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Contrasting photosynthesis, photoinhibition and oxidative damage in honeysuckle (*Lonicera japonica* Thunb.) under iso-osmotic salt and drought stresses

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ABSTRACT

Honeysuckle (*Lonicera japonica Thunb.*) is a traditional Chinese medicinal crop and belongs to the glycophyte with certain salt tolerance. This study aimed to deeply dissect its salt adaptability by contrasting photosynthesis, photoinhibition and oxidative damage under moderate and severe iso-osmotic salt (150 and 300 mM NaCl) and drought (19.3 % and 28 % PEG-6000) stresses with hydroponic protocol. Photosynthesis was more susceptible to drought stress than iso-osmotic salt stress in honeysuckle according to drought-induced greater decrease in photosynthetic rate. In contrast to salt-induced mild PSII and PSI photoinhibition, severe photosystem II (PSII) and photosystem I (PSI) photoinhibition arose upon iso-osmotic drought stress, indicated by greater decreased the maximal photochemical efficiency of PSII and PSI and remarkable loss of their reaction center proteins. However, PSII and PSI interaction hardly contributed to salt stability of photosynthetic apparatus because of salt-induced finite restriction on electron flow from PSII to PSI. Consistent with photosystems photoinhibition, leaf lipid peroxidation, H_2O_2 production and electrolyte leakage were elevated much greater by drought stress than iso-osmotic salt stress, confirming drought-induced severe oxidative stress in honeysuckle. Furthermore, the principal components analysis comprehensively showed higher salt adaptability in honeysuckle due to larger cluster separation upon drought stress than iso-osmotic salt stress. As an apparent reason, honeysuckle could prevent drought-induced tremendous leaf water loss upon iso-osmotic salt stress, and had a capacity to dispose accumulated Na^+. Therefore, honeysuckle resembles halophytes in this respect and seems appropriate for planting in coastal saline land.

1. Introduction

The problems of soil drought and salinization may become serious in the future due to regional water scarcity and unbalanced precipitation particularly in arid regions and coastal zone under the background of global change (Hussain et al., 2015; Han et al., 2018). Soil drought and salinization are two kinds of major environmental stresses and have detrimental effects on crop growth and yield. Similar to drought stress, salt stress also disrupts water homostasis in plant tissues by inducing osmotic pressure, and some drought-induced physiological symptoms such as lowered transpiration and stomatal closure exists in plants exposed to salt stress as well (Zhu, 2002; Munns and Tester, 2008; Zhu, 2016). Besides osmotic pressure, plants have to confront ionic toxicity under salt stress, and Na^+ is the dominant toxic ingredient (Zhu, 2003; Munns and Tester, 2008; Turkan and Demiral, 2009). Salt-induced ionic toxicity usually does not arise as rapidly as osmotic stress, but may lead to severe inhibition on physiological metabolisms (Munns, 2002; Allakhverdiev and Murata, 2008; Yang et al., 2014). Accordingly, some studies proved that salt stress was more hazardous to plant growth than iso-osmotic drought stress (Muranaka et al., 2002; Cha-um and Kirdmanee, 2010; Cha-um et al., 2010; Patade et al., 2011; Hossain et al., 2017). In contrary, some plant species exhibited greater susceptibility to

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iso-osmotic drought stress rather than salt stress, indicating that salt stress may be not identical to a simple combination of osmotic stress and ionic toxicity (Zhao and Harris, 1992; Zhao et al., 2003; Hassine and Lutts, 2010; Silva et al., 2010; Sucre and Suárez, 2011; Amjad et al., 2015; Lan et al., 2020; Katuwal et al., 2020).

Salt and drought stresses can influence redox equilibrium and bring about secondary oxidative stress with excess generation of reactive oxygen species (ROS) in plant cells (Gill and Tuteja, 2010; Hossain and Dietz, 2016; van Zelm et al., 2020). As a consequence, oxidative damages on biological macromolecules such as lipids and proteins will further interfere with cellular functions and seriously depress plant growth. Consistently, oxidative damage was often noted in line with declined plant growth under salt and drought stresses. In photosynthetic organisms, the major ROS generation sites are located at photosystem I and II (PSI and PSII) in chloroplast, and oxidative stress bears close relation with photoinhibition and photosynthesis (Asada, 2006; Gill and Tuteja, 2010). As a fundamental metabolism for plant survival and growth, photosynthesis is sensitive to drought and salt stresses, and plant tolerance can be evaluated by photosynthetic analysis. It has been well documented that both salt and drought stresses initially cause stomatal limitation on photosynthesis, as plants can rapidly perceive elevated osmotic pressure, and the inhibition on dark enzymatic processes can further reduce CO_2 fixation (Flexas and Medrano, 2002; Loreto et al., 2003; Yang et al., 2008; Chaves et al., 2009). Consequently, excitation pressure in chloroplast may be elevated to induce photosystems photoinhibition with excess ROS generation (Murata et al., 2007; Takahashi and Murata, 2008; Sonoike, 2011; Kalaji et al., 2018a). Photosystems photoinhibition which originates from oxidative damages on photosynthetic membrane proteins or lipids has bigger threat than stomatal limitation on photosynthesis, as the damaged photosystems cannot recover as readily as stomatal opening after the cease of stress. At present, CO_2 assimilation and PSII photoinhibition have been analyzed by detecting gas exchange and the maximal photochemical efficiency of PSII (Fv/Fm) in some studies to discern plant tolerance to iso-osmotic salt and drought stresses (Muranaka et al., 2002; Cha-um and Kirdmanee, 2010; Cha-um et al., 2010). However, oxidative stress was rarely concerned in these studies, and it is still unclear how PSII donor and acceptor sides respond to iso-osmotic salt and drought stresses, as PSII performance cannot be comprehensively reflected by Fv/Fm (Li et al., 2009a; Yan et al., 2018a,b).

To date, less attention has been paid to PSI under salt and drought stresses, much less the comparison of its performance under iso-osmotic salt and drought stresses. In our recent studies, PSI was proved to be a crucial photoinhibition site in susceptible plant species or cultivars under salt stress (Yan et al., 2015a,b; Yan et al., 2020). In contrast, PSI inactivation was only reported in some trees with tremendous leaf water deficit after long severe drought stress (Huang et al., 2013; Zhang et al., 2016). Compared with PSII, the damaged PSI is difficult to be repaired, and PSI photoinhibition can aggravate PSII photoinhibition by elevating PSII excitation pressure through feedback inhibition on electron transport. Inversely, PSII photoinhibition can reduce ROS generation at PSI acceptor side by restricting electron donation to PSI and then decline the possibility of PSI photoinhibition. Thus, PSI photoinhibition is more harmful than PSII photoinhibition, and PSII and PSI coordination is critical for protecting PSI and even the whole photosynthetic apparatus. However, it is still largely unknown the role of PSII and PSI interaction in differentiating plant tolerance to iso-osmotic salt and drought stresses.

Honeysuckle (*Lonicera japonica* Thunb.) is a traditional medicinal crop in China and has strong adaptability to adverse environmental conditions such as low temperature, drought and barren soil. Drought tolerance of honeysuckle has been elucidated in terms of photosynthesis, osmolytes and leaf morphology and anatomy (Li et al., 2009b). In our recent study, the stability of photosynthesis and photosystems was maintained without oxidative stress by restricting leaf Na^+ accumulation in honeysuckle under salt stress (Yan et al., 2015a). Moreover, honeysuckle was demonstrated as an ideal economic crop for utilizing coastal saline land because of its salt adaptability and economic value according to our field experiments (Yan et al., 2016a, b; Yan et al., 2017). Plant salt tolerance can be deeply dissected by contrasting the effects of iso-osmotic salt and drought stress, as salt sensitive glycophytes commonly exhibit more vulnerability to iso-osmotic salt stress in contrary to the halophytes (Zhao et al., 2003; Hassine and Lutts, 2010; Patade et al., 2011; Sucre and Suárez, 2011; Hossain et al., 2017). However, there is no report about the effects of iso-osmotic salt and drought stresses on honeysuckle, a salt-tolerant glycophyte.

