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Differences in ecophysiological responses of *Populus euphratica* females and males exposed to salinity and alkali stress

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ABSTRACT

Soil salinity is usually accompanied by alkalization in northwest China, and they both negatively impact plant growth and result in severe ecological problems. Some studies have reported tree responses to salinity or alkali stress alone, however, the interactive salinity and alkali effects are still unclear, especially in dioecious trees. In this study, we measured growth, morphology, leaf stomata, gas exchange, carbon isotope composition ($\delta^{13}C$), total soluble sugar and starch contents, Na^+ accumulation and allocation, oxidative stress, and antioxidants of female and male *Populus euphratica* seedlings in response to salinity, alkali and their interaction. Our study showed no significant sexual differences in studied traits under control conditions. In addition, *P. euphratica* females showed greater inhibitory and negative effects, such as bigger decreases in growth and gas exchange, lower stomatal density and water use efficiency (as described by $\delta^{13}C$), and lower levels of soluble sugars and antioxidant enzyme activities compared with males under salinity, alkali and interactive stress conditions. Furthermore, *P. euphratica* males had a greater ability of ion exclusion and Na^+ transport restriction. For example, males allocated more Na^+ to stems and roots than females, whereas females had higher Na^+ contents in leaves under stress conditions. In conclusion, our results indicated that *P. euphratica* males have superior resistance and they perform better than females under salinity, alkali and their interactive stress conditions.

1. Introduction

Soil salinization is a widespread environmental problem that limits the growth and production of most plants. According to reports by the Food and Agriculture Organization of the United Nations (FAO), the whole world's saline land area is about 954 million ha and is increasing at a rate of 1–1.5 million ha per year (Rao et al., 2008; Guo et al., 2022). During recent years, along with global warming, salinization processes associated with human activities have increased significantly (Davis et al., 2010; Polle and Chen, 2015; Acosta-Motos et al., 2020; Yan et al., 2021; Xu et al., 2022), especially in arid and semi-arid regions, such as northwest China.

Salinity usually results in physiological dryness and osmotic stress (Li et al., 2016; Yu et al., 2020). Under salinity, trees will show a series of different morphological, physiological and defense responses. For instance, the leaf mass per area (LMA) and stomatal density usually increase, and the stomatal length decreases under salinity stress (Rajput et al., 2015; Wang et al., 2021; Yu et al., 2022). Furthermore, tree species possess defense mechanisms e.g. increased antioxidant enzyme activities, which leads to reduced oxidative damage by generating reactive oxygen species (ROS) when exposed to salinity stress (Chen et al., 2010; Li et al., 2016; Yu et al., 2023). Yet, excessive salt can result in toxic effects. Thus, the ion exclusion mechanism plays a crucial role in tree species to deal with salinity stress (Chen et al., 2002; Polle and Chen, 2015). Previous studies have shown that *Populus euphratica* can limit ion transport and allocate more ions into roots and less into leaves (Sun et al., 2018; Huang et al., 2022; Wu et al., 2022).

Soil salinization is usually accompanied by alkalinization in arid and semi-arid areas (Paz et al., 2012; Parihar et al., 2015). As it is well known, alkaline stress is a very complex stress, mainly produced by $NaHCO_3$ and Na_2CO_3, which negatively affect plants through chemical damage, osmotic stress, ionic injury, nutrient deficiency and hypoxia (Bai et al., 2021; Lu et al., 2021). Alkaline stress, in addition to negative effects such as ion toxicity and osmotic stress, further increases pH and induces additional damage in plants. For example, high pH severely interferes with cellular pH stability, disrupts cell membrane integrity,

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and reduces root vigor and photosynthetic function, and inhibits plants' ion and nutrient uptake (Yang et al., 2007; Zhang et al., 2017; Sun et al., 2018; Kaiwen et al., 2020). The high pH induced by alkali stress can also directly damage the structure and function of biomolecules, membranes and organelles, and it can precipitate many nutrient ions at the inter-root level (Wang et al., 2012). In natural ecosystems, tree species usually suffer different abiotic stresses and their interactions, e.g. the interactive salinity and alkali effects. Therefore, it is important to know how tree species respond to the interactive effects of salinity and alkali stress, as these may result in unique physiological and biochemical responses.

When plants are exposed to salinity and alkali stress, reactive oxygen species (ROS) will accumulate in the leaves, inducing cell membrane damage and lipid peroxidation, thus limiting growth and photosynthetic performance (Sun et al., 2018; Zhang et al., 2022). For example, salinity and alkali stress can increase the contents of malondialdehyde (MDA) and O_2^-, which are often used to assess the damage levels in plants (Xu et al., 2008; Chen et al., 2010; Huang et al., 2022). In response to the accumulations of ROS, plants have an antioxidant defense system to deal with environmental stresses (Petrov et al., 2015; Wang et al., 2021), and the production and removal of reactive oxygen species are in a dynamic equilibrium state. The up regulation of antioxidant enzyme activities, such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), can scavenge ROS and reduce the oxidative damage to plant cells (Chen and Polle, 2010; Yu et al., 2020).

