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Populus euphratica males exhibit stronger drought and salt stress resistance than females

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ABSTRACT

Much attention has been paid on dioecious plants and their responses to environmental stresses. However, little information is available on sexual dimorphism in *Populus euphratica* Oliver, which usually grows in arid desert areas and exposes to drought and salinity stress. In this study, we investigated female and male seedlings of *P. euphratica* and their growth and anatomical structure, gas exchange, water and nitrogen use, oxidative stress and antioxidants under drought, salinity, and the combined stress. We found that there were no significant sexual differences in *P. euphratica* under control conditions (well-watered and no salt addition). However, males showed stronger resistance to drought, salinity and especially to the combined stress, as they had protective structures, higher biomass, net photosynthetic rate (P_n), water and nitrogen use, osmotic adjustment and reactive oxygen species (ROS) scavenging ability, and a lower leaf Cl^- content under stress conditions. The significant sex × drought × salinity interactions for biomass, P_n, water and nitrogen use, superoxide radicals (O_2^-), proline and free amino acids (FAA) indicated that sexual dimorphism occurs depending on the stress gradients, implying contrasting adaptive responses and life strategies in *P. euphratica* females and males under stress conditions. Due to the sexual dimorphism of *P. euphratica* under drought and salinity stress, more severe shifts in the sex ratio of *P. euphratica* forests may occur under future climate change with aggravated aridity and soil salinity.

1. Introduction

Drought negatively impacts plant growth and production, and climate change is likely to result in increasingly frequent and severe drought worldwide (Trenberth et al., 2014; Anderegg et al., 2015; Chen et al., 2015). To mitigate the drought stress, trees have different adaptive and defense strategies, including physiological adjustments and changes in biomass allocation with increased root growth, resulting in a higher root/shoot ratio (Manzoni et al., 2015; Nolan et al., 2017), which can improve the capacity of water uptake in roots (Poorter et al., 2012; Dong et al., 2016). Physiological adjustments, such as stomatal closure, can decrease transpiration and minimize water loss to prevent critically low canopy water potential (Nolan et al., 2017). In addition, under drought stress, the upregulation of antioxidant enzymes, as a defense mechanism, can mitigate the oxidative damage induced by reactive oxygen species (ROS) (Polle and Chen, 2015; Wang et al., 2021). Salinity, like drought, can result in osmotic stress and limit plant growth and development (Chaves et al., 2009; Chen et al., 2010). Additionally, salinity stress usually induces ionic toxicity, e.g., through chloride ions, which can limit the rates of enzymatic reactions and breakdown proteins, and disrupt metabolic processes (Behnke et al., 2013; Li et al., 2016). It is predicted that soil salinity will increase due to inappropriate irrigation and global warming (Kundzewicz et al., 2007; Janz et al., 2012). Salt exclusion mechanisms, as an important adaptive salinity strategy, can restrict salt transportation from roots to leaves and reduce salt uptake (Chen et al., 2002; Chen and Polle, 2010). Some studies have previously investigated drought or salinity stress alone. However, only few studies have reported the interaction of drought and salinity, which can exacerbate the influence and may induce unique physiological and biochemical responses.

Despite representing only 5–6% of total plant species, with 15,600 dioecious angiosperm species (Renner, 2014), dioecious plant species are very important for the structure and function of terrestrial ecosystems. Compared to males, females usually have greater reproduction costs to produce flowers and seeds, resulting in a higher sensitivity to stress conditions (Juvany and Munné-Bosch, 2015; Lei et al., 2017; Xia

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et al., 2020; Liu et al., 2021). Generally, females associated with a higher reproduction investment perform worse than males in growth and morphology, photosynthetic capacity and resource use efficiency, etc. under abiotic stresses (Eppley, 2005; Xu et al., 2008; Hultine et al., 2016; Melnikova et al., 2017).

Populus euphratica Oliver, as a dioecious tree species, usually grows in desert and arid regions. The total area of *P. euphratica* distribution in the Tarim River watershed accounts for approximately 90 % in China and 55 % worldwide (Wang et al., 1995). In China, *P. euphratica* usually grows along rivers (e.g., Tarim River) in Xinjiang, and it plays a key role in sand fixation, as agricultural shelterbelts and in ecological conservation. Due to its strong drought and salinity resistance, *P. euphratica* is a good model plant to study the mechanism of defending environmental stresses (Polle and Chen, 2015; Sun et al., 2018; Yu et al., 2020; Guo et al., 2021; Xia et al., 2022). However, only few studies have reported the differences between *P. euphratica* females and males, for example, sexual differences in bacterial species (Guo et al., 2021), root phenolic metabolomes and rhizosphere bacterial communities (Xia et al., 2021), and nutrient resorption (Yu et al., 2022). Previous studies have reported that the Tarim River Basin may be an area that most sensitivity to climate change (Chen et al., 2012) due to strong potential evapotranspiration aggravated by arid conditions (Su et al., 2018). Additionally, more frequent and extreme droughts are expected to occur in the future (Zhou et al., 2020). Thus, it is crucial to know, whether *P. euphratica* shows a sex-specific response to drought and salinity stress under future climate change with increasing drought events and soil salinity.