In this study, we intended to contrast photosynthesis, photoinhibition and oxidative stress in honeysuckle under moderate and severe iso-osmotic salt and drought stresses for deeply disclosing its salt adaptability. As a glycophyte, honeysuckle more likely has stronger adaptability to drought stress than iso-osmotic salt stress. Our study can enrich the understanding of salt tolerance mechanism in glycophyte and provide a reference for honeysuckle cultivation in coastal saline land.

2. Materials and methods

2.1. Plant growth and treatments

Bare-rooted honeysuckle (*Lonicera japonica* Thunb.) plants were bought from Jiujianpeng Agricultural Technology Limited Company (Pingyi, Shangdong, China). These plants are one-year old and belong to widely cultivated variety named Beihuayihao. Honeysuckle plants were planted in plastic boxes (9 plants per tank) containing Hoagland nutrient solution (pH 5.7) and placed in artificial chambers (Qiushi, China). The Hoagland nutrient solution contains KNO_3 (255 mg L^{-1}), KH_2PO_4 (67.5 mg L^{-1}), NH_4NO_3 (40 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (245 mg L^{-1}), $Ca(NO_3)_2{\cdot}4H_2O$ (590 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (905 μg L^{-1}), H_3BO_3 (1.43 mg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.11 mg L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.04 mg L^{-1}), $MoNa_2O_4$ (60.5 μg L^{-1}), $FeSO_4{\cdot}7H_2O$ (18.63 mg L^{-1}) and $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$ (13.93 mg L^{-1}). The Hoagland nutrient solution was continuously aerated and refreshed every two days. In the chambers, day/night temperature, humidity and photon flux density were set at 25/18 °C, 70 % and 400 μmolm^{-2} s^{-1} (12 h per day from 07:00 to 19:00). After 35 days, healthy and uniform plants were selected for iso-osmotic salt and drought treatments. NaCl and polyethylene glycol 6000 (PEG-6000) were used to conduct salt and drought treatments. Water potentials in nutrient solutions with 150 and 300 mM NaCl were -0.68 MPa and-1.28 MPa, and equal to those in 19.3 % and 28 % PEG-6000 (m/v) solutions. NaCl was added to nutrient solution incrementally by 50 mM step every day to the final concentrations at 150 and 300 mM for avoiding osmotic shock, and correspondingly, PEG was incrementally added to nutrient solution every day to the final concentrations at 19.3 % and 28 %. During the period, the water potential of nutrient solution with NaCl addition was measured every day to determine the daily added quantity of PEG-6000 for controlling the equal water potential throughout. Nutrient solution without PEG and NaCl was used for cultivating control plants. The newly expanded leaves were sampled from four replicate plants (one plant from one box) in each treatment for measuring the following parameters. Gas exchange and modulated chlorophyll fluorescence parameters were continuously measured at day 0, 1, 4 and 6 under severe iso-osmotic drought and salt stress at day 0, 3, 10, 15 and 20 under moderate iso-osmotic drought and salt stress. Other measurements were done after 6 d and 20 d of severe and moderate iso-osmotic drought and salt stress, respectively.

2.2. Measurements of Na^+ and relative water content

According to Song et al. (2011), dried leaf powder (0.1 g) was boiled with deionized H_2O (25 mL) for 2 h, and the supernatant was collected for measuring Na^+ content by using an atomic absorption spectrophotometer (TAS-990, Beijing, China).

Fresh leaves were harvested and weighed (fresh weight, FW), and

then were immersed in distilled water for 4 h at room temperature to determine saturated fresh weight (SW). At last, the leaves were dried completely in an oven at 70 °C and weighed (dry weight, DW). Relative water content (RWC) was calculated as: RWC = (FW − DW)/ (SW − DW) ×100 %.

2.3. Measurements of MDA and H_2O_2 contents

Leaf tissues (0.2 g) were ground under liquid nitrogen and homogenized in 4 ml 0.1 % TCA. The homogenate was centrifuged at 12,000 × *g* and 4 °C for 10 min to collect the supernatant for measurements of MDA and H_2O_2 contents. MDA content was determined by thiobarbituric acid reaction to reflect the extent of lipid peroxidation (Yan et al., 2020). The supernatant (1 ml) was mixed with 0.1 mM potassium phosphate buffer (1 ml, pH 7.0) and 1 mM KI (2 ml), and the absorbance at 390 nm was recorded for calculating H_2O_2 content (Yan et al., 2020).

2.4. Histochemical detection of H_2O_2 and leaf electrical conductivity

For histochemical detection of H_2O_2, leaf samples were vacuum infiltration in 3,3-diaminobenzidine (DAB) solution (0.1 mg ml^{-1} in 50 mM Tris-acetic acid; pH 3.8) for 3 min and incubated at room temperature for 24 h in the dark. Leaf samples were washed three times with distilled water. Thereafter, the leaves were decolorized by immersion in boiling ethanol (85 %) for 15 min and photographed (Yan et al., 2020).

Fresh leaf discs (1 cm diameter) were punched out and placed in a test tube containing 20 ml distilled deionized water. The leaf samples were vacuum infiltrated for 10 min and then rotary shaken for 3 h. The initial electrical conductivity of the solution (S1) was measured by using a conductivity meter (DDBJ-350 F, Shanghai, China). Then, the samples were boiled at 95℃ for 30 min to release all electrolytes. After cooling at room temperature, the final electric conductivity (S2) was measured. The electrolyte leakage was calculated as: EL (%) = (S1/S2) ×100 %.

2.5. Measurements of gas exchange and modulated chlorophyll fluorescence

Gas exchange and modulated chlorophyll fluorescence were simultaneously measured by using an open photosynthetic system (LI-6400XTR, Li-Cor, Lincoln, NE, USA) equipped with a fluorescence leaf chamber (6400−40 LCF, Li-Cor). All parameters were measured with 800 μmol m^{-2} s^{-1} light intensity. Temperature and CO_2 concentration were set at 25 °C and 400 μmol mol^{-1} in the leaf cuvette. Net photosynthesis rate (*Pn*), stomatal conductance (*Gs*), intracellular CO_2 concentration (*Ci*) and transpiration rate (*Tr*) were simultaneously noted. In addition, steady-state fluorescence yield was also recorded, and a saturating actinic light pulse of 8000 μmol m^{-2} s^{-1} for 0.7 s was used to produce maximum fluorescence yield by temporarily inhibiting PSII photochemistry. Subsequently, the minimum fluorescence in the steady state was detected during a brief interruption of actinic light irradiation in the presence of far-red light. Photochemical efficiency of PSII (ΦPSII) and PSII excitation pressure (1-qP) were calculated by using these parameters (Maxwell and Johnson, 2000; Yan et al., 2018a).