Dioecious plants account for 5–6% of all plant species (Renner, 2014). They are fundamental for the structure and function of terrestrial forest ecosystems, and they play an important role in maintaining species diversity and ecosystem stability (Juvany and Munné-Bosch, 2015; Hultine et al., 2016; Liu et al., 2021). Due to the high costs of reproduction, e.g. the process of flowering and seed production, dioecious plants usually show sexual dimorphism, and females tend to be more responsive and more negatively affected than males under unfavorable conditions, such as salinity and alkali stress, while females show better adaptability under favorable conditions (Xu et al., 2008; Chen et al., 2014; He et al., 2016; Yu et al., 2018; Wu et al., 2022). *P. euphratica*, which usually grows in arid and semi-arid regions, plays an important role in sand fixation and ecological environmental protection. The Tarim River watershed, as the largest area of *P. euphratica* distribution, accounts for about 90% of the distribution range of *P. euphratica* in China and 55% in the world (Wang et al., 1995). Previous studies have reported that *P. euphratica* has strong resistance to drought (Yu et al., 2020; Zhao et al., 2021), salinity (Janz et al., 2012; Polle and Chen, 2015; Jia et al., 2020) and alkali stress (Sun et al., 2018; Huang et al., 2022), and it shows sexual differences under stress conditions (Guo et al., 2021; Xia et al., 2021, 2022; Yu et al., 2022). Some earlier studies have investigated the effects of salinity or alkali stress alone on plants, but knowledge of their interactive effects on males and females remains limited.

As far as we know, our work may be the first one to investigate the interactions of salinity and alkali stress on growth and physiological responses in *P. euphratica* females and males. Additionally, the growth, leaf stomatal, gas exchange, nonstructural carbohydrate, Na^+ accumulation and allocation, antioxidant enzyme accumulation, and reactive oxygen species accumulation properties of *P. euphratica* were compared in both sexes under salinity, alkali, and the interactive stress conditions. We hypothesized that (1) salinity and alkali stress would limit growth and photosynthesis, reduce antioxidant enzyme activities and increase Na^+ in *P. euphratica*, and females and males would have different tolerances, and (2) *P. euphratica* males perform better and have higher resistance when exposed to salinity, alkali, and the interactive stress.

2. Materials and methods

2.1. Plant material and experimental design

This study was performed in a nursery, located at the northern part of the Taklamakan Desert, near the Tarim University (latitude 40°56′ N, longitude 81°25′ E, altitude 1006 m). This area has an extremely arid continental climate with an average annual rainfall of less than 50 mm, and the annual average evaporation is > 2500 mm (Yu et al., 2020, 2022).

In total, 60 female and 60 male seedlings of *P. euphratica*, all three years old and of the same size, were planted in 35-l pots (one seedling per pot) in early May 2021. The homogenized soil for planting was taken from a nearby *P. euphratica* forest. The physiochemical traits are described in Yu et al. (2023). The experimental layout was completely randomized with three factors (sex, salinity and alkali). Two sexes (female and male), two salinity levels (no salt and added salt), and two alkali levels (no alkali and added alkali) were employed. We added 150 mM sodium chloride solution (Yu et al., 2023) every second day (5 times) for salinity stress treatments. In addition, a mixture of $NaHCO_3$ and Na_2CO_3 was added ($NaHCO_3$: Na_2CO_3 = 1:1 with the total concentration of Na^+ set as 150 mM) for alkali stress treatments (Sun et al., 2018). When plants subjected to salinity or alkali stress, 0.1 L salinity or alkali solutions were added around the plant per pot every time, and no ions escape from the pot. The salinity and alkali treatments were conducted in middle May, and the seedlings were harvested in early September. In this study, there were four treatments as follows: control (CK), salinity stress, alkali stress, and the salinity and alkali stress interaction (SA).

2.2. Growth trait measurements

After all plants were harvested, they were divided into root, stem and leaf fractions, dried at 70 °C for 72 h until the weight remained constant and then weighted. The leaf area was measured by a 600 dpi resolution scanner (Cannon Scanner 5600F, Chengdu, China) and Image J imaging software (National Institutes of Health, MD, USA). The leaf mass per area (LMA) was calculated as dry mass per leaf area.

2.3. Gas exchange and carbon isotope composition measurements

The net photosynthetic rate (P_n) and stomatal conductance (g_s) of poplar plants were determined using the Li-6400 portable photosynthesis system (LI-COR Inc., Linkon, NE, USA) from 8:30 to 11:00 a.m. in early August 2021. The fourth fully expanded healthy leaf from the top to the bottom of each plant was selected for the gas exchange measurement. Before each measurement, the leaves were irradiated with saturated radiation (1000 μmol m^{-2} s^{-1} PPFD) for about 5–20 min to obtain sufficient photosynthetic induction, after which the measurement was started. The detailed measurement protocols were described by Yu et al. (2021). In addition, carbon isotope composition ($\delta^{13}C$), which generally reflects long-term water use efficiency (Chen et al., 2014; Yu et al., 2019), was measured by a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Science, Inc., Waltham, MA, USA).

2.4. Na^+ content measurement

The dried and ground samples (about 0.2 g) of roots, stems and leaves were placed in a 100 ml Erlenmeyer flasks. We added 5 ml of concentrated nitric acid and mixed thoroughly overnight, then added 1 ml of perchloric acid, covered with a short-necked funnel and heated on an electric heating plate (slow heating) until white smoke was exhausted, after cooling added 5 ml of 1:1 hydrochloric acid, boiled and dissolved again, after cooling added 10 ml of 5% $SrCl_2$ and fixed the volume to 100 ml. Then, the sodium ion content was determined by atomic absorption spectrometer (Spectr AA-200).

