PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2023.012 PHOTOSYNTHETICA 61 (1): 124-134, 2023

Morphological and physiological responses of two *Osmanthus fragrans* cultivars to salt stress

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Abstract

We examined the morphological and physiological responses of *Osmanthus fragrans* 'Yingui' (Yin) and *O. fragrans* 'Jingui' (Jin) to different NaCl concentrations. NaCl concentrations significantly affected plant height and leaf mass per area. Total biomass decreased by 22.8–41.8% under moderate and high NaCl which inhibited *O. fragrans* growth. The ratio of root to shoot biomass in Yin was 44.3% higher than that in Jin at high NaCl concentrations which suggested that Yin possesses conservative resource acquisition strategies to resist salt stress. Compared to Yin, Jin showed higher net photosynthesis, stomatal conductance, and intercellular CO_2 concentration under high NaCl treatment. Jin exhibited also relatively higher proline, soluble sugar, K^+ content, and K^+/Na^+ under the treatments implying that acquisitive resource acquisition may be the main strategy for salt resistance in Jin. Our results demonstrated that Yin and Jin could be cultivated in saline land in a short time and the two cultivars respond to salinity by different morphological and physiological mechanisms.

Keywords: gas exchange; leaf dry mass content; membrane permeability; plant biomass; water-use efficiency.

Introduction

Soil salinity is a major global environmental factor limiting plant growth and crop productivity (Yang and Guo 2018), which has been aggravated by poor irrigation practices, rising population, and industrial pollution (Ouhibi *et al.* 2014). Almost 950 million hectares of agricultural land are affected by salinity worldwide (Shabala *et al.* 2015). Because of the occurrences of salt-affected soils and their vast impact on plant growth and crop production,

Highlights

- The moderate and high NaCl stresses inhibited *Osmanthus fragrans* growth
- Cultivar Yin allocated a higher percentage of biomass to roots to resist salt stress
- Cultivar Jin exhibited acquisitive resource acquisition strategies for salt resistance

Received 18 October 2022
Accepted 23 March 2022

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Abbreviations: C_i – intercellular CO_2 concentration; DM – dry mass; E – transpiration rate; EC – electrical conductivity; FM – fresh mass; g_s – stomatal conductance; Jin – *Osmanthus fragrans* cultivar 'Jingui'; LDMC – leaf dry mass content; LMA – leaf mass per area; P_N – net photosynthetic rate; REL – rate of electrolyte leakage; RWC – relative water content; SS – soluble sugar; TM – turgid fresh mass; WUE – water-use efficiency (= P_N/E); Yin – *Osmanthus fragrans* cultivar 'Yingui'.

Acknowledgments: The research was supported by the China Postdoctoral Science Foundation, grant no. 2017M623224, the Shaanxi Natural Science Foundation Program, grant no. 2020JQ-887, and the Special Funds of Science and Technology of Shaanxi Academy of Sciences: Rural Revitalization Project.

Conflict of interest: The authors declare that they have no conflict of interest.

responses to salt stress in a variety of plant species have been intensively studied over the last century (Flowers and Colmer 2008).

Osmanthus fragrans (Thunb.) Lour is a kind of noteworthy horticultural plant belonging to the Oleaceae family. *O. fragrans* is an evergreen shrub native to southwestern China, which is now widely cultivated in the Yangtze River Basin (Zang and Xiang 2004, Wang *et al.* 2006). As one of the most widely exploited species in Oleaceae, *O. fragrans* is used for vegetation restoration landscaping, and perfume making (Zang *et al.* 2003). It has also great potential in pharmacology and the food industry (Wei *et al.* 2020). China is a country whose soils are severely affected by soil salinization, which, due to its extensive area, and wide distribution poses a serious threat to regional agricultural and forestal development. The total area of saline soil in China is about 3.6×10^7 ha, accounting for 4.88% of the country's total available land base (Li *et al.* 2014). Salt stress is one of the major environmental stresses limiting *O. fragrans* growth and productivity (Yang *et al.* 2018). Therefore, it is urgent to understand the mechanisms underlying the salt tolerance of *O. fragrans.*

Plants have developed defense mechanisms that enable them to adapt and survive under salt conditions in their life cycle (Mansour and Hassan 2022). Salt stress impacts on plants are ascribed to osmotic and ionic effects as well as induction of oxidative damage as secondary stress (Munns and Tester 2008). Under salt stress, early osmotic stress is induced by lowering the soil solution water potential. After a long time of salt stress, ionic imbalance and toxicity occur as a result of high salt concentration within the cytoplasm. Salt-induced oxidative stress leads to intensive reactive oxygen species production which causes ultimately cell death (Munns and Tester 2008, Guo *et al*. 2022). It is worth noting that plant response to salinity is a complex phenomenon involving morphological, physiological, and biochemical alterations. These responding mechanisms include the regulation of osmotic balance under the osmotic stress caused by high salinity and the adjustment of ionic balance to avoid ionic toxicity (Guo *et al*. 2022). Soil salinization usually leads to an increase in the osmotic pressure of the soil, which causes osmotic stress in the plant cells. However, the plant can also adapt to stress through osmotic regulation. Accumulation of organic and inorganic solutes in the cell reduces the water potential of the cells and ensures water uptake under salt stress conditions. Soluble sugar and proline are common in nature and are effective osmotic regulators; they have an important role in mediating saline resistance in plants (Vendruscolo *et al.* 2007, Guo *et al.* 2015). Accumulated proline was the main osmolyte responsible for the osmotic adjustment of ornamental plants in response to saline conditions (Adamipour *et al.* 2019, Geng *et al.* 2019). Inorganic ion accumulation is also a way to reduce the osmotic potential. As Na^+ and $Cl^–$ mainly accumulate in the vacuole, they can be used for osmotic adjustment of the plant cell (Chen and Jiang 2010).