2.6. Measurements of chlorophyll fluorescence and modulated 820 nm reflection transients

Chlorophyll fluorescence and modulated 820 nm reflection transients were analyzed with a multifunctional plant efficiency analyzer (MPEA, Hansatech, Norfolk, UK), and the instrument has been described in detail (Strasser et al., 2010; Kalaji et al., 2012, 2018b). The leaves were adapted in dark for 30 min before the measurement. Thereafter, the leaves were orderly illuminated with 1 s red light (627 nm, 5000 μmol photons m^{-2} s^{-1}), 10 s far red light (735 nm, 200 μmol photons m^{-2} s^{-1}) and 2 s red light (627 nm, 5000 μmol photons m^{-2} s^{-1}) (Strasser et al., 2010; Dabrowski et al., 2016). Chlorophyll fluorescence and modulated 820 nm reflection were simultaneously recorded during the illumination. Monitoring modulated reflection change near 820 nm is a very convenient way to follow redox state of PSI. Relative value of the maximal difference of 820 nm reflection during the last 2 s red illumination was used to indicate the maximal photochemical efficiency of PSI ($\Delta MR/MR_0$) (Schansker et al., 2003; Yan et al., 2013). MR_0 is the value of 820 nm reflection at 0.7 ms (the first reliable MR measurement). ΔMR is the value of the maximal difference of 820 nm reflection at the last 2 s red light illumination. Chlorophyll fluorescence transients were quantified by JIP test to calculate the following parameters according to previous studies (Strasser et al., 2010), Fv/Fm, total performance index (PItotal), PSII performance index (PIabs), ratio of relative variable fluorescence at K step to that at J step (W_k), primary quinone (Q_A) reducing reaction centers per PSII antenna chlorophyll (RC/ABS) and probability with which an electron moves beyond Q_A (ETo/TRo) and from the intersystem electron carriers to reduce PSI end electron acceptors (REo/ETo).

2.7. Isolation of thylakoid membranes and western blot

According to the method of Yan et al. (2018a), leaf discs (4 g) were ground under liquid nitrogen and homogenized in 400 mM sucrose, 50 mM HEPES-KOH (pH 7.8), 10 mM NaCl, and 2 mM $MgCl_2$. The homogenate was filtered through two layers of cheesecloth and then centrifuged at 5000*g* and 4 °C for 10 min for collecting thylakoid pellets. At last, the pellets were resuspended in the homogenization buffer. The homogenate (20 μL) was mixed with 96 % ethanol (2.98 mL) and then centrifuged at 14,000*g* and 4 °C for 1 min. Liquid supernatant was used to measure the absorbance at 649 and 665 nm for calculating chlorophyll content (Yan et al., 2018b).

Thylakoid membranes containing 10 μg chlorophyll were separated by a 12 % (w/w) SDS-PAGE gel. Proteins from the gel were transferred onto polyvinylidene fluoride membrane by semi dry method. After blocking with 5% skimmed milk for 1 h, the membrane was incubated for 2 h with the primary anti-PsbA and anti-PsaA antibodies (PhytoAB, USA), respectively and then incubated with horseradish peroxidase conjugated anti-rabbit IgG antibody (PhytoAB, USA) for 2 h. The BeyoECL Plus substrate (Beyotime Biotechnology, China) was applied to test immunoreaction, and the chemiluminescence was recorded by using a Tanon-5500 cooled CCD camera (Tanon, Shanghai, China).

2.8. Statistical analysis

One-way ANOVAs Two-way ANOVAs were carried out by using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) to examine the effects of treatment time, treatment patterns and their interactions on photosynthetic and fluorescence parameters. The values presented are the mean of samples collected from four replicate plants. The comparisons of means were determined using a least significant difference test, and the differences were considered significant at $P < 0.05$.

Principal component analysis (PCA) was performed to show the integrated responses of honeysuckle to iso-osmotic drought and salt stresses. Beforehand, KMO and Bartlett's spherical tests were conducted by using SPSS 17.0 to examine data correlation for determining the feasibility of PCA. In this study, KMO value of the data reached 0.81 (KMO > 0.6), and the data also passed Bartlett's spherical test ($P < 0.01$). Then, PCA was performed and graphed by using Canoco 4.5.

3. Results

3.1. Gas exchange, PSII actual quantum yield and excitation pressure

Pn, *Gs*, *Tr* and ΦPSII tremendously decreased after 1 day of severe salt and drought stresses, and the decrease persisted until day 6 (Fig. 1A, C, G, I). Significant decrease in *Pn*, *Gs*, *Tr* and ΦPSII was also found under moderate drought and salt stresses (Fig. 1B, D, H, J). *Pn*, *Gs*, *Tr* and ΦPSII