In the present study, we investigated the growth, anatomical structure, photosynthetic capacity, Cl^- accumulation, water and nitrogen use, oxidative stress and antioxidants in female and male seedlings of *P. euphratica* under drought, salinity and the combined stress. We hypothesized that: (1) sex-specific growth and physiological traits will occur along environmental gradients, and (2) *P. euphratica* males have a stronger resistance to drought, salinity and the combined stress.

2. Materials and methods

2.1. Plant material and experimental design

This study was performed in a nursery, located on the northern edge of the Taklimakan Desert (latitude 40°56´N, longitude 81°25´E, altitude 1006 m). This region has a warm temperate and extremely arid continental climate with annual average rainfall is < 50 mm, and the annual average evaporation is > 2500 mm (Yu et al., 2020, 2022).

Early May 2021, 120 female and male seedlings of *P. euphratica* were planted in 35 -L pots (one seedling per pot). All seedlings were three years old and approximately the same size. The planted soil was obtained from a nearby *P. euphratica* forest with pH 8.35 ± 0.03, soil organic matter content 26.28 ± 0.72 mg g^{-1}, and total N content 0.98 ± 0.06 mg g^{-1}. The experimental design was completely randomized with two sexes (female and male), two watering patterns (well-watered and drought), and two salinity levels (no salt and added salt). Under salt stress, we added 150 mM sodium chloride solution (Wang et al., 2008) every second day for 5 times. The other groups were provided with the same amount of water. The time domain reflectometer (Yu et al., 2018) was used to measure the soil water content under different watering regimes. The soil water content of the well-watered group ranged from 27 % to 32 %, and in the drought group it ranged from 7 % to 11 %. Thus, there were four treatments as control (well-watered, WW), drought stress (D), salinity stress (S), and combined drought and salinity stress (DS). In drought conditions, plants were protected from rainfall by a transparent plastic sheet. We adjusted the watering regime according to this standard of the soil water content.

2.2. Determination of growth and leaf anatomical structure

All plants were harvested and separated into leaves, stems and roots, then dried at 70 °C to a constant mass at the end of the experiment. The root/shoot ratio (R/S ratio) was calculated as root biomass/aboveground biomass (the sum of leaf biomass and stem biomass). Five fresh leaves from each treatment were randomly chosen, cut into tissue sections of 8–10 μm thickness and stained with sarranine-fast green. We observed and photographed the blades, and measured leaf thickness, palisade tissue thickness and spongy tissue thickness with a Nikon Eclipse Ni-U microscope (Nikon Corporation).

2.3. Determination of gas exchange and Cl^- content

In early August 2021, we measured the net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration (E) of the fourth fully expanded and intact leaf of five randomly selected seedlings in each treatment using the LI-COR 6400 portable photosynthesis measuring system (LI-COR, Lincoln, NE, USA). Prior to starting the measurements, the target leaves were illuminated with saturating irradiance (1000 μmol m^{-2} s^{-1} PPFD) for about 5–20 min to achieve full photosynthetic induction. The details about conditions and protocols of measurements were described by Li et al. (2020) and Yu et al. (2021). Intrinsic water use efficiency (WUE) was calculated as the net photosynthetic rate (P_n)/stomatal conductance (g_s), and the photosynthetic nitrogen use efficiency (PNUE) was calculated as the net photosynthetic rate (P_n)/leaf N concentration (Yu et al., 2021). Dry powdered leaf samples were used for Cl^- analyses followed the modified silver titration method by Chen et al. (2010).

2.4. Determination of whole plant hydraulic conductance

The whole plant hydraulic conductance (Wp) was calculated as follows (Tyree, 2003; Liu, 2020a): $Wp = E_L/(\Psi_{soil} - \Psi_{leaf})$. E_L was the whole plant transpiration (mmol m^{-2} s^{-1}) and measured as the weight lost at midday (Mitchell et al., 2013). Ψ_{soil} and Ψ_{leaf} were estimated as leaf water potential at predawn (06:00–7:00) and midday (13:00–14:00), respectively (Mitchell et al., 2013). We used a pressure chamber (PMS Instruments, Albany, OR, USA) to measure the leaf water potential.

2.5. Determination of reactive oxygen species and enzyme activities

Malondialdehyde (MDA) and superoxide radicals (O_2^-) were determined following Chen et al. (2011). Briefly, the fresh leaf samples were ground with 10% trichloroacetic acid (TCA) and centrifuged at 12,000 g for 10 min. Then, MDA was measured by a spectrometer (UV-330, Unicam) at 450, 532, and 600 nm, and O_2^- was measured by a spectrometer at 540 nm. The measurements of Catalase (CAT) and glutathione reductase (GR) were described by Chen et al. (2011) and Liu et al. (2020b). Additionally, the determination of proline and free amino acids (FAA) were described by Li et al. (2016) and Wang et al. (2021). Briefly, fresh leaf samples were ground in 3% aqueous sulfosalicylic acid solution, and proline and FAA were measured by a spectrometer at 520 nm and 570 nm, respectively.