The most common poisonousness in saline soil is Na^+ which disrupts the ion and water balance of the cells if excessive Na^+ enters a plant (Hasegawa 2013). Because K^+ and Na^+ have the same hydrated ionic radius, they inhibit each other's absorption (Blumwald 2000). Maintaining a higher K^+/Na^+ in plant cells under salt stress could promote normal physiological metabolism (transpiration, photosynthesis, and growth) in the plant (Kopittke 2012, Guo *et al.* 2015). At high salinity, salts can build up in the chloroplast and exert a direct toxic effect on photosynthetic processes (Munns and Tester 2008, Ahanger *et al.* 2018). Some plants showed higher tolerance to salinity in photosynthetic processes by higher K^+ and chlorophyll contents (Soroori *et al*. 2021). Lower Na^+ in the leaves than roots ensured normal photosynthesis in the plant (Acosta-Motos *et al*. 2015). Inhibited photosynthetic efficiency in the plant will no longer be able to supply the carbohydrate requirement of the leaves, which further reduces their growth rate. At the same time, because of the competition of Na^+ and $Cl^–$ with other nutrients such as K^+, Ca^{2+}, and $NO_3^–$, which reduce plant growth by affecting the availability, transport, and partitioning of nutrients, high salinity stress causes nutrient imbalances (Hu and Schmidhalter 2010), decreased leaf area and dry mass accumulation (Kumar *et al.* 2000), and increased root-to-shoot biomass ratio (Liu *et al.* 2015) in plants. The major effect of salinity was the curtailing of the size of individual leaves or the number of branches. Root growth was usually less affected than leaf and shoot growth by NaCl stress. The explanation is that a reduction in leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve soil moisture and prevent an escalation in the salt concentration in the soil (Munns and Tester 2008).

At present, researchers are focusing on the color and fragrance of *O. fragrans* cultivars (Han *et al.* 2015, Fu *et al.* 2019). There are also reports on its stress resistance, such as low temperature, drought, and heavy metal stress (Liao *et al.* 2018, Qian *et al.* 2018, Li *et al.* 2020). However, mechanisms of morphological and physiological responses to salt stress in *O. fragrans* are still unknown. In this study, the seedlings of two *O. fragrans* cultivars were subjected to NaCl treatments and their physiological and growth responses to the salt stress were examined. We hypothesized that both cultivars would tolerate low and moderate NaCl stresses and high NaCl concentrations would significantly inhibit plant growth. We further hypothesized that there would be differences in salt resistance mechanisms between the two cultivars under NaCl stress. Results from this study would be valuable for a better understanding of the physiological responses in *O. fragrans* to salt stresses and would be highly relevant to its proper management and application in salinized soil areas.

Materials and methods

Seed collection and germination: Seeds of *O. fragrans* 'Yingui' (Yin) and *O. fragrans* 'Jingui' (Jin) were collected from Xi'an University located in the Shaanxi province in China (34°13.35'N, 108°54.47'E), in May 2017. The seeds were stored in wet sand at 4°C before germination. The mean annual air temperature is about 13.3°C, with monthly values ranging from –4°C in January to 32°C in July. Annual rainfall ranges from 500–750 mm, of which 78% falls between May and October. Before the start of the experiment, the seeds were surface-sterilized with 0.52% sodium hypochlorite solution for 1 min and rinsed three times with distilled water to avoid fungal infection. Then the seeds were placed on threefold filter paper on 30 Petri dishes (100 mm diameter and 15 mm height, ten seeds per dish) and kept at a photon flux density of 350–400 μmol m^{-2} s^{-1} with 12/12-h photoperiod at a temperature of 25/18°C (day/night) in a phytotron (*PQX 330H*, *Ningbo Life Technology Co.*, Zhejiang, China) in October 2017. Seeds started germinating 15 d after sowing.

Experimental design: Seedlings of each cultivar were transplanted into 72 plastic pots (15 cm diameter and 20 cm height) filled with washed sand and positioned in a sunlit greenhouse. Pots were well-watered with Hoagland solution every two days. The temperature was maintained at 25/18°C (day/night) by three air conditioners and relative humidity was kept at about 50%. The average photon flux density was 654 μmol m^{-2} s^{-1} in the daytime. When the seedlings had six leaves of a uniform size, the pots were divided into six 12-pot groups for each cultivar. Each group of pots corresponded to one of the NaCl concentrations: C (control), S2 (low), S4, S6 (moderate), S8, and S10 (high). The selection of the NaCl concentration range was based on soil salt components in the Weibei region [0–6 g(NaCl) kg^{-1}(soil)] in Shaanxi province (Fan *et al.* 2017). NaCl stress was initiated 50 d after sowing (DAS), which corresponded to the sixth-leaf stage. Plants under stress were watered with Hoagland solution which differed only in the amount of additional NaCl. Pots were arranged in a randomized complete block design with six blocks and rotated twice a week during the experiment to minimize location effects.

Stress level	NaCl [g(NaCl) kg^{-1}(soil)]	Treatment
Control	0	C
Low	2	S2
Moderate	4	S4
	6	S6
High	8	S8
	10	S10

Gas exchange: After three weeks of salt treatment (71 DAS), measurements of net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) on fully expanded leaves were taken on the same day using *Li-6400* portable photosynthesis system (*LI-COR Inc.*, Lincoln, Nebraska, USA) at PPFD of about 800 μmol m^{-2} s^{-1}, provided by an external halogen lamp. The ambient temperature in the measuring cuvette was maintained at 25°C with a relative humidity of about 65%. A CO_2 cartridge had been used to maintain a CO_2 concentration of 360 μmol mol^{-1} during measurements. Three measurements were carried out in one plant. Steady-state values from each leaf were recorded after the 200-s equilibration period. Leaf water-use efficiency (WUE) was calculated as follows: WUE = P_N/E.

Plant height and mass: Plant heights were measured after gas-exchange measurements. Plants were separated into roots and shoots. All separated samples were placed in perforated paper bags, at 80°C for 48 h and then weighed for the dry mass. The ratio of root-to-shoot biomass (root-to-shoot ratio) was calculated for each treatment.