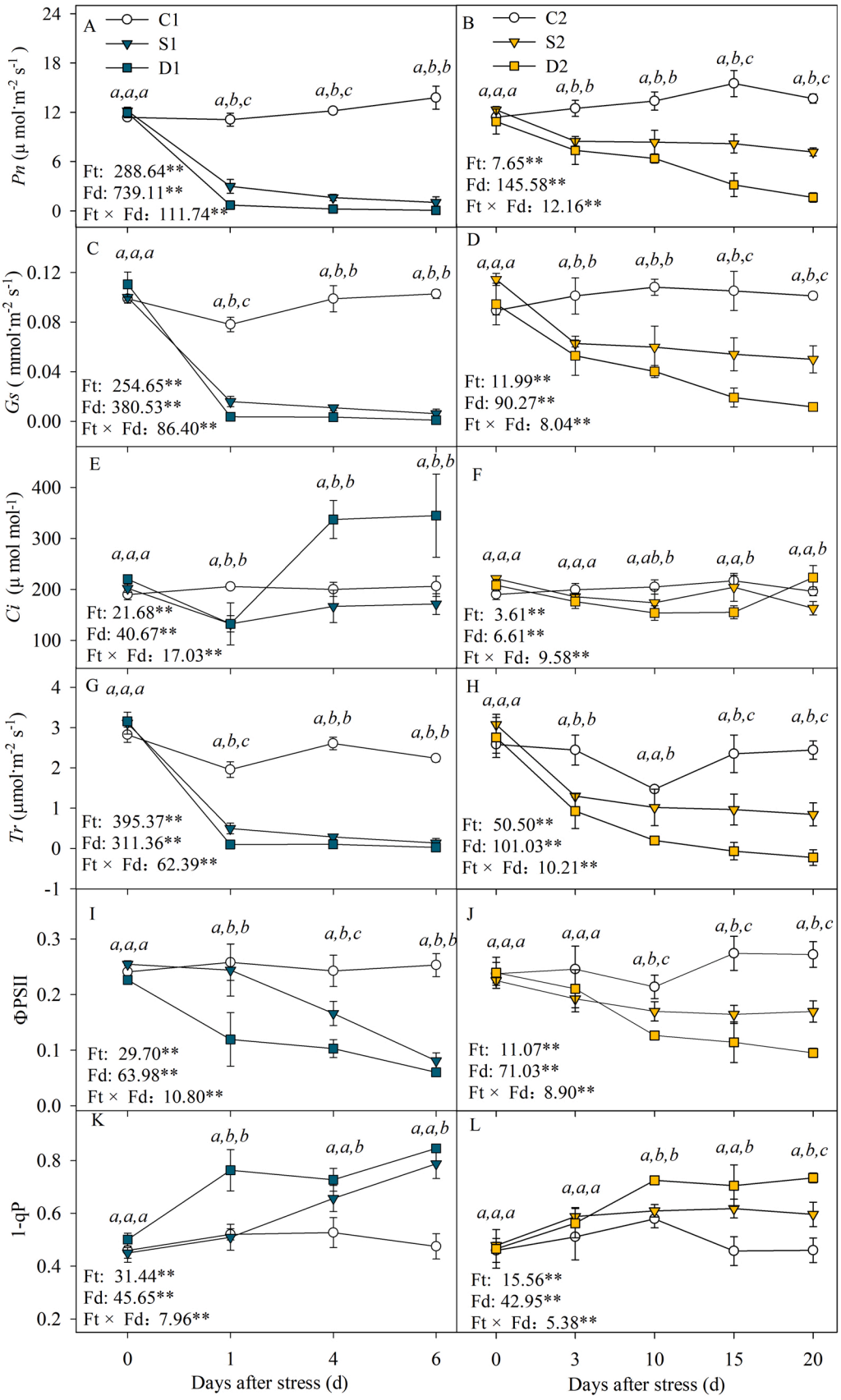

Fig. 1. Changes in photosynthetic rate (*Pn*, A, B), stomatal conductance (*Gs*, C, D) and intercellular CO_2 concentration (*Ci*, E, F), transpiration (*Tr*, G, H), actual photochemical efficiency of PSII (ΦPSII, I, J) and PSII excitation pressure (1-qP, K, L) in honeysuckle under iso-osmotic salt and drought stress. C1 and C2 indicate two groups of control plants without salt or drought stress. S1 and D1 indicate plants exposed to severe iso-osmotic stresses with 300 mM NaCl and 28 % PEG, respectively. S2 and D2 indicate plants exposed to moderate iso-osmotic stresses with 150 mM NaCl and 19.3 % PEG, respectively. Data in the figure indicate mean of four replicates (±SD), and different letters on error bars indicate significant difference at $P < 0.05$. Ft, Fd and Ft×Fd, respectively, indicate treatment time effect, treatment pattern effect and interaction effect between treatment time and pattern. The effect at the significant level of 0.05 is indicated by *, and the effect at the significant level of 0.01 is indicated by **. The symbols, Ft, Fd, Ft×Fd, C1, S1, D1, C2, S2 and D2, are also used in the following figures.

were at a relatively higher level under iso-osmotic salt stress because of drought-induced greater decrease in them (Fig. 1A-D, G–J). *Ci* was declined initially but increased to a significant higher level after 4 days of severe drought stress, and similarly, the initial decrease in *Ci* was also reversed after 20 days of moderate drought stress (Fig. 1E, F). During iso-osmotic salt stress, only lowered *Ci* could be observed (Fig. 1E, F). Severe and moderate drought stress significantly elevated 1-qP at day 1 and day 10 respectively, whereas the marked elevation of 1-qP occurred later and was less under iso-osmotic salt stress (Fig. 1K, L). Besides treatment pattern, treatment time also significantly influenced the above parameters, and there was a significant interactive effect between treatment pattern and treatment time (Fig. 1).

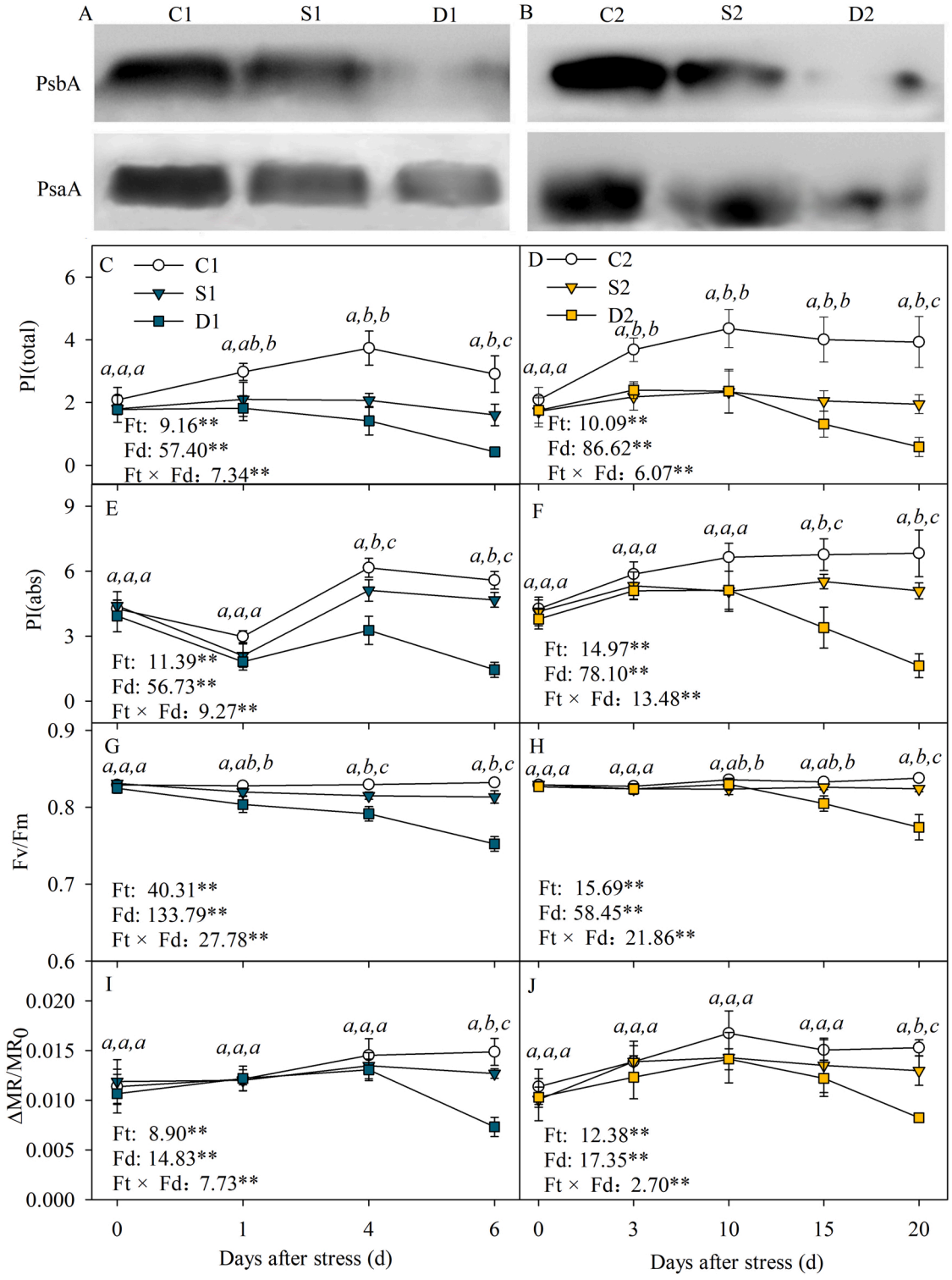

Fig. 2. Immunoblot analysis of reaction center proteins of PSI (PsaA, A) and PSII (PsbA, B) in the leaves of honeysuckle after 6 days of severe and 20 days of moderate iso-osmotic salt and drought stresses. Changes in total performance index (PI(total), C, D), PSII performance index (PI(abs), E, F), the maximal photochemical efficiency of PSII (Fv/Fm, G, H) and the maximal photochemical efficiency of PSI ($\Delta MR/MR_0$, I, J) in honeysuckle under iso-osmotic salt and drought stresses. Data in the figure indicate mean of four replicates (±SD), and different letters on error bars indicate significant difference at $P < 0.05$.

