concentrations ranging from 2.5 to 10 mM (Table 2).

Correlationship between growth traits and other physical traits

Pearson correlation analysis showed that the chlorophyll content, P_{max} and LSP were significantly positively correlated with the number of leaves, while F_0 was significantly negatively correlated with the width of the largest leaf (Table 3). LCP was significantly negatively correlated with the plant height, leaf number, width of the largest leaf and canopy diameter (Table 3).

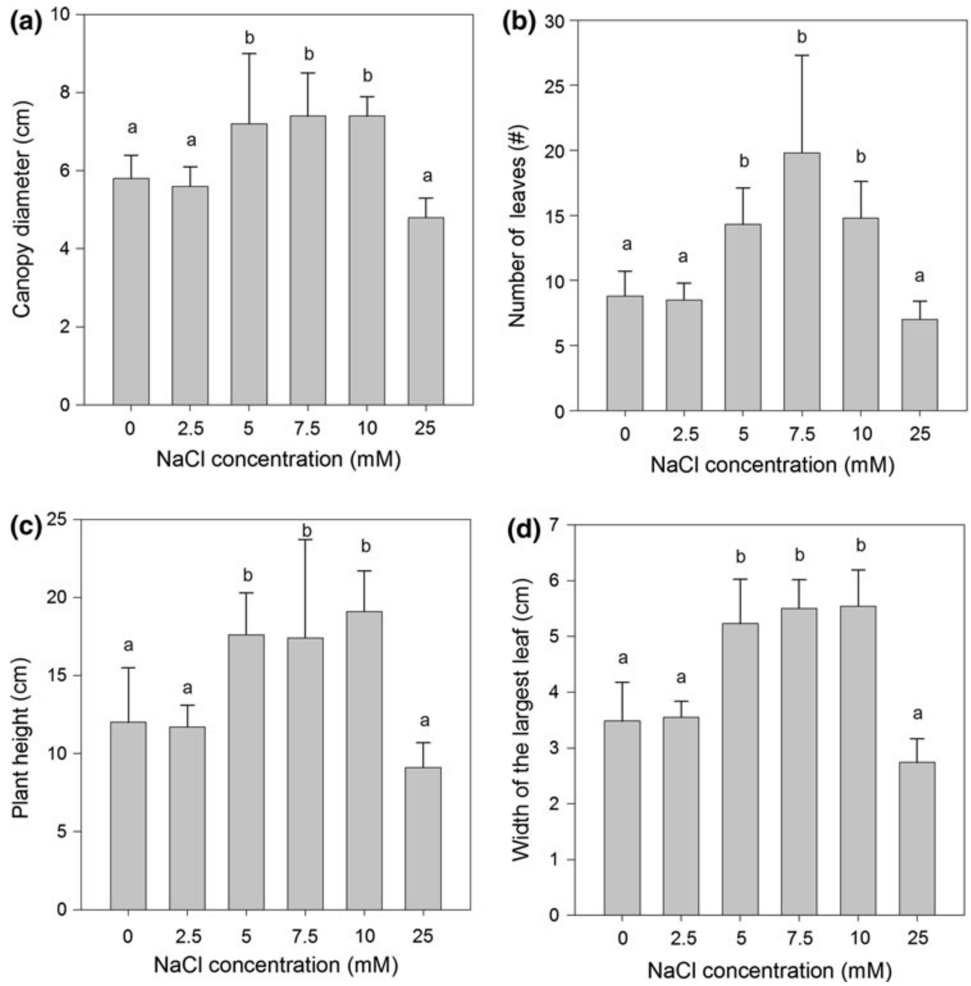

Fig. 4 Effect of NaCl concentration on the canopy diameter (**a**), number of leaves (**b**), and plant height (**c**) and width of the largest leaf (**d**) of *Hibiscus hamabo*. Different *lowercase letters* indicate the significant difference among different treatments