LMA and LDMC: The second fully expanded leaves (2nd leaf from shoot top, hereafter) of the two cultivars were sampled randomly from six plants in each stress level. The fresh mass (FM) for each leaf was measured immediately with a *SE202F* electronic balance (*Ohaus Corp.*, Parsippany, NJ, US). Leaf area was measured using a flatbed scanner connected to a personal computer running image analysis software. All leaf samples were placed in perforated paper bags separately and oven-dried at 80°C for 24 h, then weighed to have dry mass (DM). Leaf mass per area (LMA) denotes leaf dry mass per area (Cornelissen *et al.* 2003). Leaf dry mass content (LDMC) was calculated as DM/FM (Chai *et al.* 2015).

Membrane permeability and RWC: Approximately 0.5 g of a fresh top leaf was taken from a plant in each treatment and cut into 0.5 cm long segments before being washed three times with deionized water to remove surface-adhered electrolytes. Leaf samples were divided into two equal portions and placed separately into two closed vials, each containing 20 ml of deionized water. One vial was incubated at 25°C on a rotary shaker for 24 h before the electrical conductivity (EC1) of the solution was determined using a conductivity gauge (*DDSJ-319L*, *Leici*, China). The other vial was autoclaved at 120°C for 20 min and electrical conductivity (EC2) was measured after equilibration to 25°C. The rate of electrolyte leakage (REL [%]) was calculated as EC1/EC2 × 100 (Guo *et al.* 2015).

The 2nd fully expanded leaves were sampled at about 09:00 h and then weighed immediately to obtain fresh mass (FM) in the field. The leaves were placed into plastic bags filled with distilled water overnight. The turgid fresh mass (TM) of each leaf was measured the next morning. Leaf samples were placed in perforated paper bags, oven-dried at 80°C for 24 h, and weighed to measure dry mass (DM). Relative water content (RWC) was calculated as RWC = (FM – DM)/(TM – DM) (Barrs and Weatherley 1962).

Proline, soluble sugar, Na^+, and K^+ contents: Dry leaves were hand-ground using mortar and pestle to a size small enough to pass through a 100-mesh screen. A ground dry leaf sample (0.1 g) was used to determine proline content according to the ninhydrin method (Bates *et al.* 1973). Leaf powders of 50 mg of each sample were subjected to soluble sugar analysis using the anthrone–sulfuric acid method (Ebell 1969). About 0.02 g of ground samples (leaves and roots) were used to determine concentrations of K$^+$ and Na$^+$ by flame photometry (*Flame Photometer 410*, *Corning*, Halstead, UK). Six measurements were made for each treatment to determine proline and soluble sugar content, [K$^+$], and [Na$^+$].

Statistical analysis: All results are presented as the means ± standard errors (SE). Analysis of variance (*ANOVA*) was performed on data of plant height, biomass, leaf area, LMA, LDMC, gas-exchange parameters (P_N, E, WUE, g_s, and C_i), proline and soluble sugar content, [K$^+$], and [Na$^+$] to determine divergence in the two cultivars under different salt treatments. Two-way analysis of variance (*ANOVA*) with NaCl concentrations and cultivars as the grouping factors was used to compare all experimental parameters with *Tukey*'s HDS multiple comparison test. All statistical analyses on the data were performed using *SPSS 17.0* (*SPSS* for *Windows*, Chicago, IL, USA), and all figures were processed by *Origin 10.0* (*OriginLab Corporation*, USA). A difference was considered significant if $p<0.05$.

Results

Plant height and leaf area: NaCl concentrations significantly affected plant height and leaf area (Table 1). Plant height in *O. fragrans* Yin and Jin exhibited remarkable variations along the NaCl gradient (Fig. 1*A*). Low NaCl concentrations showed a small effect on plant height in either Yin or Jin. The plant height of Yin and Jin decreased significantly when NaCl concentrations increased to S4–S10. Plant height dropped by 29.1% in Yin and 29.7% in Jin at S10 compared with C. Low NaCl concentrations showed a small effect on leaf area in Yin (Fig. 1*B*). High salt stress, however, reduced leaf area significantly. The leaf area in Yin at S10 was 66.9% of that in C. Average leaf area in Jin dropped by 26.9% at high salt concentrations compared to that in C.

Leaf mass per area (LMA) and leaf dry mass content (LDMC): NaCl concentrations significantly affected plant LMA and LDMC (Table 1). The two cultivars presented a similar pattern of LMA in response to salt (Fig. 1*C*). LMA of Yin and Jin at the moderate NaCl concentrations (S4 to S6) were about 7.1 and 4.9% higher than that at the control (S0), respectively. LMA of Yin and Jin decreased significantly under S8. Compared to C, the LMA of Yin and Jin dropped by 25.4 and 25.9% at high salt concentrations, respectively. LDMC of Yin and Jin at first increased, peaking in S6, then decreasing with further increase of NaCl concentrations (Fig. 1*D*).

Biomass allocation: Salt stress also had significant effects on plant biomass allocation (Table 1, Fig. 2). In general, shoot biomass and total biomass decreased significantly with the NaCl gradient. Compared with C, shoot biomass of Yin and Jin were reduced by 3.3 and 7.1% in the low,

Table 1. Results of multiple comparisons by *ANOVA* through GLM for repeated measurements of NaCl concentrations, cultivars, and their interaction (NaCl concentrations × cultivars) for leaf morphological and physiological traits. (**$p<0.01$, *$p<0.05$, ns – not significant).