3.2. Immunoblot analysis, total and PSII performance index and the maximal photochemical efficiency of PSII and PSI

The abundance of PSI reaction center protein (PsaA) and PSII reaction center protein (PsbA) were obviously decreased after drought stress and slightly affected after iso-osmotic salt stress (Fig. 2A, B). Significant decline in PI(total) and PI(abs) occurred during both salt and drought stresses, and iso-osmotic drought stress caused greater decrease in them (Fig. 2C-F).

Fv/Fm was significantly decreased after 1 day of severe drought stress, and the decrease was up to 9.61 % at day 6 (Fig.2G). Severe drought stress initially had no obvious effect on $\Delta MR/MR_0$ and remarkably decreased it by 46.32 % at day 6 (Fig. 2I). Under moderate drought stress, Fv/Fm and $\Delta MR/MR_0$ were significantly decreased at day 15, and the decrease reached 6.48 % and 49.47 % at day 20 (Fig. 2H, J). Moderate iso-osmotic salt stress also induced significant decrease in Fv/Fm and $\Delta MR/MR_0$, but the decrease was much less than those upon drought stress (Fig. 2H, J). Besides treatment pattern, treatment time also significantly influenced these parameters, and there was a significant interactive effect between treatment pattern and treatment time (Fig. 2C-J).

3.3. Characterization of photosynthetic electron transport

ETo/TRo was significantly decreased after 4 days of severe drought stress, while decreased RC/ABS and increased W_k became remarkable after 6 days of severe drought stress (Fig. 3A, E, G). After 15 days of moderate drought stress, remarkable decrease in ETo/TRo and RC/ABS occurred with significant increase in W_k, however, these parameters were not obviously affected by iso-osmotic salt stress (Fig. 3B, F, H). Inconsistently, REo/ETo was significantly declined after 1 day of severe salt stress and 3 days of moderate salt stress, and salt-induced decrease

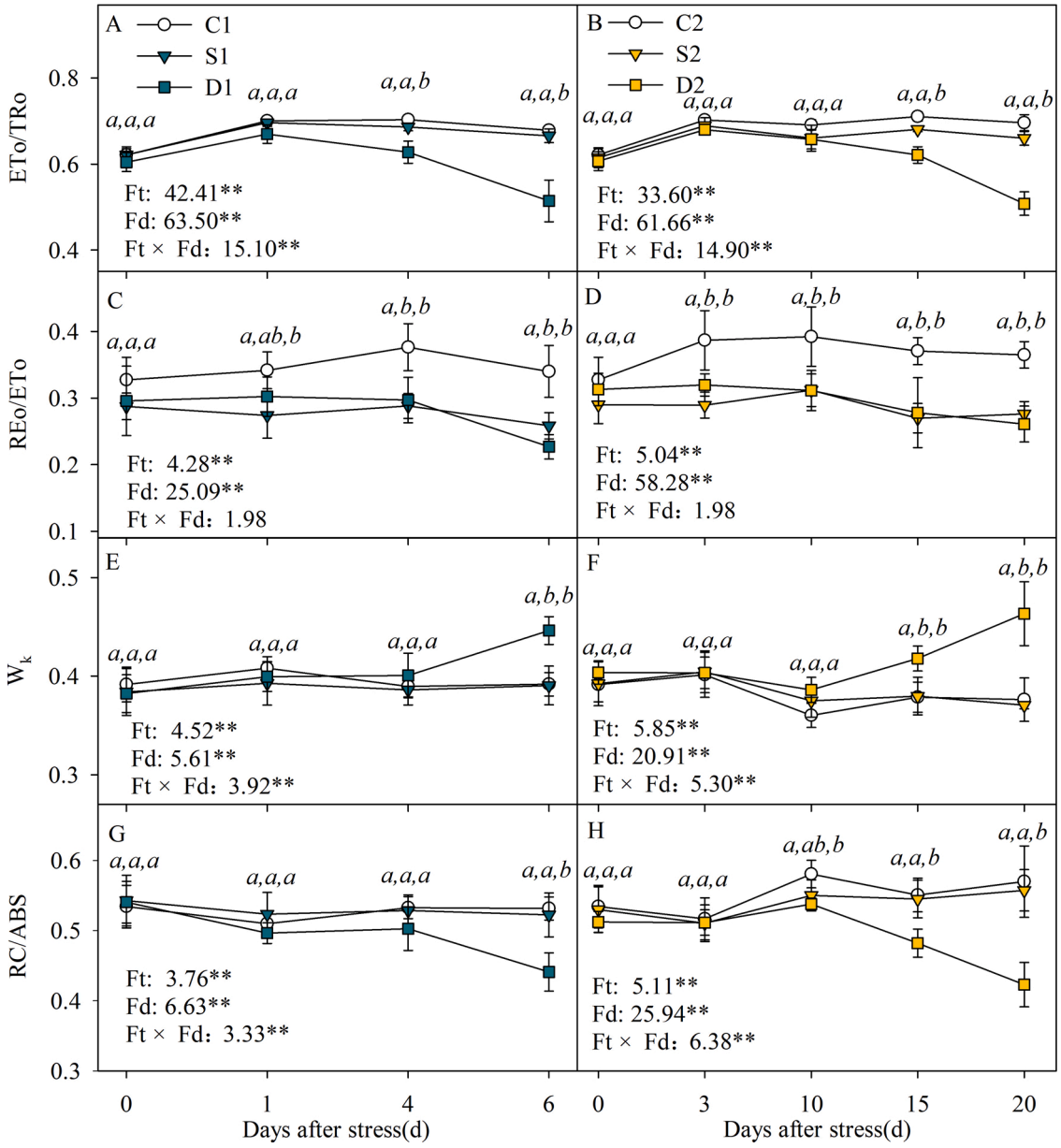

Fig. 3. Changes in the probability that an electron moves further than Q_A (ETo/TRo, A, B), probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (REo/ETo, C, D), ratio of relative variable fluorescence at K step to that at J step (W_k, E, F) and primary quinone (Q_A) reducing reaction centers per PSII antenna chlorophyll (RC/ABS, G, H) in honeysuckle under iso-osmotic salt and drought stress. Data in the figure indicate mean of four replicates (±SD), and different letters on error bars indicate significant difference at $P < 0.05$.

in REo/ETo was approximate to that upon iso-osmotic drought stress (Fig. 3C, D). Besides treatment pattern, treatment time also had significant effect on these parameters, and the significant interactive effect between treatment pattern and treatment time was also found apart from REo/ETo (Fig. 3).

3.4. Chlorophyll *a* fluorescence and modulated 820 nm reflection transients

J and I steps appear due to the accumulated reduced Q_A and PQ. After drought stress, J and I steps were significantly elevated, indicating that electron transport at PSII acceptor side beyond Q_A and PQ were inhibited (Fig. 4A, C). The appearance of K step represents the injury on OEC at PSII donor side (Fig. 4A, C). After drought stress, elevated K step indicated that PSII donor side was impaired. In contrast, I step was obviously elevated under both drought and salt stresses (Fig. 4A, C).

MR signals are presented by MR/MR_0, and the initial decrease in 820 nm reflection signal indicated PSI oxidation, and the subsequent increase suggested that PSI was gradually re-reduced. Both salt and drought stresses evidently restricted PSI oxidation, however, PSI re-reduction was inhibited by drought stress rather than iso-osmotic salt stress (Fig. 4B, D).

3.5. Lipid peroxidation, H_2O_2 content and electrolyte leakage

Lipid peroxidation extent was indicated by malondialdehyde (MDA) content in plant tissue. Salt stress significantly increased leaf H_2O_2 content, lipid peroxidation and electrolyte leakage, and comparatively, greater increase appeared under iso-osmotic drought stress (Fig. 5C-H). Correspondingly, histochemical staining with 3,3-diaminobenzidine also suggested the greater leaf H_2O_2 generation under drought stress than iso-osmotic salt stress according to drought-induced intense brown colour in leaf discs (Fig. 5A, B).