2.6. Statistical analysis

All analyses were performed with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 18.0. Before analyses, data were checked for normality and homogeneity of variances and log-transformed to correct for deviations from these assumptions when needed. Tukey's tests of one-way ANOVAs were used to compare individual traits among treatments at a significance level of $P < 0.05$. Three-way ANOVAs were used to test the effects of sex, drought, salinity and their interactions. A principal component analysis (PCA) was used to test coordination among studied traits with Canoco 5.0 (Microcomputer Power, USA).

Table 1
Biomass accumulation and allocation in *P. euphratica* females and males under drought and salinity stress.

Sex	Treatment	Leaf biomass (g)	Stem biomass (g)	Root biomass (g)	Total biomass (g)	R/S ratio
Female	WW	7.67(0.24)a	12.02(0.22)a	8.97(0.14)a	28.65(0.59)a	0.46(0.00)c
	D	5.51(0.13)c	7.84(0.12)c	6.51(0.08)c	19.85(0.19)c	0.49(0.00)b
	S	5.61(0.12)c	7.72(0.20)c	6.51(0.08)c	19.83(0.32)c	0.49(0.00)b
	DS	3.87(0.20)d	4.92(0.18)e	4.67(0.16)d	13.47(0.25)e	0.53(0.02)a
Male	WW	8.06(0.19)a	12.22(0.37)a	9.03(0.16)a	29.32(0.52)a	0.45(0.01)c
	D	6.59(0.09)b	9.13(0.16)b	7.55(0.04)b	23.26(0.15)b	0.48(0.00)b
	S	6.70(0.09)b	8.95(0.18)b	7.63(0.04)b	23.28(0.28)b	0.49(0.01)b
	DS	5.38(0.13)c	5.98(0.15)d	6.11(0.09)c	17.47(0.29)d	0.54(0.01)a
P-values for the significance of different treatment effects						
	F_{Sex}	**0.000**	**0.000**	**0.000**	**0.000**	0.644
	F_{D}	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
	F_{S}	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
	$F_{Sex\times D}$	**0.018**	0.137	**0.000**	**0.002**	0.715
	$F_{Sex\times S}$	**0.016**	0.190	**0.000**	**0.002**	0.394
	$F_{D\times S}$	0.197	**0.017**	0.063	**0.012**	0.309
	$F_{Sex\times D\times S}$	0.541	**0.041**	**0.042**	**0.035**	0.857

Each value is the mean ± SE (n = 5). Different letters denote significant differences according to Tukey's test at a significance level of P < 0.05. Three-way ANOVAs were applied to evaluate the effects of different factors and their interactions. WW, well-watered; D, drought, S, salinity; DS, drought and salinity. F_{Sex}, sex effect; F_{D}, drought effect; F_{S}, salinity effect; $F_{Sex\times D}$, sex × drought interaction effect; $F_{Sex\times S}$, sex × salinity interaction effect; $F_{D\times S}$, drought × salinity interaction effect; $F_{Sex\times D\times S}$, sex × drought × salinity interaction effect.

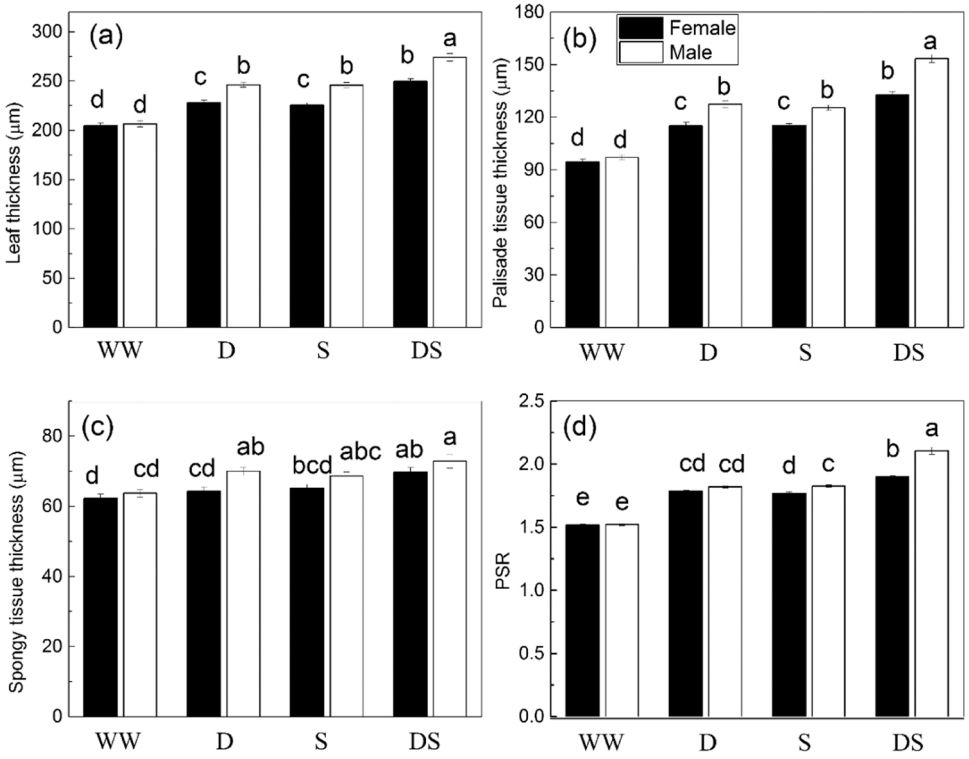

Fig. 1. Effect of drought and salinity stress on (a) leaf thickness, (b) palisade tissue thickness, (c) spongy tissue thickness and (d) spongy tissue thickness/palisade tissue thickness ratio (PSR) in *P. euphratica* females and males. Each value is the mean ± SE ($n = 5$). Different letters above bars denote significant differences according to Tukey's test at a significance level of $P < 0.05$. WW, well-watered; D, drought, S, salinity; DS, drought and salinity.