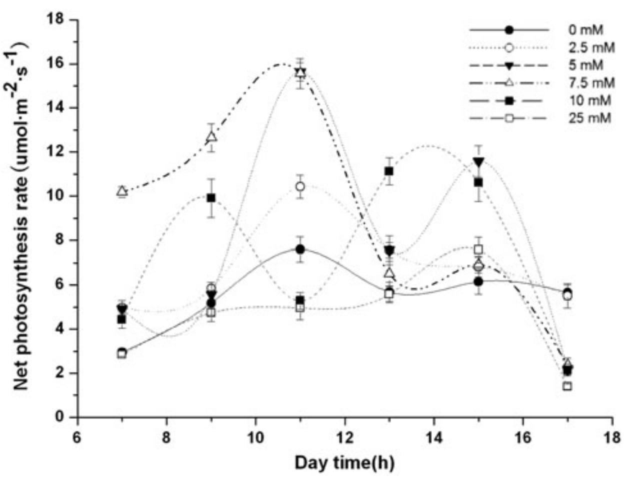

Fig. 5 Effect of NaCl concentration on the net photosynthetic rate of *Hibiscus hamabo*. The *error bar* indicates standard error

Discussion

Many studies have verified that salinity has adverse effects on seed germination and seedling growth (e.g., Khan et al. 1999; Abogadallah et al. 2010; Cambrollé et al. 2010). However, salinity at low concentrations has a stimulating effect on halophytes, but completely inhibits germination and growth at high concentrations (Rawat and Banerjee 1998; Kurban et al. 1999; Nedjimi 2009; Sun et al. 2011). In our study, the optimal concentration for germination in the halophyte *H. hamabo* was 25 mM NaCl, and the optimal concentration for survival and growth ranged from 5 to 10 mM NaCl. Growth traits, such as plant height, canopy diameter, number of leaves and the width of the largest leaf, were not significantly different from the control conditions than at 25 mM NaCl. In our study, 100 % of the *H. hamabo* seeds germinated at 50 mM NaCl, 50 % germinated at 100 mM NaCl, none germinated at 500 mM NaCl and survival was completely inhibited at 100 mM NaCl. The stimulation or inhibition effect of salt stress at low or high concentrations likely depends on the plant species (Bell et al. 1994; Rawat and Banerjee 1998; Nasim et al. 2009). Rawat and Banerjee (1998) found that a 40 mM salt concentration stimulated growth and biomass production of *E. camaldulensis* and *D. sissoo*, while Kurban et al. (1999) reported that total plant weight increased at low salinity (50 mM NaCl) in *Alhagi pseudoalhagi*. Nedjimi (2009) found