3.6. Na^+ content and relative water content

Leaf Na^+ content was significantly elevated by 89.20 % and 71.87 % after severe and moderate salt stresses, but iso-osmotic drought stress had no significant effect on leaf Na^+ content (Fig. 6A, C). Leaf relative water content was markedly declined after salt and drought stresses, and the decrease by 46.64 % and 23.88 % upon severe and moderate drought stresses were greater than those under iso-osmotic salt stresses (Fig. 6B, D).

3.7. Principal component analysis

The first and second principal components (PCs), respectively, reflect 63.50 % and 25.70 % variation of the experimental data, and altogether explain 89.20 % variation. Leaf Na^+ content majorly contribute to PC2 formation, and PC1 formation covers leaf water content, photosynthetic rate, oxidative parameters and photochemical indices, indicating a close relation of oxidative damage and photosynthesis with leaf water status rather than Na^+ content (Fig. 7). This finding conformed to the greater oxidative damage, photosynthetic depression and leaf water loss under drought stress than iso-osmotic salt stress. The samples distribution within PC1/PC2 plane is heterogeneous, and can be positioned into six separated clusters in accordance with six different treatments (Fig. 7). PC2 clearly separated salt-treated plants from control and drought-treated plants because of leaf Na^+ accumulation under salt stress (Fig. 7). In particular, PC1 obviously separated all clusters in the series

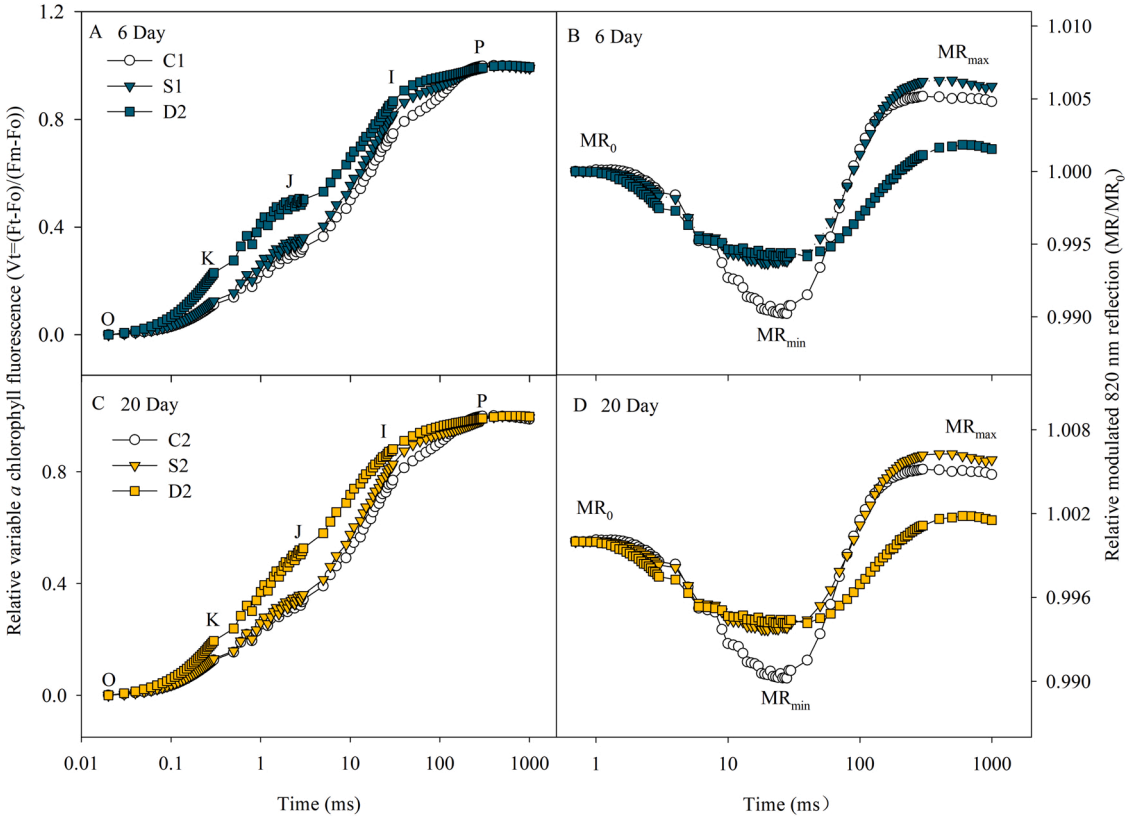

Fig. 4. Transients of prompt chlorophyll fluorescence and modulated 820 nm reflection during the first 1 s red illumination in the leaves of honeysuckle after 6 days of severe (A, B) and 20 days of moderate (C, D) iso-osmotic salt and drought stress. O, K, J, I and P indicate the specific steps in chlorophyll *a* fluorescence transient. MR_0 is the value at the onset of actinic illumination (at 0.7 ms), and MR is the reflection signal during the 1 s of red illumination. MR_{min} and MR_{max} indicate the maximal point of PSI oxidation and the maximal point of PSI re-reduction. Data in the figure indicate mean of four replicates (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