3. Results

3.1. Sexual differences in growth and anatomical traits of leaves

When compared to the control treatment, the leaf, stem, root and total biomass of *P. euphratica* females decreased by 28 %, 35 %, 27 % and 31 %, respectively, under drought stress, by 27 %, 36 %, 27 % and 31 %, respectively, under salinity stress, and by 50 %, 59 %, 48 % and 53 %, respectively, under the combined stress (Table 1). However, in males, when compared to the controls, the leaf, stem, root and total biomass decreased by 18 %, 25 %, 16 % and 21 %, respectively, under drought stress, by 17 %, 27 %, 16 % and 21 %, respectively, under salinity stress, and by 33 %, 51 %, 32 % and 40 %, respectively, under the combined stress (Table 1). All stress conditions significantly increased the stem and R/S ratio in both *P. euphratica* females and males. In addition, sex × drought × salinity interactions were significant for stem, root and total biomass (Table 1).

There were no significant sexual differences in leaf anatomical traits in *P. euphratica* under control conditions. However, males had significantly higher palisade tissue thickness, spongy tissue thickness and total thickness than females under stress conditions (Fig. 1). Additionally, the palisade tissue thickness/spongy tissue thickness ratio (PSR) increased

Table 2
P values of three-way ANOVAs conducted for anatomical structure, gas exchange, water and nitrogen use, and oxidative stress and antioxidants traits in *P. euphratica* females and males under drought and salinity stress.

Parameters	*P* value						
	F_{Sex}	F_D	F_S	$F_{Sex\times D}$	$F_{Sex\times S}$	$F_{D\times S}$	$F_{Sex\times D\times S}$
LT	**0.000**	**0.000**	**0.000**	**0.016**	**0.004**	0.193	0.134
PT	**0.000**	**0.000**	**0.000**	**0.000**	**0.003**	0.262	0.878
ST	**0.001**	**0.000**	**0.000**	0.300	0.898	0.901	0.220
PSR	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.003**
P_n	**0.000**	**0.000**	**0.000**	**0.008**	**0.005**	**0.000**	**0.000**
g_s	**0.000**	**0.000**	**0.000**	0.327	0.155	**0.000**	0.060
E	**0.000**	**0.000**	**0.000**	**0.007**	**0.006**	**0.000**	**0.007**
Leaf Cl^- content	**0.000**	**0.000**	**0.000**	0.842	**0.000**	**0.000**	0.131
PNUE	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	**0.000**
WUE	**0.000**	**0.000**	**0.000**	**0.001**	**0.006**	**0.006**	**0.005**
Wp	**0.000**	**0.000**	**0.000**	**0.006**	**0.007**	**0.002**	**0.003**
MDA	**0.000**	**0.000**	**0.000**	0.116	**0.013**	0.721	0.126
O_2^-	**0.000**	**0.000**	**0.000**	**0.006**	0.093	**0.005**	**0.049**
CAT	**0.000**	**0.000**	**0.000**	0.063	0.082	0.253	0.211
GR	**0.000**	**0.000**	**0.000**	**0.000**	0.052	**0.007**	0.277
Proline	**0.000**	**0.000**	**0.000**	**0.000**	**0.026**	**0.001**	**0.034**
FAA	**0.000**	**0.000**	**0.000**	**0.009**	**0.005**	0.694	**0.014**

LT, leaf thickness; PT, palisade tissue thickness; ST, spongy tissue thickness; PSR, spongy tissue thickness/palisade tissue thickness ratio; Wp, whole plant hydraulic conductance. F_{Sex}, sex effect; F_D, drought effect; F_S, salinity effect; $F_{Sex\times D}$, sex × drought interaction effect; $F_{Sex\times S}$, sex × salinity interaction effect; $F_{D\times S}$, drought × salinity interaction effect; $F_{Sex\times D\times S}$, sex × drought × salinity interaction effect.