Dependent variables	Independent variables NaCl concentrations	cultivars	NaCl concentrations × cultivars
Plant height	40.16^{**}	1.85^{ns}	0.07^{ns}
Leaf area	11.15^{**}	0.71^{ns}	0.08^{ns}
LMA	13.53^{**}	1.62^{ns}	0.13^{ns}
LDMC	8.35^{**}	0.00^{ns}	0.47^{ns}
Shoot biomass	28.71^{**}	0.31^{ns}	0.63^{ns}
Root biomass	4.53^{**}	7.75^{**}	2.68*
Total biomass	26.01^{**}	0.01^{ns}	0.22^{ns}
Root-to-shoot ratio	11.69^{**}	8.24^{**}	4.21^{**}
P_N	34.51^{**}	0.46^{ns}	2.50^{*}
E	14.19^{**}	7.39^{**}	1.02^{ns}
g_s	28.5^{**}	0.38^{ns}	1.87^{ns}
C_i	17.74^{**}	10.84^{**}	1.22^{ns}
WUE	0.84^{ns}	4.85^{*}	0.35^{ns}
RWC	16.15^{**}	2.53^{ns}	1.19^{ns}
Membrane permeability	87.73^{**}	39.41^{**}	4.33^{**}
Proline	25.54^{**}	2.57^{ns}	10.16^{**}
Soluble sugar	61.07^{**}	72.03^{**}	3.71^{**}
Leaf [Na$^+$]	34.31^{**}	16.00^{**}	2.69^{*}
Leaf [K$^+$]	51.98^{**}	3.79^{ns}	2.29^{ns}
Root [Na$^+$]	12.63^{**}	5.27^{*}	0.55^{ns}
Root [K$^+$]	33.62^{**}	15.76^{**}	2.48^{*}

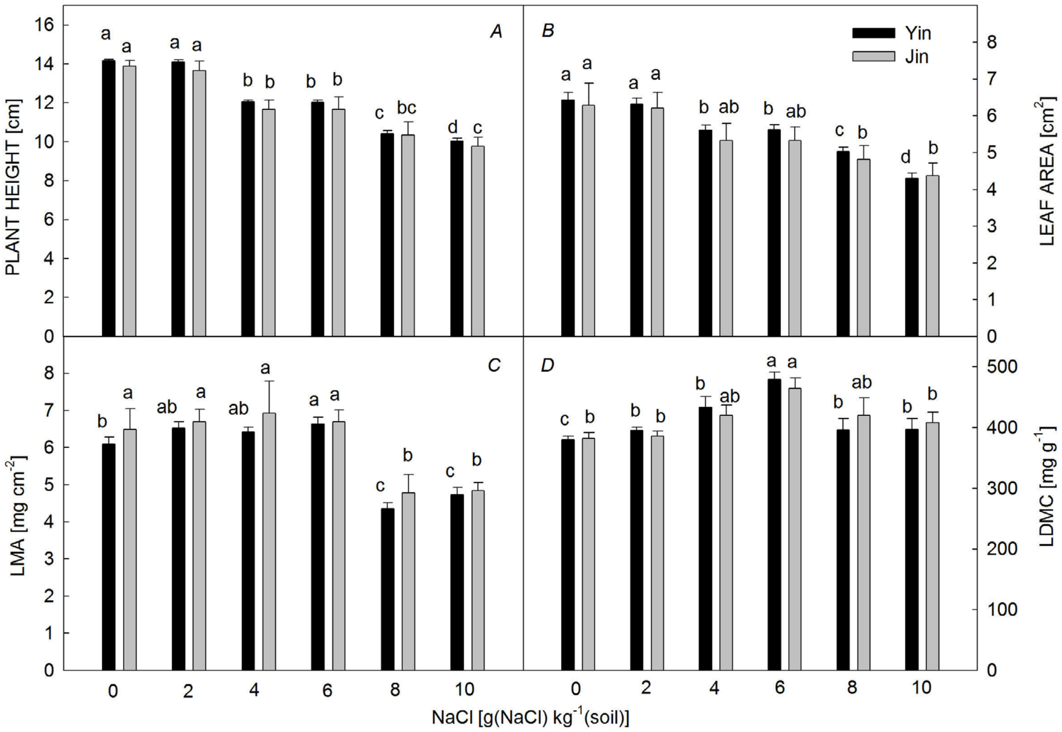

Fig. 1. Plant height (*A*), leaf area (*B*), leaf mass per area (LMA) (*C*), and leaf dry mass content (LDMC) (*D*) in *Osmanthus fragrans* 'Jingui' (Jin) and 'Yingui' (Yin) grown under different salt stress levels. Data are means ± SE ($n = 12$), *different lowercase letters* indicate statistically significant differences at the $p<0.05$ level.