2.5. Soluble sugar and starch measurements

Each dried leaf sample (about 50 mg) was transferred into 10 ml centrifuge tube, 4 ml of 80% (v/v) ethanol was added, followed by incubation in 80 °C water bath for 30 min, centrifugation at 5000 r for 10 min, and transfer of the supernatant into a 10 ml centrifuge tube. Then, 2 ml of 80% (v/v) ethanol was added to the precipitate, followed by centrifugation at 5000 r for 10 min. The above process was repeated, the supernatant was mixed and 80% (v/v) ethanol was added to make the total volume to 10 ml. The transferred supernatant was used for the determination of total soluble sugars. Centrifuge tubes with residues were placed in a fume hood overnight to evaporate the residual ethanol for starch determination. The determination of total soluble sugars and starch contents was conducted by Anthranilic Acid Method (Yemm and Willis, 1954). Detailed assay methods are provided by Song et al. (2017).

2.6. Determination of leaf anatomical structures

One clean fresh leaf was sampled from each plant, a layer of nail polish was applied to the abaxial surface of the leaf, the leaf was photographed under a light microscope, the length and width of stomata were measured (Casson et al., 2009), and the stomatal density was measured and calculated according to Royer (2001).

2.7. Determination of antioxidant enzyme activities

An amount of 0.2 g of fresh poplar leaves were placed in a pre-chilled mortar, 2 ml of extraction medium was added, leaves were homogenized in an ice bath, the mortar was rinsed with extraction medium and the volume was fixed to 5 ml, followed by centrifugation at 12000 rpm for 20 min at 4 °C. The obtained supernatant was the enzyme solution to be measured, and it was kept in a refrigerator at 4 °C. The content of superoxide dismutase (SOD) was measured by the nitrogen blue tetrazolium photochemical reduction method, and the content of peroxidase (POD) was measured by the guaiacol method (details described in Zhao et al. (2022)). Superoxide anion radicals (O_2^-) were determined by the hydroxylamine oxidation method (Wang, 1990), and catalase (CAT), glutathione reductase (GR) and Malondialdehyde (MDA) were measured according to the instructions given in the kit (Beyotime, Shanghai, China).

2.8. Statistical analysis

Prior to analysis, data were checked for normality and homogeneity of variances, and log-transformed if necessary to correct for bias from these assumptions. Individual characteristics between treatments were compared using Tukey's tests of one-way ANOVAs at a significance level of $P < 0.05$. The effects of sex, alkali, salinity and their interaction were examined using three-way ANOVAs. All analyses were performed with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 18.0. The synergy among the studied characteristics was analyzed by principal component analysis (PCA) method using Canoco 5.0 (Microcomputer Power, USA).

3. Results

3.1. Plant growth and stomatal anatomy

Compared with the control (CK), salinity, alkali and the interactive stress conditions significantly reduced the leaf, stem, root, and total biomass in both sexes of *P. euphratica* (Fig. 1). In addition, the leaf, root and total biomass of female plants were significantly lower than those of male plants under salinity, alkali and their interactive stress, thus indicating that females suffered greater inhibitory effects. Furthermore, sex × salinity, sex × alkali and salinity × alkali interactions were significant for leaf, root and total biomass (Fig. 1).

Salinity, alkali and their interactive stress significantly increased the stomatal density but decreased the stomatal width and length (Fig. 2). There were significant sexual differences in stomatal density of *P. euphratica* leaves, as the stomatal density of male leaves was higher than that of female leaves under stress treatments, while stomatal width and length showed no sexual differences under any treatment. In addition, sex × salinity and sex × alkali interactions were significant for stomatal density (Fig. 2).

3.2. Gas exchange and carbon isotope composition

The net photosynthetic rate (P_n) and stomatal conductance (g_s) of *P. euphratica* were significantly reduced under salinity, alkali and their interactive stress, while specific leaf mass per area (LMA) and carbon isotope composition ($\delta^{13}C$) increased in both sexes of *P. euphratica* (Fig. 3). Additionally, there were no sexual differences in P_n, g_s, LMA and $\delta^{13}C$ under control (CK) treatment, but these four parameters were significantly higher in males than in females under salinity, alkali and their interactive stress, except g_s under alkali stress. Furthermore, sex × salinity, salinity × alkali and sex × salinity × alkali interactions had significant effects on P_n and LMA, and salinity × alkali interactions had significant effects on g_s and $\delta^{13}C$ (Fig. 3).