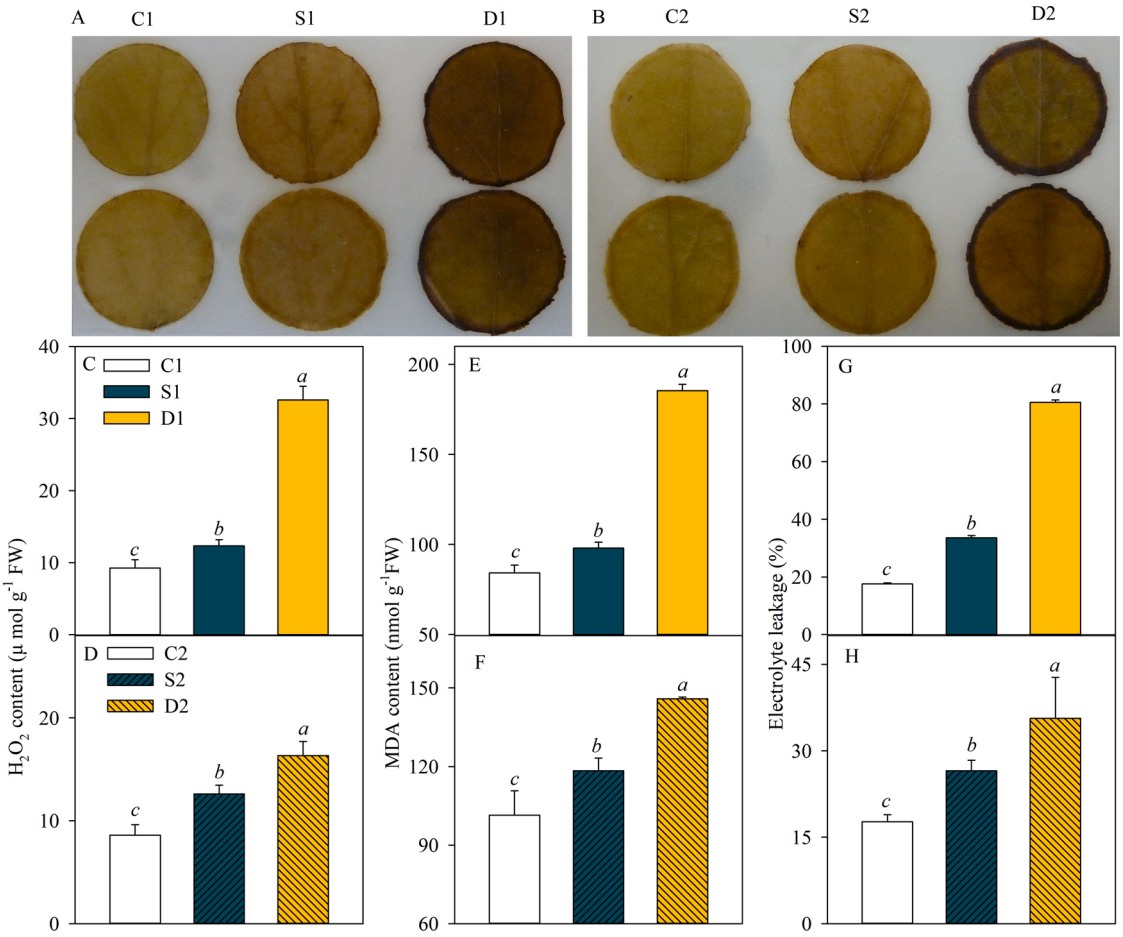

Fig. 5. Histochemical detection of H_2O_2 (A, B), H_2O_2 (C, D) and malondialdehyde (MDA) contents (E, F) and electrolyte leakage (G, H) in leaves of honeysuckle after 6 days of severe and 20 days of moderate of iso-osmotic salt and drought stress. Data in the figure indicate mean of four replicates (±SD), and different letters on error bars indicate significant difference at $P < 0.05$.

of severe and moderate iso-osmotic salt and drought stresses, and drought-induced cluster separation from control plants was greater than the separation induced by iso-osmotic salt stress, suggesting that honeysuckle endured severe oxidative stress with more detrimental effects on photosynthesis and photosystems under drought stress (Fig. 7).

4. Discussion

Photosynthesis in honeysuckle exhibited more susceptibility under drought stress than iso-osmotic salt stress according to drought-induced greater decrease in *Pn*. Stomatal closure serves as an important self-protective way by reducing water loss from transpiration under osmotic pressure, however, as a side effect, this protective way inevitably blocks CO_2 diffusion into leaf tissue and leads to stomatal limitation on photosynthesis (Chaves et al., 2009; Yan et al., 2018c). The concomitant decrease in *Pn*, *Gs*, *Ci* and *Tr* illuminated the positive and negative effects of stomatal closure in honeysuckle under salt stress (Fig. 1A-H). In contrast, drought-induced greater decrease in *Gs*, *Tr* and *Pn* suggested that honeysuckle encountered bigger osmotic pressure and had to further reduce water loss from transpiration with the sacrifice of CO_2 assimilation (Fig. 1A-D, G–H). Actually, leaf water status was interfered more seriously in honeysuckle under iso-osmotic drought stress (Fig. 6B, D). Depressed CO_2 assimilation can elevate excitation pressure in chloroplast, and threaten photosynthetic apparatus by elevating the possibility of ROS generation (Murata et al., 2007; Sonoike, 2011; Yan et al., 2020). Thus, higher PSII excitation pressure appeared with lower PSII electron transport in honeysuckle under drought stress than iso-osmotic salt stress, and could augment the risk of oxidative damage on photosynthetic apparatus (Fig. 1I-L). Notably, non-stomatal factors rather than stomatal limitation were mainly responsible for decreased *Pn* at later period of drought stress due to inverse variation of *Ci* and *Gs* (Fig. 1A-F), implying photosynthetic apparatus probably had been damaged.

PSII and PSI photoinhibition arose in honeysuckle exposed to drought stress, indicated by lowered Fv/Fm and $\Delta MR/MR_0$, and decreased PI(total) corroborated drought-induced deleterious effects on photosynthetic apparatus (Fig. 2C-D, G–J). Particularly, large loss of PsaA and PsbA proteins confirmed severe PSII and PSI photoinhibition in honeysuckle after drought stress (Fig. 2A, B). Oppositely, iso-osmotic salt stress had smaller influence on photosynthetic apparatus and led to mild PSII and PSI photoinhibition and slight decrease in PsaA and PsbA abundance (Fig. 2A–B, G–J). In parallel with lowered Fv/Fm, drought stress reduced the amount of active PSII reaction centers and depressed electron generation from PSII donor side and electron transport at PSII acceptor side according to increased W_k with elevated K step and decreased ETo/TRo with elevated J step respectively, and consequently, PSII entire performance was greatly declined by drought stress (Figs. 2C, D, 3 A, B, E, F and 4A, C). Recently, the large suppression on PSII photosynthetic activity has been reported in some salt-sensitive cultivars of glycophytes such as sweet sorghum and barley (Kalaji et al., 2018b; Rastogi et al., 2020). However, PSII entire performance and PSII components in honeysuckle showed greater tolerance to salt stress than iso-osmotic drought stress (Fig. 3). PSII was more vulnerable than PSI according to earlier decrease in Fv/Fm than $\Delta MR/MR_0$ under severe drought stress (Fig. 2G, I), and rapid PSII photoinhibition restricted electron flow to PSI, and even led to PSII and PSI disconnection at day 6

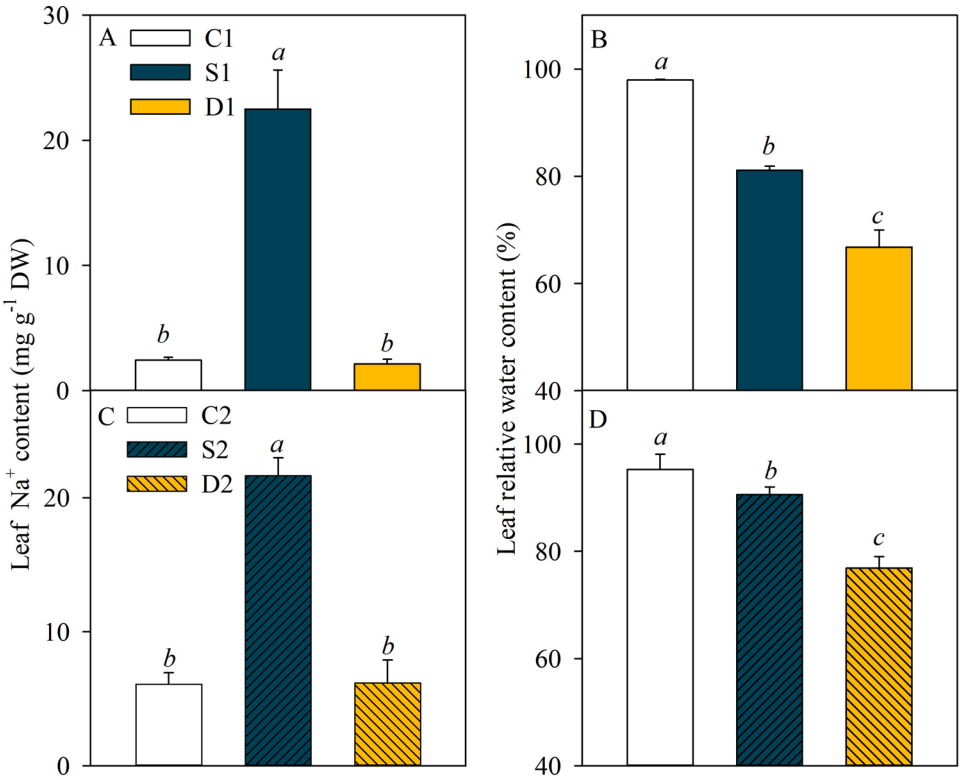

Fig. 6. Leaf Na^+ and relative water contents after 6 days of severe (A, B) and 20 days of moderate (C, D) iso-osmotic salt and drought stresses. Data in the figure indicate mean of four replicates (±SD), and different letters on error bars indicate significant difference at $P < 0.05$.