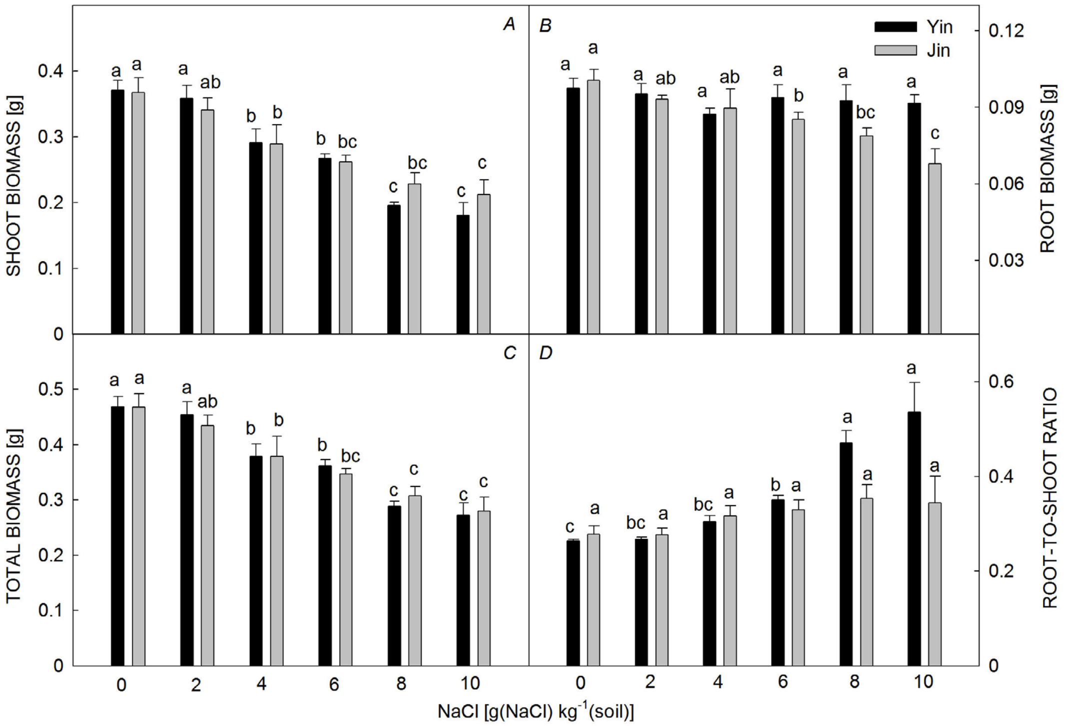

Fig. 2. Shoot biomass (*A*), root biomass (*B*), total biomass (*C*), and the ratio of root to shoot biomass (root-to-shoot ratio) (*D*) in *Osmanthus fragrans* 'Jingui' (Jin) and 'Yingui' (Yin) grown under different salt stress levels. Data are means ± SE ($n = 6$), *different lowercase letters* indicate statistically significant differences at the $p<0.05$ level.

24.6 and 25% in the moderate, and by 49.1 and 40% in the high NaCl concentrations, respectively (Fig. 2*A*). Compared with C, the total biomass of Yin and Jin was reduced by 3.1 and 7.2% in S2, 22.8 and 25.8% in S6, and by 41.8 and 40.2% in S10 (Fig. 2*C*).

Both NaCl concentrations and cultivars significantly affected root biomass and root-to-shoot ratio (Table 1). Root biomass of Yin and Jin varied considerably with NaCl concentrations (Fig. 2*B*). Root biomass of Jin decreased significantly from S6 to S10; compared to C, the reductions were from 15.3% in S6 to 32.6% in S10. NaCl stress had no significant effect on root biomass in Yin. Root biomass in Yin was significantly higher than that in Jin at S10. The root biomass of Yin was 17.5 and 35% higher than that of Jin at S8 and S10, respectively. The root-to-shoot ratio increased with the NaCl gradient, from 0.26 to 0.54 in Yin (Fig. 2*D*). The root-to-shoot ratio of Yin was 33.2 and 55.4% higher than that of Jin at S8 and S10, respectively.

Gas exchange: Low NaCl stress did not affect P_N in the two cultivars, but P_N decreased significantly at moderate and high NaCl stress (Fig. 3*A*, Table 1). P_N of the two cultivars at moderate and high NaCl stress maintained about 38 to 85% of the P_N of plants under C conditions, respectively. P_N in Yin was 14% higher than that of Jin at moderate NaCl concentrations, but P_N in Jin was 31.1% higher than that of Yin at high NaCl concentrations. The g_s followed a similar pattern as that of P_N (Fig. 3*C*), g_s in Jin were 22.2 and 140.3% higher than that of Yin at S8 and S10, respectively. Both NaCl concentrations and cultivars significantly affected *E* and C_i (Table 1). *E* was higher in Yin than that in Jin at low NaCl stress, however, it decreased more rapidly at moderate and high NaCl stress than that in Jin (Fig. 3*B*). C_i was unaffected at low NaCl stress but started to decline at moderate stress for the two cultivars (Fig. 3*D*). Compared with C, C_i of Yin and Jin were reduced by 17.6 and 23.2% in the moderate, and by 41.0 and 31.8% in the high NaCl concentrations, respectively. The C_i of Jin was 43.0 and 19.2% higher than that of Yin at S8 and S10, respectively. Only cultivars had a significant effect on WUE (Table 1). WUE was consistently higher in Yin than in Jin at all NaCl treatments (Table 2).

Relative water content (RWC) and membrane permeability: RWC differed more with the increasing NaCl stress in the two cultivars (Fig. 4*A*). RWC decreased significantly at moderate NaCl stress in Yin and at high NaCl stress in Jin. RWC of Yin and Jin at S10 maintained about 91.2 and 92.5% of the RWC of plants under C conditions. Both NaCl concentrations and cultivars significantly affected membrane permeability (Table 1). Yin at moderate and high stresses exhibited 129.9 and 342.5% higher REL than those in C (Fig. 4*B*). S2 and S4 had no significant effect on REL in Jin. Jin at S6, S8, and S10 showed 180.8, 248.2, and 312.5% higher REL compared with C, respectively. Compared to Jin, Yin had 48.0, 28.9, and 39.7% higher REL at low, moderate, and high-stress treatments, respectively.