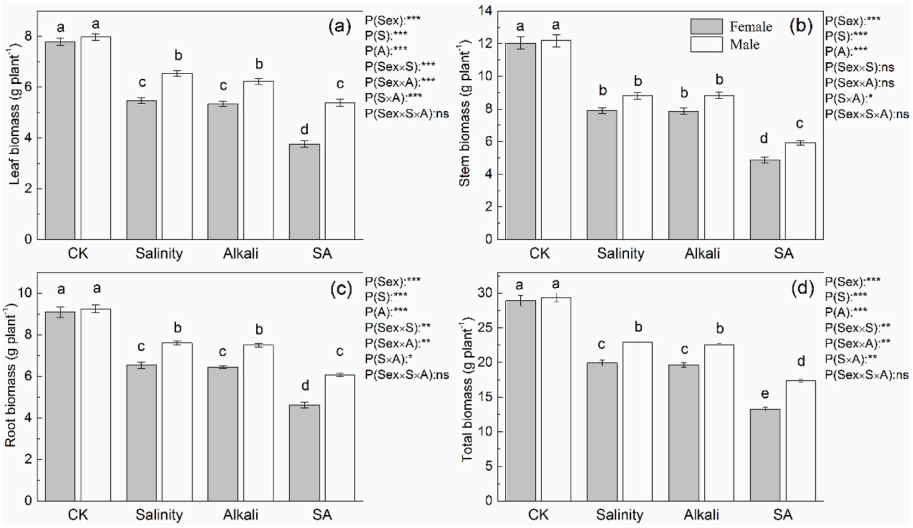

Fig. 1. (a) Leaf biomass, (b) stem biomass, (c) root biomass, and (d) total biomass in *P. euphratica* females and males under salinity, alkali and their interactive stress. Each value is the mean ± SE ($n = 5$). Different letters above bars denote significant differences according to Tukey's test at a significance level of $P < 0.05$. Three-way analyses of variance (ANOVA) were applied to evaluate the effects of different factors and their interactions. Sex, sex effect; S, salinity effect; A, alkali effect; Sex × S, sex × salinity effect; Sex × A, sex × alkali effect; S × A, salinity × alkali effect; Sex × S × A, sex × salinity × alkali effect. ns, not significant; *$0.01 < P < 0.05$; **$0.001 < P \leq 0.01$; and ***$P \leq 0.001$. CK, control treatment; Salinity, salinity treatment; Alkali, alkali treatment; SA, salinity and alkali stress.

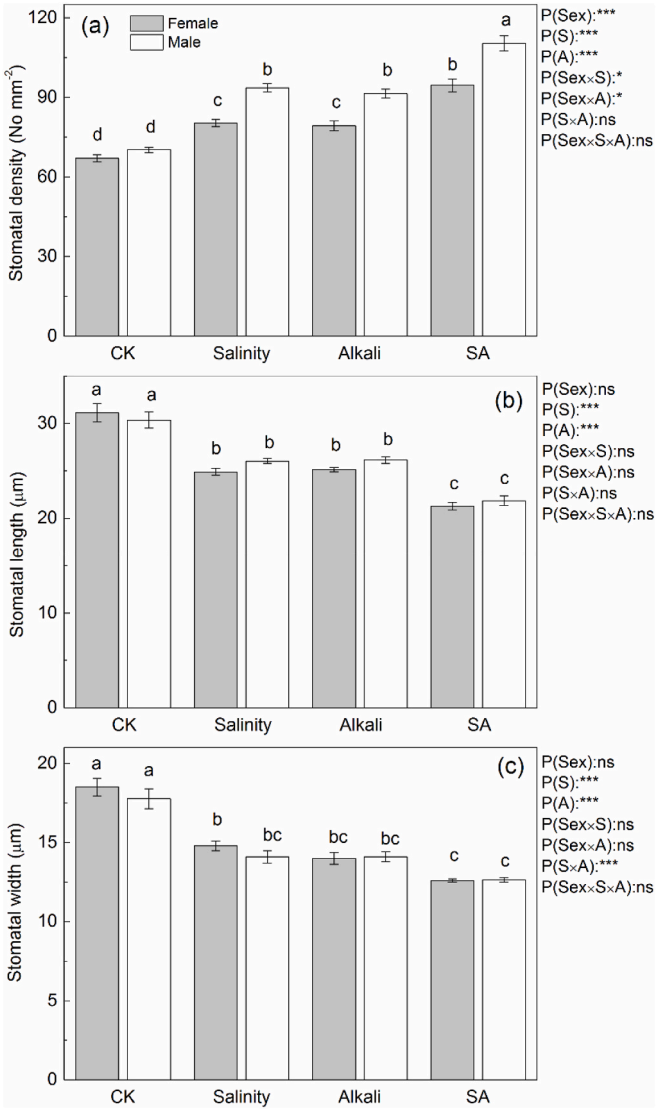

Fig. 2. (a) Stomatal density, (b) stomatal length, and (c) stomatal width in *P. euphratica* females and males under salinity, alkali and their interactive stress. Each value is the mean ± SE (n = 5). Treatment codes and statistical analyses as in Fig. 1.

3.3. Soluble sugar and starch contents

Compared to the control, soluble sugars of leaves significantly increased, but starch decreased in both sexes under salinity, alkali and their interactive stress. There were no sexual differences in starch contents, but males had higher leaf soluble sugar levels than females under all stress treatments (Fig. 4). In addition, sex × salinity, sex × alkali and salinity × alkali interactions were significant for soluble sugar contents of leaves (Fig. 4).

3.4. Na^+ accumulation and allocation

Under salinity, alkali and their interactive stress, Na^+ contents in different organs were significantly higher than those in the control treatment (CK), and roots had significantly higher Na^+ contents than leaves and stems in both sexes of *P. euphratica* (Fig. 5). In addition, Na^+ contents showed sexual differences under salinity, alkali and their interactive stress (Fig. 5). The Na^+ contents in the stems and roots of males were significantly higher than those of females, whereas *P. euphratica* females had significantly higher Na^+ contents in leaves compared to males. Furthermore, sex × salinity, sex × alkali and salinity × alkali interactions were significant for Na^+ contents in leaves, stems and roots, except the sex × salinity interaction for the leaf Na^+ content (Fig. 5).