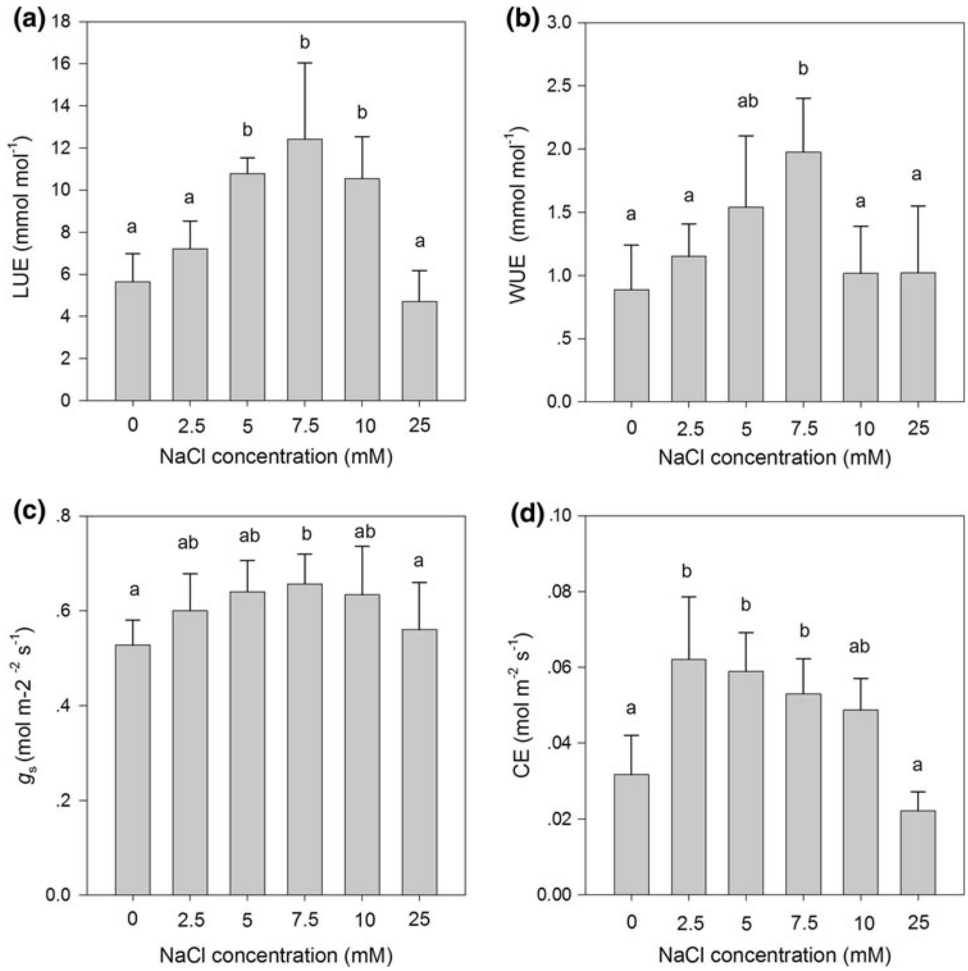

Fig. 6 Effect of NaCl concentration on the light utilization efficiency (LUE, **a**), water utilization efficiency (WUE, **b**), stomatal conduction (g_s, **c**) and carboxylation efficiency (CE, **d**) of *Hibiscus hamabo*. Different *lowercase letters* indicate the significant difference among different treatments

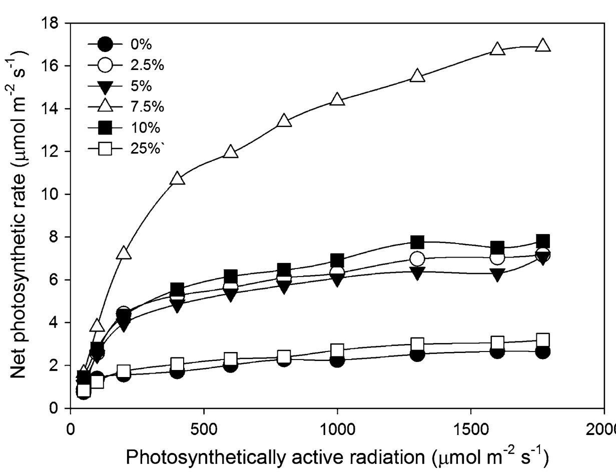

Fig. 7 Effect of NaCl concentration on the light response curve of *Hibiscus hamabo*

that the growth of *Lygeum spartum* was reduced at 60 and 90 mM NaCl, but was not significantly lower than the controls at 30 mM NaCl. Abogadallah et al. (2010) found that 46 % of fows A3, a salt-tolerant mutation of barnyard grass (*Echinochloa crusgalli*), germinated in 300 mM of NaCl, while Cambrollé et al. (2010) found that the seedlings of *Glaucium flavum* could not survive at 600 mM NaCl.

Photosynthetic traits, chlorophyll content and chlorophyll fluorescence are used to evaluate the salt tolerance of plants (Ashraf 2004; Sun et al. 2011). Salinity increased the net photosynthetic rate, transpiration rate, stomatal conductance and chlorophyll content at low salinity (Heuer and Plaut 1981; Locy et al. 1996; Ashraf 2004; Sun et al. 2011) and reduced them at high salinity in certain plant species (Rawat and Banerjee 1998; Tezara et al. 2002; Ashraf 2004; Chartzoulakis 2005; Sun et al. 2011). LUE, WUE and CE increased at low salinity and decreased at high salinity (Sun et al. 2011). NaCl affects photosynthetic electron transport and inhibits PSII activity as a consequence of the accumulation of salts in the chloroplasts (Sudhir and Murthy 2004). Chlorophyll fluorescence yields, such as F_0 and F_v, can be used for observing stress and damage of the photosynthetic apparatus and characterizing the environment where plants grow (Ranjbarfordoei et al. 2006). F_v/F_m indicates the efficiency and stability of PSII, the major component of the photosynthetic apparatus (Netto et al.