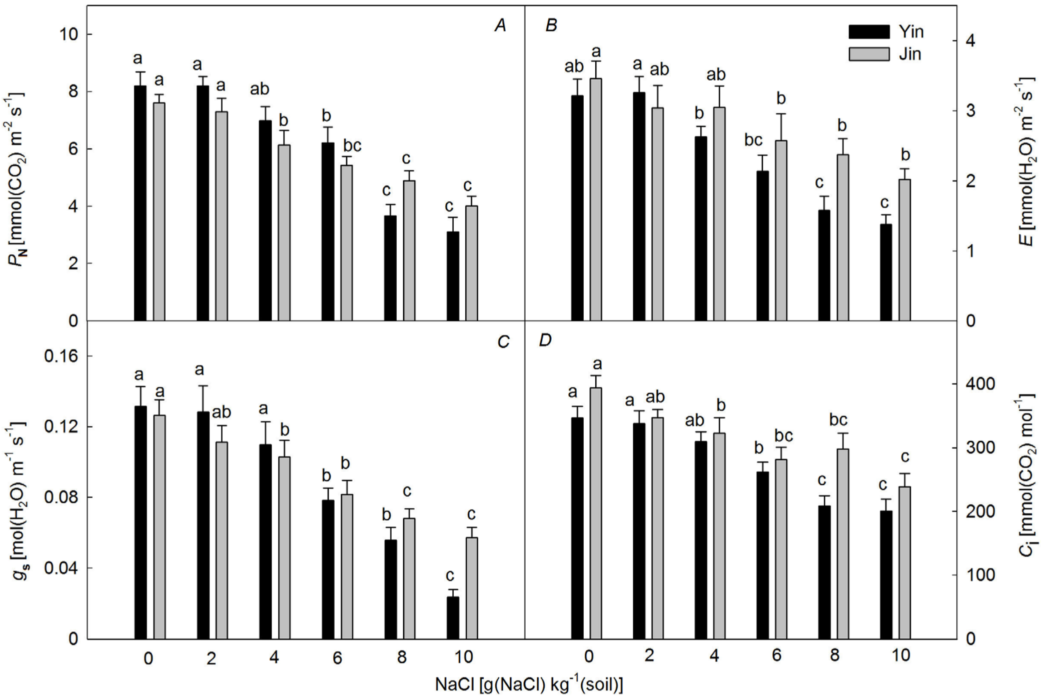

Fig. 3. The net photosynthetic rate (P_N) (*A*), transpiration rate (*E*) (*B*), stomatal conductance (g_s) (*C*), and intercellular CO$_2$ concentration (C_i) (*D*) in *Osmanthus fragrans* 'Jingui' (Jin) and 'Yingui' (Yin) grown under different salt stress levels. Data are means ± SE ($n = 6$), *different lowercase letters* indicate statistically significant differences at the $p<0.05$ level.

Proline and soluble sugar contents: High NaCl stress had a significant effect on proline content in Yin (Fig. 4*C*). Low NaCl stress did not affect proline content in Jin, but proline content at moderate and high stresses was about 2.5 and 3.8 times higher than that in C. Proline content in Jin was 45.6% greater than that in Yin at high-stress treatment. NaCl concentrations and cultivars affected significantly the soluble sugar contents in the two cultivars (Table 1, Fig. 4*D*). The soluble sugar contents in Yin and Jin increased with NaCl stress. The soluble sugar contents of Jin were about 22.1, 52.6, and 41.4% higher than those of Yin at low, moderate, and high-stress levels, respectively.

Table 2. Effects of NaCl concentrations on the water-use efficiency (WUE) in leaves of *Osmanthus fragrans* 'Jingui' (Jin) and 'Yingui' (Yin). Data are means ± SE ($n = 6$), *different lowercase letters* indicate statistically significant differences at the $p<0.05$ level.

NaCl [g(NaCl) kg^{-1}(soil)]	WUE [μmol(CO_2) mmol(H_2O)$^{-1}$]	
	Yingui	Jingui
0	2.57 ± 0.09^{a}	2.27 ± 0.19^{a}
2	2.56 ± 0.16^{a}	2.49 ± 0.25^{a}
4	2.71 ± 0.25^{a}	2.16 ± 0.32^{a}
6	3.07 ± 0.38^{a}	2.35 ± 0.32^{a}
8	2.41 ± 0.28^{a}	2.18 ± 0.29^{a}
10	2.31 ± 0.39^{a}	2.06 ± 0.27^{a}

Concentrations of Na^+ and K^+: Both NaCl concentrations and cultivars significantly affected [Na^+] (Table 1). The two cultivars exhibited similar patterns in the response of [Na^+] to increasing NaCl stress (Fig. 5*A*,*C*). Compared with C, [Na^+] in leaves and roots of Yin increased by 59.6 and 19.9% at S4 and as high as 171.8 and 68.8% at S10, respectively. Similarly, leaf [Na^+] and root [Na^+] in Jin increased by 22.1 and 40.0% at S4 and 77.9 and 65.6% at S10, respectively. Leaf [Na^+] and root [Na^+] in Yin were 9.9 and 10.0, 18.2 and 3.2, and 32.2 and 16.5% higher than that in Jin at low, moderate, and high-stress levels, respectively.

NaCl stress affected significantly [K^+] in the two cultivars (Table 1, Fig. 5*B*,*D*). For Yin, leaf [K^+] and root [K^+] started to decrease at S6 and S4; it dropped by 42.2 and 53.1% at S6, 47.4 and 61.0% at S8, and 72.0 and 79.1% at S10, respectively. Leaf [K^+] and root [K^+] in Jin started decreasing significantly when NaCl stress reached S4 and S6. Leaf [K^+] and root [K^+] were reduced by 33.5 and 19.2% at S6, 43.1 and 31.1% at S8, and 50.1 and 55.1% at S10, compared to C, respectively. The root [K^+] was significantly different between the two cultivars. Jin exhibited about 2.3 and 25.7% higher root [K^+] than that in Yin at S2 and S4, but the difference in root [K^+] between the two cultivars was much greater (59.4%) at S6, S8, and S10.

Discussion

Salinity is one of the major environmental stresses affecting *O. fragrans* as well as other salt-sensitive crop