3.5. MDA and O_2^- contents and antioxidant enzyme activities

Compared with the control (CK), salinity, alkalinity and their interactions increased the levels of MDA, O_2^-, POD, SOD, CAT and GR in the female and male leaves of *P. euphratica* (Figs. 6 and 7). There were no sexual differences in MDA and O_2^- contents, and antioxidant enzyme activities under CK treatment, but these parameters showed sex-specific responses to stress conditions. For instance, the levels of MDA and O_2^- of females were significantly higher than those in males, whereas males possessed higher POD, SOD, CAT, and GR than females under salinity, alkali and their interactive stress (Figs. 6 and 7). In addition, sex × alkali and sex × salinity × alkali interactions had significant effects on MDA and O_2^- contents, and sex × salinity, and sex × alkali interactions had significant effects on POD, SOD, CAT, and GR.

3.6. PCA analyses

Principal Component Analysis (PCA) of the first two axes explained 94.8% of the total variance of the studied parameters in both sexes of *P. euphratica* under salinity and alkali stress (Fig. 8). Under stress treatments, females and males were better separated along the second PCA axis. Furthermore, PC1 was strongly influenced by leaf, stem, root and total biomass, P_n, g_s, starch content, stomatal length and width, LMA, Na^+ contents in stems and roots, stomatal density, CAT, GR, and SOD. PC2 was strongly influenced by POD (Fig. 8). In addition, P_n, g_s, starch content, and stomatal length and width were significantly positively correlated with leaf, stem, root and total biomass.

4. Discussion

4.1. Salinity and alkali stress affect growth and stomatal anatomy

Our data showed that the growth and biomass in both sexes of *P. euphratica* decreased under salinity and alkali stress, which was consistent with previous studies (Meena et al., 2016; Sun et al., 2018; Jia et al., 2020; Yu et al., 2020). Additionally, we found that *P. euphratica* had sexual differences under salinity, alkali, and especially under their interactive stress. We found that the leaf, root and total biomass of *P. euphratica* females were significantly lower than those of males, indicating that the growth of *P. euphratica* females was more inhibited than that of males under salinity, alkali and their interactive stress (Fig. 1). These results agreed with previous studies indicating that poplar males show stronger resistance to salinity and alkali stress (Chen et al., 2010; Li et al., 2016). Furthermore, sex × salinity and sex × alkali interactions had significant effects on the leaf, root and total biomass of *P. euphratica*, indicating that *P. euphratica* males had a higher leaf, root and total biomass under salinity or alkali stress conditions. The higher root biomass of *P. euphratica* males when suffering stress implied that males have a stronger ability to absorb nutrients and water through the root system (Brunner and Godbold, 2007; Wang et al., 2021; Yu et al., 2023).

The reduction in leaf gas exchange may relate to stomatal restriction, mainly in the form of stomatal closure and reduced stomatal area (Zhuang et al., 2010; Hamani et al., 2021). Earlier studies have reported that the leaf stomatal area and stomatal opening length are significantly reduced and stomatal density increased in poplar plants under salt stress (Abbruzzese et al., 2009; Rajput et al., 2015). Our results are in line with the above studies showing that all stress treatments significantly increase the stomatal density in both sexes of *P. euphratica*, but decrease the stomatal length and width (Fig. 2). Previous studies have found that changes in stomatal density are closely related to plants' water-use

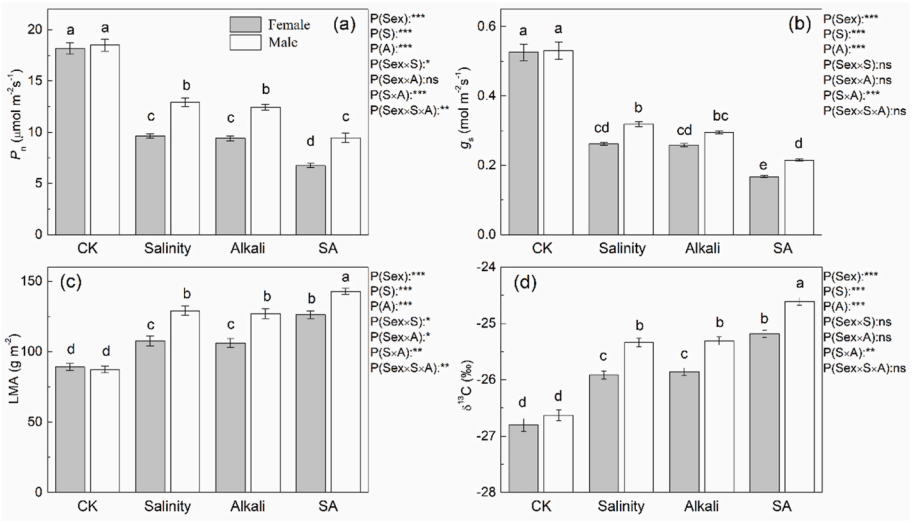

Fig. 3. (a) Net photosynthetic rate (P_n), (b) stomatal conductance (g_s), (c) leaf mass per area (LMA) and (d) carbon isotope composition ($\delta^{13}C$) in *P. euphratica* females and males under salinity, alkali and their interactive stress. Each value is the mean ± SE ($n = 5$). Treatment codes and statistical analyses as in Fig. 1.