Table 1 Effect of NaCl concentration on the photosynthetic response to light in *Hibiscus hamabo*

NaCl concentration (mM)	P_{max} (μmol CO_2 m^{-2} s^{-1})	LCP (μmol m^{-2} s^{-1})	LSP (μmol m^{-2} s^{-1})
0	3.41 ± 0.16a	25.94	306.39
2.5	8.83 ± 0.25a	21.43	346.84
5	8.57 ± 0.28a	21.36	332.87
7.5	21.93 ± 0.83b	14.85	457.04
10	9.56 ± 0.25a	14.74	339.21
25	4.19 ± 0.19a	25.34	313.56

Different lowercase letters in the same column indicate the significant difference among different treatments at $P < 0.05$

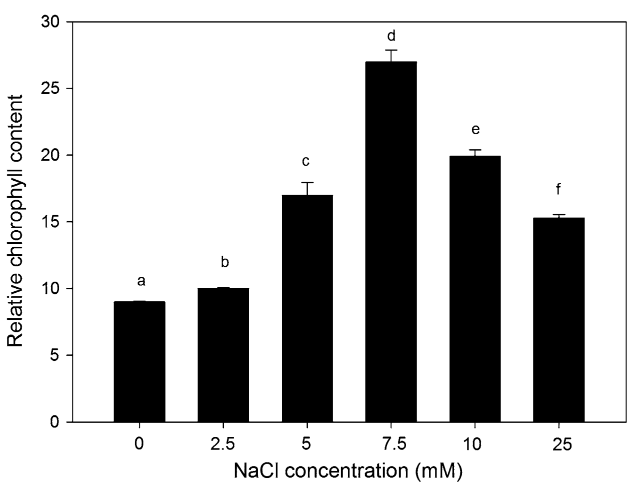

Fig. 8 Effect of NaCl concentration on the relative chlorophyll content of *Hibiscus hamabo*. Different *lowercase letters* indicate the significant difference among different treatments

2009; Ranjbarfordoei et al. 2011). F_0 indicates the impairment of the light-harvesting complex of PSII (Krause and Weis 1991). The most sensitive salt stress indicators seem to be the ratio F_v/F_0 and the chlorophyll content and are thus best suited for early salt stress detection (Ranjbarfordoei et al. 2006). Salinity increased the value of F_0 and decreased the values of F_m and F_v/F_m in certain plants (Ranjbarfordoei et al. 2006). In this study, P_n, LUE, g_s, WUE, LSP, P_{max} and chlorophyll content were highest at 7.5 mM NaCl. F_v/F_m and F_v/F_0 were significantly higher at 5 and 7.5 mM, while F_0 was significantly lower. F_m and F_v were significantly higher at NaCl concentrations ranging from 2.5 to 10 mM. The results suggested that *H. hamabo* at 7.5 mM had higher photosynthetic rate, more efficient and stable PSII and less impairment of PSII, which could promote the growth of seedlings.

In this study, Pearson correlation analysis showed that the chlorophyll content, P_{max} and LSP were significantly positively correlated with the number of leaves, while F_0 was significantly negatively correlated with the width of the largest leaf. LCP was significantly negatively correlated with the plant height, number of leaves, width of the largest leaf and canopy diameter. Due to the significant correlations observed between the physiological and growth traits, the chlorophyll content, P_{max}, LSP, F_0 and, in particular, LCP might be good indicators for the salt tolerance of *H. hamabo*.

The lower germination rate of *H. hamabo* at 200 mM NaCl (<20 %) was similar to the previous case (Bo et al. 2008) and the interesting thing indicated in this study was that the threshold for the seedling survival (50 mM) was lower than that corresponding to maximal salt tolerance reported for *H. hamabo* (188–256 mM NaCl, Yang et al. 2008). The seedling survival rate of *H. hamabo* was only 8.33 and 0 % at 25 and 50 mM, respectively, where the highest germination rate and the quickest germination time were found. The maximum quantum yield of PSII photochemistry, expressed through F_v/F_m, is almost constant for different plant species measured under non-stressful conditions ($0.8 \leq F_v/F_m \leq 0.86$) (Björkman and Demmig 1987). In our study, F_v/F_m of *H. hamabo* grown at 0 mM NaCl was only 0.6190 ± 0.0217, indicating 25 mM NaCl had no significantly inhibitory effect on the physiological traits of *H. hamabo*. The mechanism behind the contradiction is unknown. One possible mechanism might be the shortage of suitable arbuscular mycorrhizal fungi in this sand culture experiment. Turjaman et al. (2006) found that arbuscular mycorrhizal fungi significantly increased the survival rate of the seedlings of two plant species