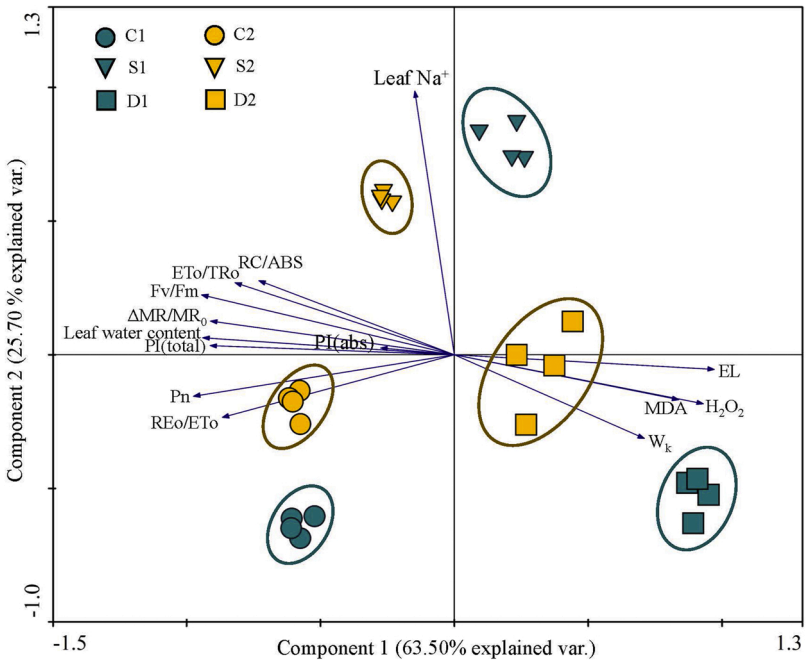

Fig. 7. Principal components analysis of the whole data involving photosynthesis, photosystem performance, oxidative stress, leaf water and Na^+ contents in honeysuckle after 6 days of severe and 20 days of moderate iso-osmotic salt and drought stresses.

day because of the uncompleted PSI re-reduction in 820 nm reflection transients (Fig. 4B). However, besides the lowered PsaA abundance and $\Delta MR/MR_0$, shortened PSI oxidation in 820 nm reflection transients also denoted that PSI was injured after 6 days of severe drought stress (Figs. 2A, I and 4 B). Likewise, PSI was also damaged in spite of PSII inactivation after prolonged moderate drought stress. Therefore, PSI oxidative damage could not be prevented by active photosystems interaction upon greatly elevated excitation pressure in chloroplast, although rapid PSII photoinhibition might postpone the occurrence of PSI photoinhibition. In contrast, less limitation on photosynthetic electron flow to PSI resulted from PSII stability under iso-osmotic salt stress, and then PSII and PSI connection could be sustained according to the

normal PSI re-reduction in 820 nm reflection transients, but importantly, PSI never encountered big oxidative threat in absence of severe PSII photoinhibition. Notably, elevated I step and significant decrease in REo/ETo suggested that PQ re-oxidation was inhibited under iso-osmotic drought and salt stresses (Figs. 3C, D and 4). The inhibited PQ re-oxidation conformed to PSI oxidative damage under drought stress, whereas it seemed elusive under iso-osmotic salt stress given the mild PSI photoinhibition. PQ situated at cytochrome b6f complex can combine with the primary PSI acceptor, ferredoxin, for promoting PSI cyclic electron flow (Joliot and Joliot, 2006). PSI cyclic electron flow acts as a critical mechanism to protect photosynthetic apparatus in plants by discharging electron flow at the downstream of PSI and producing ATP for the repair of damaged PSII (Sun et al., 2017; Yang et al., 2018; Huang et al., 2018; Wu et al., 2019). We presumed that inhibited PQ re-oxidation might depend on rapid enhancement of PSI cyclic electron transport under salt stress. Overall, photosynthetic apparatus in honeysuckle was vulnerable to drought stress than iso-osmotic salt stress, and the differential vulnerability hardly depended on photosystems interaction.

Photosystems photoinhibition closely relates to ROS generation and oxidative stress in plants (Takahashi and Murata, 2008; Oukarroum et al., 2015). Compared with iso-osmotic salt stress, greater elevation of leaf H_2O_2 concentration and lipid peroxidation conformed to severe photosystems photoinhibition and resulted in serious oxidative destruction of leaf cell membrane integrity under iso-osmotic drought stress (Fig. 5). Furthermore, principal component analysis comprehensively showed stronger salt adaptability in honeysuckle in terms of photosynthesis, photosystems performance and oxidative stress, according to drought-induced greater cluster separation than iso-osmotic salt stress (Fig. 7). Oxidative damage is a secondary stress following osmotic pressure or ionic toxicity under salt stress (Gill and Tuteja, 2010; Hossain and Dietz, 2016). Na^+ is the primary toxic ion under salt stress, and Na^+ toxicity may not occur as rapidly as osmotic pressure but can induce worse effects on crops such as rice, lentil, sugarcane, eucalypt, wheat, but noticeably, these crops belong to salt sensitive glycophytes (Muranaka et al., 2002; Munns and Tester, 2008; Cha-um and Kirdmanee, 2010; Cha-um et al., 2010; Patade et al., 2011; Hossain et al., 2017). On the contrary, halophytes are capable to effectively maintain water homostasis and dispose of accumulated Na^+ under salt stress, and can better adapt to salt stress than iso-osmotic drought stress (Zhao and Harris, 1992; Zhao et al., 2003; Hassine and Lutts, 2010; Sucre and Suarez, 2011). Similarly, drought-induced tremendous leaf water loss was mitigated in honeysuckle exposed to iso-osmotic salt stress (Fig. 6B, D), which contributed to protecting against severe oxidative damage. It has been reported that succulent halophytes such as *Salicornia europaea* and *Suaeda salsa* can effectively conserve a large of amount of Na^+ in the vacuoles to reduce its toxicity in cytoplasm, and Na^+ functions as a cheap osmolyte to potentiate water absorption capacity by lowering water potential in tissues (Zhao et al., 2003; Lv et al., 2012). Accordingly, we also can suppose this positive role of leaf accumulated Na^+ in salt-tolerant glycophyte such as honeysuckle under salt stress.

In disagreement with our hypothesis, honeysuckle has stronger adaptability to salt stress in terms of relatively higher photosynthetic capacity, lower photosystem photoinhibition and slighter oxidative injury under salt stress than iso-osmotic drought stress. Therefore, honeysuckle resembles a halophyte rather than a salt-sensitive glycophyte in this respect, and in future, it is worthy to investigate saline water irrigation for honeysuckle because of the deficiency of fresh water in coastal zone.

Authors' contributions

Wenjun He performed the experiments and wrote the manuscript. Kun Yan designed the experiment, performed the experiments and revised the manuscript. Yue Zhang provided the plant materials and participated in plant culture. Lanxing Bian and Huimin Mei participated in the experiments. Guangxuan Han reviewed the manuscript. All authors have read the manuscript and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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