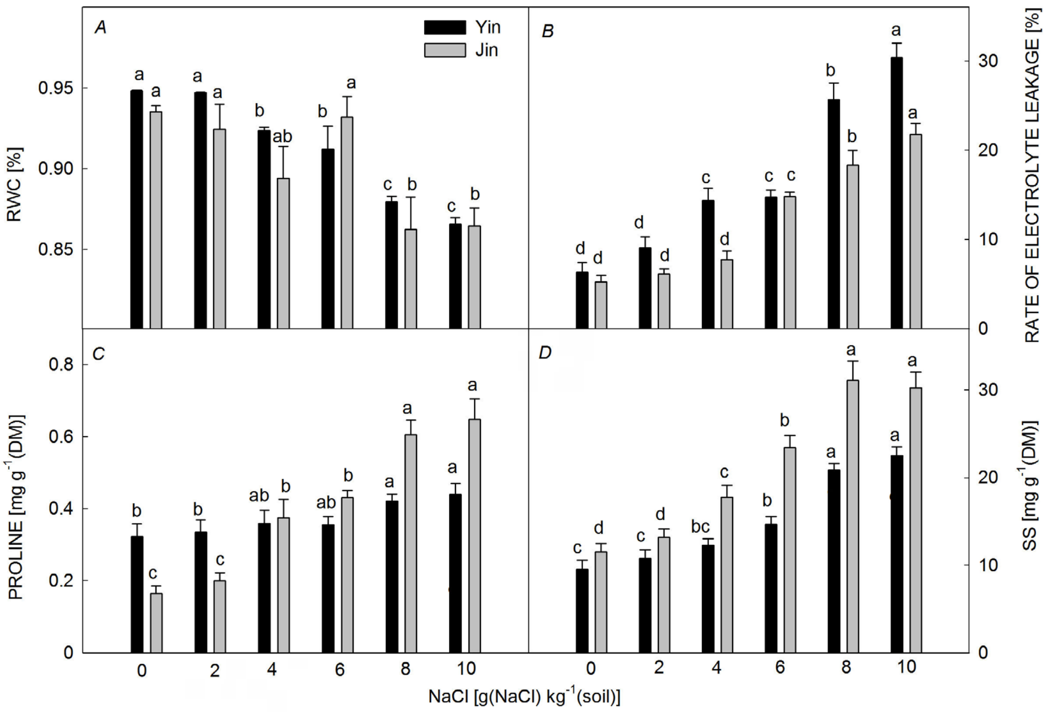

Fig. 4. Leaf relative water content (RWC) (*A*), rate of electrolyte leakage (membrane permeability) (*B*), proline (*C*), and soluble sugar (*D*) contents in *Osmanthus fragrans* 'Jingui' (Jin) and 'Yingui' (Yin) grown under different salt stress levels. Data are means ± SE ($n = 6$), *different lowercase letters* indicate statistically significant differences at the $p<0.05$ level.

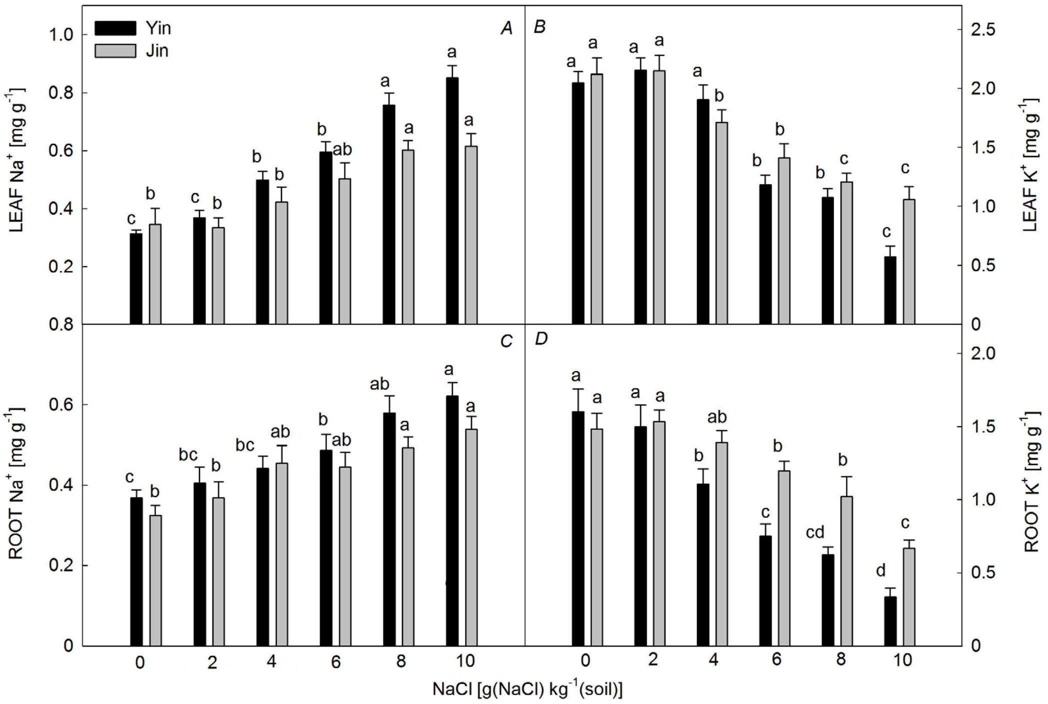

Fig. 5. Contents of Na$^+$ and K$^+$ in leaf and root of *Osmanthus fragrans* 'Jingui' (Jin) and 'Yingui' (Yin) grown under different salt stress levels. Data are means ± SE ($n = 6$), *different lowercase letters* indicate statistically significant differences at the $p<0.05$ level.

species worldwide. Moderate and high NaCl stresses were found to inhibit *O. fragrans* growth (Fig. 2), in general, shoot biomass and total biomass decreased significantly with the NaCl gradient. Like other environmental stresses, salinity causes oxidative stress as a result of water deficit (Gama *et al.* 2009). During oxidative stress, biomass is considerably reduced because of low CO_2 concentration in chloroplasts and reduced photosynthetic efficiency (Tourneux and Peltier 1995). At the whole plant level, there was an increase in root-to-shoot ratio with NaCl gradient in the two cultivars, as similar results had been found for other plants, such as in tomato and chickpea (Tejera *et al.* 2005, Tuna *et al.* 2007), indicating that roots grow faster to reduce to water deficit and increase the resource acquisition. This is a common response in salt and water stress, probably due to factors associated with water stress (osmotic effect) rather than a salt-specific effect (Hsiao and Xu 2000, Fernández-García *et al.* 2014). Reductions in shoot biomass (canopy leaf surface) have been considered as an avoidance mechanism that minimizes water loss under stress conditions (Munns and Tester 2008). A relatively higher root-to-shoot ratio found in Yin in comparison to Jin suggested that the Yin possesses conservative resource acquisition strategies to resist salt stress (Fig. 2). This supports our hypothesis that the two *O. fragrans* cultivars differ in their resistance to salt stress.