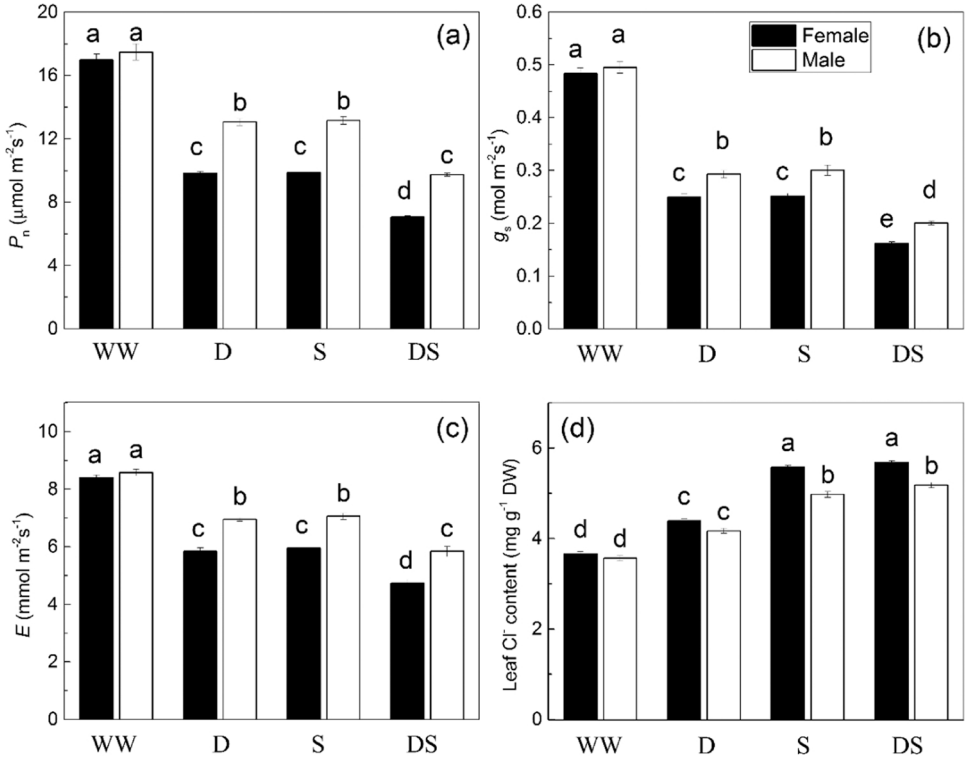

Fig. 2. Effect of drought and salinity stress on (a) net photosynthetic rate (P_n), (b) stomatal conductance (g_s), (c) transpiration (E) and (d) leaf Cl^- content in *P. euphratica* females and males. Each value is the mean ± SE ($n = 5$). Treatment codes and statistical analyses as in Fig. 1.

under drought stress and salinity stress, especially under the combined stress. Furthermore, sex × drought and sex × salinity interactions were significant for leaf thickness and palisade tissue thickness (Table 2).

3.2. Sexual differences in gas exchange and Cl^- content

When compared to the control treatment, stress conditions significantly decreased the net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E). In addition, *P. euphratica* females had significantly lower P_n, g_s and E than males under drought, salinity and the combined stress, while these gas exchange traits showed no sexual differences under control conditions (Fig. 2). Stress conditions increased the leaf Cl^- content in both sexes compared to control conditions. Under salinity stress, the leaf Cl^- content of females and males increased by 52 % and 40 %, respectively, but 55 % and 45 % under the combined stress, respectively (Fig. 2). *P. euphratica* females had significantly higher leaf Cl^- contents under salinity and the combined stress. Additionally, sex × salinity and drought × salinity interactions were significant for P_n, E, and leaf Cl^- content (Table 2).

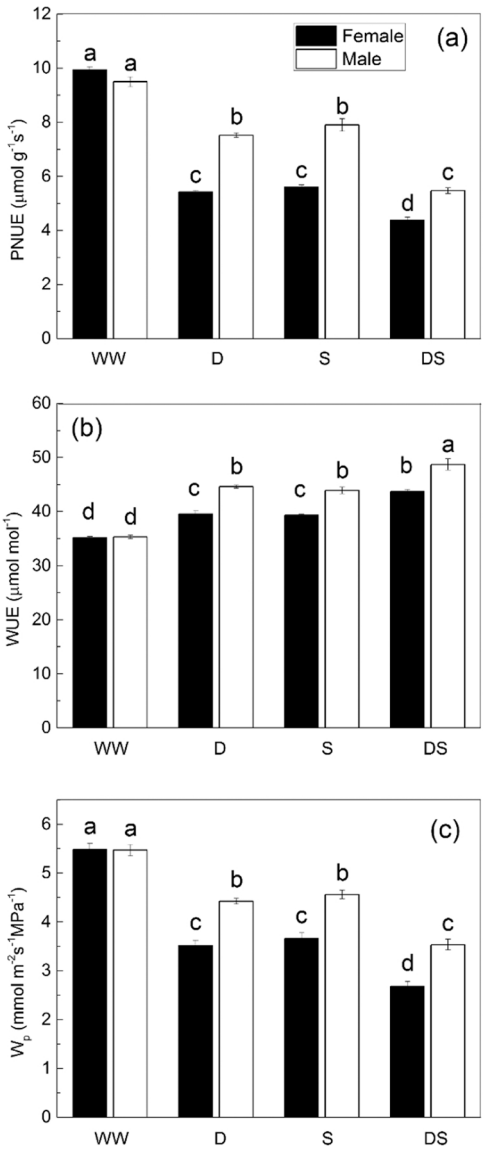

Fig. 3. Effect of drought and salinity stress on (a) photosynthetic nitrogen use efficiency (PNUE), (b) water use efficiency (WUE) and (c) whole plant hydraulic conductance (Wp) in *P. euphratica* females and males. Each value is the mean ± SE ($n = 5$). Treatment codes and statistical analyses as in Fig. 1.