Table 2 Effect of NaCl concentration on chlorophyll fluorescence parameters in *Hibiscus hamabo*

NaCl concentration (mM)	F_v/F_m	F_0	F_m	F_v	F_v/F_0
0	0.2650 ± 0.0093a	168.4 ± 4.6152a	229.4 ± 7.5033a	61.0 ± 3.6742a	0.3621 ± 0.0174a
2.5	0.6624 ± 0.0209b	136.2 ± 6.7602b	404.4 ± 7.4027b	268.2 ± 12.872b	1.9768 ± 0.1990b
5.0	0.7198 ± 0.0093c	114.0 ± 3.2404c	407.6 ± 7.3348b	293.6 ± 7.8930b	2.5777 ± 0.1159c
7.5	0.7306 ± 0.0313c	112.8 ± 11.4324c	420.2 ± 8.0125b	307.4 ± 18.9947b	2.7650 ± 0.4957c
10	0.6728 ± 0.0163b	137.8 ± 5.0695b	426.6 ± 6.8411b	288.8 ± 10.8259b	2.1000 ± 0.1489b
25	0.6190 ± 0.0217d	150.0 ± 7.7460d	378.4 ± 39.5133c	228.4 ± 39.0231c	1.5266 ± 0.2837d

Different lowercase letters in the same column indicate the significant difference among different treatments at $P < 0.05$

Table 3 Pearson correlation coefficients and the significance between the growth traits and the other physical traits of *Hibiscus hamabo*

Factors	Canopy diameter		Height		Width of the largest leaf		Number of leaves	
	r	Sig.	*r*	Sig.	*r*	Sig.	*r*	Sig.
Chlorophyll content	0.6980	0.1230	0.6730	0.1429	0.7408	0.0921	**0.8866**	**0.0186**
P_{max}	0.6598	0.1539	0.5868	0.2209	0.6861	0.1323	**0.8764**	**0.0220**
LCP	**−0.8248**	**0.0434**	**−0.8312**	**0.0404**	**−0.8636**	**0.0267**	**−0.8570**	**0.0292**
LSP	0.5539	0.2541	0.4681	0.3492	0.5776	0.2299	**0.8121**	**0.0496**
F_0	−0.6823	0.1354	−0.6573	0.1560	−0.6573	0.1028	**−0.7655**	**0.0760**

Values in bold are significant at $P < 0.05$

Sig. significance

Dyera polyphylla and *Aquilaria filarial* used as non-timber forest products. It has been verified that arbuscular mycorrhizal fungi in the soil could improve the salt tolerance of plants (Hajiboland et al. 2010) by enhancing photosynthesis and water status and increasing the nutrient uptake of plants under salt stress (Sheng et al. 2008; Porras-Soriano et al. 2009; Talaat and Shawky 2011). Further study should be focused on the factors that influence the survival of *H. hamabo* in salty condition, such as addition of arbuscular mycorrhizal fungi inferred by Hajiboland et al. (2010).

In our study, F_v/F_m of *H. hamabo* grown at 0 mM NaCl was only 0.2650 ± 0.0093, which is significantly lower than 0.8, indicating that suitable salt concentration was necessary for the growth of halophytic *H. hamabo*. Our studies suggest that *H. hamabo* would be an ideal candidate for the afforestation of salt-affected lands when salinity is moderate or low. The contradiction between the physiological traits and survival rate indicates that the seedling survival rate might be the bottleneck for the dispersion of *H. hamabo*. Thus, transplanting seedlings, rather than sowing seeds, has been suggested when *H. hamabo* was used in an afforestation project of reclamation areas.

Author contribution Junmin Li and Engfeng Wang conceived and designed the experiments. Junmin Li, Jingjing Liao and Jing Zhang performed the experiments. Junmin Li, Jingjing Liao and Ming Guan analyzed the data. Junmin Li and Engfeng Wang contributed reagents/materials/analysis tools. Junmin Li and Ming Guan wrote the paper.

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Conflict of interest The authors declare that no competing interests exist.

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