Reductions in the biomass of *O. fragrans* under salinity were indicative of growth limitations as reflected in plant height, leaf area, and leaf mass per area (LMA). The LMA is a key trait in plant growth and an important indicator of plant strategies (Poorter *et al.* 2009). *O. fragrans* cultivars adapted to low and moderate salinity by increasing LMA, but this trait failed to adapt at high salinity levels (Fig. 1). The increase in LMA in salt-stressed plants may imply a greater investment in assimilatory tissue as a strategy to maximize photosynthesis potential (Fernández-García *et al.* 2014). Some studies also demonstrated that high LMA enables plants to maintain high leaf water content under stress (Wang *et al.* 2011). Higher LMA, contributing to higher tolerance in unfavorable conditions, was proved to be a main adaptive strategy to moderate salt stress in the two *O. fragrans* cultivars.

The response of the stomatal conductance (g_s) plays a major role in plant performance under salt stress (Fernández-García *et al.* 2014). g_s is an indicator of water, energy, and CO_2 cycles between plants and the atmosphere (Saradadevi *et al.* 2016). Stomatal conductance in the two cultivars declined with the increasing salt stress (Fig. 3). Lower g_s reduced the capacity of the plants to take up CO_2 from the atmosphere and subsequently reduced photosynthetic carbon assimilation (Lawlor and Cornic 2002, Guo *et al.* 2015). However, Koyro (2006) suggests that reductions in g_s represent adaptive mechanisms to cope with excessive salt, by reducing the salt loading into the leaves and helping to increase longevity by maintaining salts at subtoxic levels longer than it would occur if transpiration rates were not diminished (Everard *et al.* 1994, Koyro 2006). Relatively higher P_N and g_s in Jin than that in Yin at high salt treatments suggested that Jin gains a greater photosynthetic capacity to resist high salt stress than that in Yin. The greater photosynthetic capacity in Jin could also be accounted for the increasing proline and soluble sugar contents and the greater K^+/Na^+ in leaves at high salt treatments. The proline and soluble sugar are used to protect the structure of cells and macromolecular substances, and lower Na^+ in the leaves ensured normal photosynthesis (Acosta-Motos *et al.* 2015).

The increase of proline and soluble sugar contents in Yin and Jin indicated the capacity for dehydration tolerance and the osmotic adjustment was enhanced with the increase of salinity gradient (Fig. 4; Table 1S, *supplement*). Osmotic adjustment is considered one of the crucial processes for plants to adapt to water deficit, decreasing cell water potential, and allowing the maintenance of the water absorption and cell turgor under salt and water stresses (Chaves *et al.* 2003). Relative high proline and soluble sugar content for Jin in moderate and high NaCl stress levels indicated its abilities for osmotic adjustment and the dehydration tolerance was much stronger than that of Yin. Many studies suggested that the accumulation of proline and soluble sugar (the most common compatible osmoprotectant molecule) in plant leaves is one of the most common and direct biochemical adaptive strategies to salt and water stress because there are positive correlations between the capacity for proline and soluble sugar accumulation and dehydration tolerance (Hare and Cress 1997, Wang *et al.* 2011, Ayaz *et al.* 2021).

It is known that one of the most damaging consequences of salt stress in plants is the influx of Na^+ and a decline in K^+ contents in tissues (Cheeseman 1988, Garthwaite *et al.* 2005, Guo *et al.* 2015). The best strategy to cope with salt stress in plants is to keep away Na^+ ion from leaves and sequester it within the cells of other organs and use limited water efficiently (Ayaz *et al.* 2021). Increases in $[Na^+]$ and decrease in $[K^+]$ in leaves and roots for both cultivars with rising NaCl concentrations demonstrated that the plasma membrane was severely compromised by salt stress, especially in Yin at high NaCl concentrations. Higher $[Na^+]$ in leaves and roots in Yin in comparison to Jin under salt stress could inhibit the growth of Yin, because the high $[Na^+]$ in leaves can cause salt toxicity, leading to a decline in growth and biomass production (Shi and Yin 1993, Guo *et al.* 2015). $[K^+]$ plays a major role in several physiological processes, such as osmotic regulation, protein synthesis, and enzyme activation (Garthwaite *et al.* 2005), and relatively greater salt tolerance in Jin might be mainly due to high $[K^+]$ in its leaves and roots. Higher $[K^+]$ and chlorophyll contents in some plants showed higher tolerance to salinity in photosynthetic processes and ensured normal photosynthesis (Acosta-Motos *et al.* 2015, Soroori *et al.* 2021). Normal photosynthetic efficiency in a plant will be able to supply the carbohydrate requirement of the leaves, which further promotes their growth rate. Jin exhibited the acquisitive strategies with greater osmotic adjustment and photosynthetic capacity which resulted in greater aboveground biomass to resist salt stress (Balachowski *et al.* 2016). The greater K^+/Na^+ ratios or K^+ and Na^+ selectivity and distribution of ions in plants are important criteria for salt tolerance assessment (Gu *et al.* 2016). K^+/Na^+ in the leaves and roots of Jin were 6.1–150 and 22–130% higher than those in Yin at moderate and high NaCl concentrations. It is suggested that Jin may belong to a group of cultivars that are reported as Na^+ excluders and tolerate NaCl stress (Acosta-Motos *et al.* 2017). Thus, the K^+/Na^+ ratio could be used as a salt tolerance indicator in *O. fragrans* cultivars, as it is in sunflower cultivars (Umar *et al.* 2019).

Conclusion: Our experimental study indicated that moderate and high NaCl stresses inhibited *O. fragrans* growth. The shoot biomass and total biomass decreased significantly with the NaCl gradient in the two cultivars. A relatively higher root-to-shoot ratio found in Yin suggested that the Yin possesses conservative resource acquisition strategies to resist salt stress. Jin exhibited acquisitive resource acquisition strategies with greater osmotic adjustment capacity, photosynthetic capacity, and greater shoot biomass to cope with salt stress. This finding is of great significance for short-term cultivating of *O. fragrans* in saline land.

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