3.3. Sexual differences in water and nitrogen use

When compared to the control treatment, stress conditions significantly decreased PNUE and the whole plant hydraulic conductivity (Wp), but increased WUE in both sexes. Under drought stress, PNUE and Wp decreased by 45 % and 36 % in females, respectively, whereas by only 21 % and 19 % in males, respectively (Fig. 3). Under salinity stress, PNUE and Wp decreased by 44 % and 33 % in females, respectively, whereas by only 17 % and 17 % in males, respectively. Under combined stress, PNUE and Wp decreased by 56 % and 51 % in females, respectively, whereas by only 42 % and 35 % in males, respectively (Fig. 3). In addition, under drought stress, WUE in females and males increased by 12 % and 26 %, respectively, and by 12 % and 24 % under salinity stress, respectively, but by 24 % and 38 % under the combined stress, respectively. Furthermore, sex × drought × salinity interactions were significant for PNUE, Wp and WUE (Table 2).

3.4. Sexual differences in oxidative stress and antioxidants

The levels of MDA, O_2^-, CAT, GR, proline and FAA significantly increased in *P. euphratica* females and males under all stress conditions (Fig. 4). Females had significantly higher levels of MDA and O_2^- than males under stress conditions. For example, under the combined stress, MDA and O_2^- increased by 71 % and 81 % in females, respectively, whereas by only 53 % and 58 % in males, respectively. In addition, males had significantly higher levels of CAT, GR, proline and FAA than females under all stress conditions. For instance, under the combined stress, CAT, GR, proline and FAA increased by 85 %, 101 %, 113 % and 43 % in males, respectively, whereas by only 65 %, 60 %, 68 % and 30 % in females, respectively (Fig. 4). Furthermore, sex × drought × salinity interactions were significant for O_2^-, proline and FAA (Table 2).

3.5. Relationships among all traits under drought and salinity stress

The principal component analysis (PCA) of first two axes explained 78.7 % of the total variance of all parameters in *P. euphratica* females and males in response to drought and salinity stress (Fig. 5). The control treatment and stress treatments were separated along the first PCA axis. Under stress conditions, females and males were well separated along the second PCA axis (Fig. 5). In addition, PC1 was strongly influenced by leaf, stem, root and total biomass, P_n, g_s, E, PNUE, Wp, spongy tissue thickness, palisade tissue thickness, CAT, GR, FAA, and proline. PC2 was strongly influenced by R/S ratio, leaf thickness, WUE, PSR, leaf Cl^-, MDA, and O_2^-.

4. Discussion

4.1. The effects of drought and salinity on growth and leaf anatomy

We found that the biomass of both sexes of *P. euphratica* decreased significantly under stress conditions, but males had a higher biomass than females under drought, salinity and particularly under the combined stress. The above statements implied that males can survive better and maintain higher growth under stress conditions, which is consistent with earlier studies (Chen et al., 2010, 2014; Juvany and Munné-Bosch, 2015; Liu et al., 2020b). In addition, sex × drought × salinity interactions were significant for the total biomass indicating that, under salinity, the total biomass of *P. euphratica* females declined more when suffering from drought stress.

In adaption to drought and salinity stress, leaves usually show structural changes, such as increased leaf thickness and palisade tissue thickness, and decreased leaf area and mesophyll (England and Attiwill, 2006; Beniwal et al., 2011; Han et al., 2013). Furthermore, increased leaf thickness and palisade tissue thickness can extend the distance of water diffusion from leaf veins to epidermis, which prevents excessive water transpiration and enhances the water use efficiency (Fahmy, 1997). In this study, we found that leaf thickness and palisade tissue thickness of both sexes increased significantly under stress conditions. Compared to females, males showed a higher leaf thickness and palisade tissue thickness under stress conditions (Fig. 1). These results indicated that males have a superior drought resistance structure in leaves that is associated with higher WUE to cope with stress conditions.

4.2. The effects of drought and salinity on gas exchange, and water and nitrogen use

Earlier studies have reported sexual differences in gas exchange parameters of dioecious plants (Chen et al., 2010; Yu et al., 2018; Liu et al., 2020a). For example, females have showed a lower photosynthetic capacity compared to males under stress conditions. In agreement with these studies, we found that *P. euphratica* males have higher P_n, g_s, and E than females under drought, salinity and particularly under the combined stress (Fig. 2). According to the PCA analysis, females and males