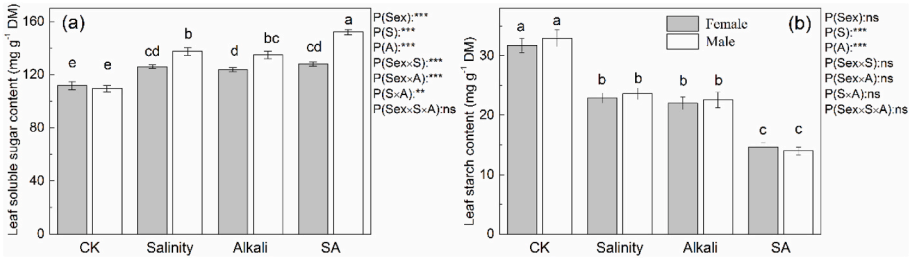

Fig. 4. (a) Leaf soluble sugar content, and (b) leaf starch content in *P. euphratica* females and males under salinity, alkali and their interactive stress. Each value is the mean ± SE ($n = 5$). Treatment codes and statistical analyses as in Fig. 1.

traits, e.g. water use efficiency (Ceulemans et al., 1995; Abbruzzese et al., 2009). In this study, under salinity, alkali and their interactive stress, the stomatal density of *P. euphratica* males was significantly higher than that of females, implying that males possess superior micromorphological adaptations in leaves and a better water-conservation strategy under stress conditions.

4.2. Salinity and alkali stress affect gas exchange and non-structural carbohydrate

In the present study, we found that salinity, alkali and their interactive stress limited gas exchange and reduced the net photosynthetic rate (P_n) and stomatal conductance (g_s) in both sexes of *P. euphratica* (Fig. 3). Furthermore, there were sexual differences in P_n and g_s of *P. euphratica* when exposed to salinity, alkali and their interactive stress. These results were consistent with previous studies indicating that poplar males exhibit strong tolerance to salinity and alkali stress (Chen et al., 2010; Li et al., 2016; Yu et al., 2022). The PCA analysis showed that *P. euphratica* females and males were better separated along the second PCA axis under stress treatments. Furthermore, P_n, g_s, starch content, and stomatal length and width were significantly positively correlated with leaf, stem, root and total biomass (Fig. 8). Plant water-use efficiency (WUE) plays a key role in growth and survival, and indicates the adaptation strategy of plants to cope with environmental changes. Many studies have reported that the carbon isotope composition ($\delta^{13}C$) of leaves acts as an indicator for long-term water use efficiency in plants (Li et al., 2007; Chen et al., 2014). In our study, salinity and alkali stress increased $\delta^{13}C$ in both sexes, and *P. euphratica* males had higher levels of $\delta^{13}C$ than females under stress treatments (Fig. 3d). The salinity and alkali stress resulted in stomatal closure (declined g_s), decreased stomatal length and width (Fig. 2), and increased fixation of ^{13}C, which induced increased $\delta^{13}C$ and indicated a conservative water use strategy (Farquhar et al., 1989; Li, 1999, 2016; Yu et al., 2018), Leaf mass per area (LMA) is an important leaf functional trait in the leaf economic spectrum, and it generally reflects a plant's efficiency of resource utilization and adaption strategies (Niinemets, 1999; Wright et al., 2004). Plants with high LMA generally show strong tolerance to stress (Poorter et al., 2009; Liu and Liang, 2016; Yu et al., 2022). Our results were consistent with the above statements that LMA of *P. euphratica* males is significantly higher than that of females, implying that males possess a stronger stress tolerance.

Some studies have reported that soluble sugars are essential for plant survival and stress resistance, and closely related to physiological adjustments (Martínez-Vilalta et al., 2016; Kannenberg et al., 2018; Tomasella et al., 2019; Wang et al., 2021). In this study, the levels of soluble sugars of *P. euphratica* males were higher than those of females under salinity, alkali and their interactive stress (Fig. 4), implying that males may possess a greater ability of osmotic adjustment when suffering stress. On the other hand, starch storage acts as an energy buffer that promotes plant survival and growth under stress conditions (Sala et al., 2012; Wiley et al., 2013, 2019; Yu et al., 2019). We found that the levels of starch in both sexes of *P. euphratica* decreased significantly under salinity, alkali and their interactive stress (Fig. 4), which agreed with previous studies showing that salinity and alkali stress decrease starch content (Yu et al., 2020; Wang et al., 2021; Huang et al., 2022).