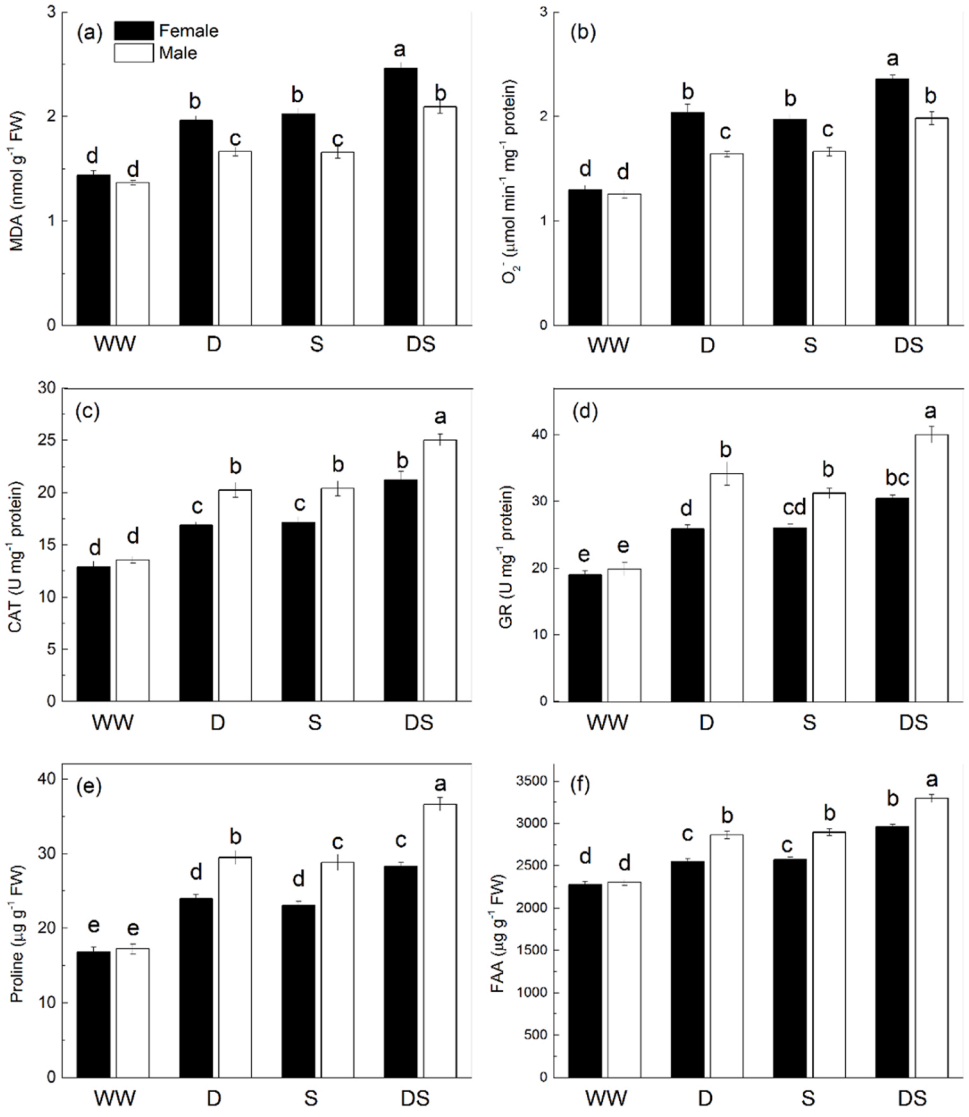

Fig. 4. Effect of drought and salinity stress on (a) malondialdehyde (MDA), (b) superoxide radicals (O_2^-), (c) catalase (CAT), (d) glutathione reductase (GR); (e) proline and (f) free amino acids (FAA) in *P. euphratica* females and males. Each value is the mean ± SE ($n = 5$). Treatment codes and statistical analyses as in Fig. 1.

were well separated along the second PCA axis under stress conditions. There were positive relationships among biomass, P_n, g_s, E, PNUE, Wp and WUE (Fig. 5).

Water use efficiency (WUE) is fundamental for plants' survival and growth, and it is strongly affected by environment stress, for instance drought and salinity (Zhang et al., 2005; Chen et al., 2014; Li et al., 2016). Drought and salinity represent osmotic stress, which results in declined P_n, stomatal closure and increased WUE (Li et al., 2016; Yu et al., 2018; Liu et al., 2020a). Our results were in line with these statements indicating that stress conditions decrease P_n, along with stomatal closure and increased WUE, in both sexes of *P. euphratica* (Figs. 2, 3). PNUE is a key plant functional trait and closely related to the leaf ecology and economics strategy (Bown et al., 2009; Hidaka and Kitayama, 2009). We found that stress environments (particularly the combined stress) decrease PNUE in *P. euphratica* females and males (Fig. 3), which may be induced by the decline of P_n under stress (Yu et al., 2018). Interestingly, we found a trade-off between PNUE and WUE (Fig. 3), implying that *P. euphratica* may maximize the use efficiencies of limited resources, e.g., water and nitrogen (Field et al., 1983; Yu et al., 2018). In addition, drought stress usually decreases the hydraulic conductance of plants (e.g. Wp), which may result from the stomatal closure, reduced transpiration, and down-regulated water transport from roots to leaves (Mitchell et al., 2013; Savi et al., 2016). In accordance with the above statements, we found that stress conditions decreased Wp in both sexes, but males had higher Wp than females under drought and salinity stress. These results suggested that there are sex-specific adaptive strategies in hydraulic traits under stress environments, which was further supported by the significant interactive effect of sex × drought × salinity on Wp.

Earlier studies have reported that the exclusion of Cl^- is fundamental for salt tolerance in trees, such as *Populus* (Chen et al., 2002; Chen and Polle, 2010). In poplars, Cl^- is mainly sequestered in the leaf tissues (Ottow et al., 2005; Zalesny et al., 2007), and a greater proportion of Cl^- may be allocated into vacuoles, inducing diluted salt concentrations along greater tolerance (Ottow et al., 2005). In this study, *P. euphratica* females had a higher leaf Cl^- content under salinity conditions, implying that males can avoid the accumulation of Cl^- in leaves more effectively, which is agreement with previous studies (Chen et al., 2010; Li et al., 2016). Additionally, the significant interactive effect of sex × salinity on leaf Cl^- content, indicating that the leaf Cl^- content showed sexual dependence, and this may result in sexual differences in salt tolerance (Chen et al., 2010).

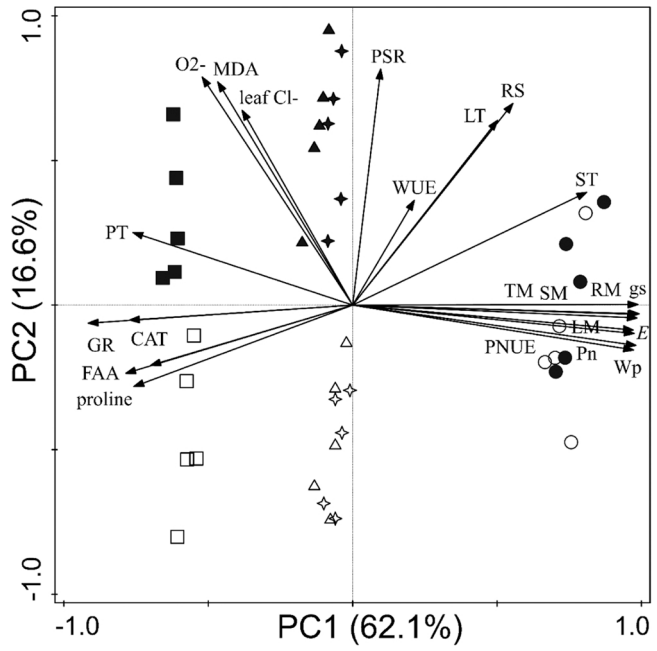

Fig. 5. Principal component analysis (PCA) based on all studied traits in *P. euphratica* females and males under drought and salinity stress. Filled symbols: females, open symbols: males. Circles, stars, triangle arrows and squares indicate WW, D, S and DS treatments, respectively. LM, leaf biomass; SM, stem biomass; RM, root biomass; TM, total biomass; RS, root/shoot ratio; P_n, net photosynthetic rate; g_s, stomatal conductance; *E*, transpiration; PNUE, photosynthetic nitrogen use efficiency; Wp, whole plant hydraulic conductance; LT, leaf thickness; ST, spongy tissue thickness; PT, palisade tissue thickness; PSR, spongy tissue thickness/palisade tissue thickness ratio; WUE, water use efficiency; MDA, malondialdehyde; O_2^-, superoxide radicals; CAT, Catalase; GR, glutathione reductase; FAA, free amino acids. Treatment codes as in Fig. 1.

4.3. The effects of drought and salinity on oxidative stress and antioxidants

Drought and salinity stress are usually accompanied by oxidative stress, which results in ROS accumulation and oxidative damage on plant cells (Chen et al., 2010; Li et al., 2016; Liu et al., 2020b). MDA and O_2^- are closely related to oxidative damage and membrane lipid peroxidation (Cao et al., 2014; Wang et al., 2021). Under stress conditions, females had higher MDA and O_2^- than males, which indicated that females suffer more serious oxidative damage. Antioxidants, such as CAT and GR, play a key role in scavenging ROS and act as a crucial tolerance mechanism to cope with abiotic stress (Petrov et al., 2015). In the present study, males had higher CAT and GR compared to females under stress conditions (Fig. 4), implying that males may have a better capacity for oxygen-scavenging and stress tolerance (Li et al., 2011, 2016; Liu et al., 2020b). In addition, proline plays a critical role in regulating the osmotic stress and preventing membrane damage in plants under stress conditions (Cao et al., 2014; Wang et al., 2021). FAA is related to osmoregulation and to the maintenance of osmotic potential in cells (Boldizsar et al., 2013). In the present study, males had higher proline and FAA levels compared to females under drought, salinity and especially under the combined stress (Fig. 4). These results showed that compared to females, *P. euphratica* males have a better capacity to osmoregulation and to the maintenance of the osmotic potential in cells under stress conditions.

5. Conclusions

The present study demonstrated that certain growth and leaf structure traits change, and physiological and biochemical differences between *P. euphratica* females and males increase along an environmental gradient. Our study revealed that *P. euphratica* males showed stronger resistance to drought, salinity and especially to the combined stress compared to females. Males have a protective leaf structure, and higher biomass, net photosynthetic rate, water and nitrogen use efficiencies, osmoregulation and ROS scavenging ability, and a lower leaf Cl^- content under stress conditions. Thus, our results provided important theoretical knowledge for the ecological restoration, afforestation and protection of *P. euphratica* forests under climate change with aggravated aridity and soil salinity.

Author contribution statement

Lei Yu had the main responsibility for data collection, analysis and writing, Zongdi Huang and Shuanglei Tang 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 conflict of interest.

Data availability

Data will be made available on request.

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