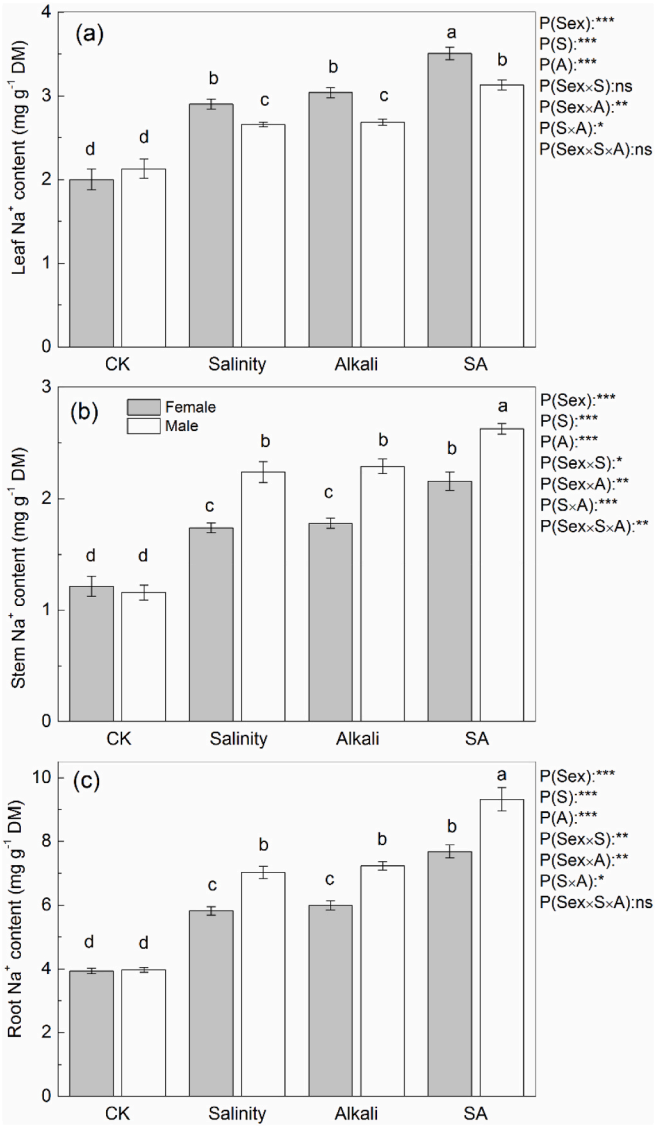

Fig. 5. (a) Leaf Na^+ content, (b) stem Na^+ content, and (c) root Na^+ content in *P. euphratica* females and males under salinity, alkali and their interactive stress. Each value is the mean ± SE (n = 5). Treatment codes and statistical analyses as in Fig. 1.

4.3. Salinity and alkali stress affect Na^+ accumulation and allocation

As documented extensively, many plants (including *P. euphratica*) can effectively prevent Na^+ uptake, and allocate Na^+ mainly into roots (Janz et al., 2012; Wu et al., 2015; Huang et al., 2022). The results of our study were consistent with these earlier studies showing that the Na^+ content of roots is significantly higher than that of leaves and stems in both sexes of *P. euphratica* under salinity, alkali and their interactive stress (Fig. 5). In addition, the Na^+ contents in stems and roots of *P. euphratica* males were significantly higher than those in females, whereas the Na^+ contents in female leaves were higher than those in males under stress treatments. This is in agreement with previous studies indicating that *Populus* males inhibit Na^+ transport from roots to leaves more efficiently compared with females (Chen et al., 2011; Wu et al., 2022). Therefore, our data showed that *P. euphratica* males with higher resistance can effectively exclude and restrict Na^+ transport and allocate more Na^+ into roots and less Na^+ into leaves, which may diminish the damage to plants under salinity and alkali stress.

4.4. Salinity and alkali stress affect oxidative stress and antioxidant

Malondialdehyde (MDA) is commonly used to assess lipid peroxidation levels in plants, and increased MDA is mainly due to the accumulation of reactive oxygen species (ROS), indicating that plants under abiotic stress are more susceptible to oxidative damage (Xu et al., 2008; Wang et al., 2022; Yu et al., 2023). Additionally, O_2^- is used to indicate the levels of oxidative damage under stress, e.g. salinity and alkali stress (Yu et al., 2020; Huang et al., 2022). In our study, salinity and alkali stress significantly increased the MDA and O_2^- contents in both sexes of *P. euphratica*, and the MDA and O_2^- contents of *P. euphratica* females were higher than those of males under salinity, alkali and their interactive stress (Fig. 6). The above statements show that females suffer more oxidative damage to membrane lipid peroxidation than males under stress treatments, which is consistent with previous results (Xu et al., 2008; Chen et al., 2010; Liu et al., 2020).

Peroxidase (POD) and superoxide dismutase (SOD) can protect cells from oxidative damage, and they play important roles in oxygen-scavenging (Chen and Polle, 2010; Petrov et al., 2015; Li et al., 2016). In this study, the SOD and POD activities in the leaves of *P. euphratica* males were significantly higher than those of female leaves under all stress treatments (Fig. 7a and b). CAT and GR are important antioxidants and closely related to scavenging ROS, and they usually increase when plants suffer stress (Chen et al., 2010; Xia et al., 2022). In our study, the activities of CAT and GR of both sexes *P. euphratica* were significantly increased, and the increased levels of CAT and GR of *P. euphratica males* were higher than those of females (Fig. 7c and d). Overall, *P. euphratica* males showed a lower degree of oxidative damage, higher antioxidant enzyme activities and more effective antioxidant defense system under salinity, alkali, and the interactive stress.

5. Conclusions

In summary, *P. euphratica* females and males display different growth, physiological, and biochemical responses to salinity, alkali, and their interactive stress, while no sexual differences were observed under control conditions. Compared with females, *P. euphratica* males showed greater growth and gas exchange, higher stomatal density and water use efficiency, and higher soluble sugar and antioxidant enzyme activities

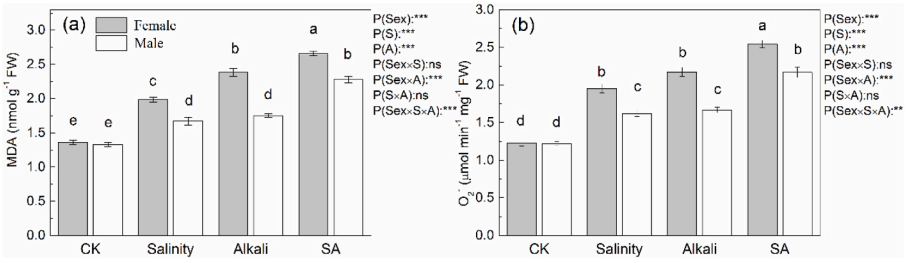

Fig. 6. (a) Malondialdehyde (MDA), and (b) superoxide radicals (O_2^-) in *P. euphratica* females and males under salinity, alkali and their interactive stress. Each value is the mean ± SE (n = 5). Treatment codes and statistical analyses as in Fig. 1.

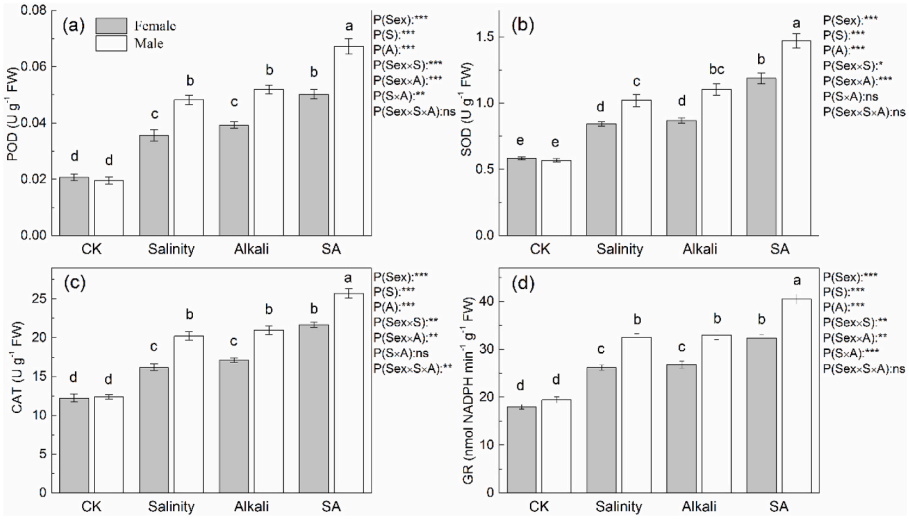

Fig. 7. (a) Peroxidase (POD), (b) superoxide dismutase (SOD), (c) catalase (CAT), and (d) glutathione reductase (GR) in *P. euphratica* females and males under salinity, alkali and their interactive stress. Each value is the mean ± SE ($n = 5$). Treatment codes and statistical analyses as in Fig. 1.

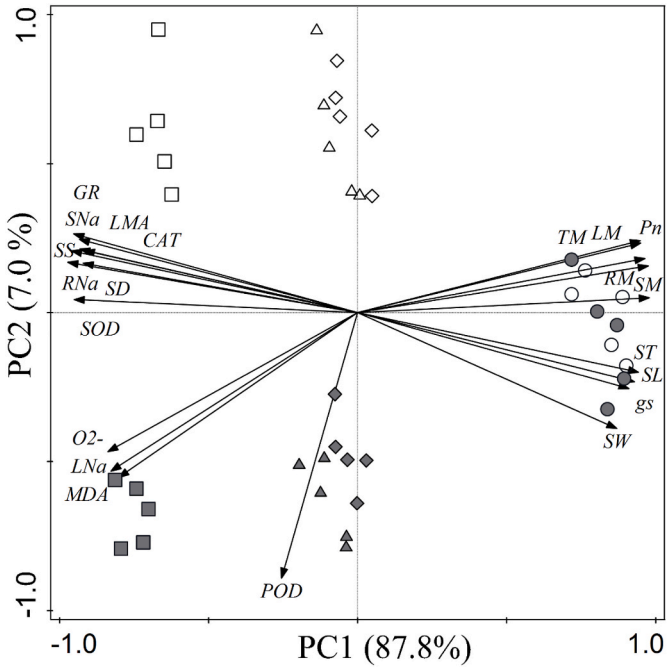

Fig. 8. Principal component analysis (PCA) based on eco-physiological traits in *P. euphratica* females and males under salinity, alkali and their interactive stress. Open symbols: *P. euphratica* males, filled symbols: *P. euphratica* females, Circles, diamonds, triangles and squares indicate CK, salinity, alkali and SA treatments, respectively. LM, leaf biomass; SM, stem biomass; RM, root biomass; TM, total biomass; P_n, net photosynthetic rate; g_s, stomatal conductance; ST, starch content; SL, stomatal length; SW, stomatal width; SD, stomatal density; SS, soluble sugar content; LNa, Leaf Na^+ content; SNa, stem Na^+ content; RNa, root Na^+ content; LMA, leaf mass per area. Treatment codes as in Fig. 1.

under salinity, alkali and their interactive stress. Furthermore, *P. euphratica* males had a greater ability of ion exclusion and Na^+ transport restriction. These data indicated that *P. euphratica* males perform better and have a stronger tolerance to salinity, alkali and their interactive stress. Considering the increasing soil salinization and alkaline levels, our results are important for the ecological restoration and afforestation in relation to *P. euphratica*.

Author contributions

Lei Yu had the main responsibility for data collection, analysis and writing, Shuanglei Tang and Chengjin Guo had a significant contribution to data collection, Helena Korpelainen had a significant contribution to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for the experimental design and project management.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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