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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 grows in arid and semi-arid regions worldwide. In this study, we investigated female and male seedlings of *P. euphratica* and their growth and anatomical structure, gas exchange, water and nitrogen use, reactive oxygen species (ROS) accumulation and osmotic regulation under drought, salinity, and the combined stress. Our results showed that there were no significant differences between *P. euphratica* females and males under control conditions. 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, osmoregulation and 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. 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.

Keywords: dioecy, gas exchange, drought, salinity, water and nitrogen use, osmotic regulation

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; Choat et al., 2018). To mitigate the drought stress, tree species 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, the upregulation of antioxidant enzymes, as a defense mechanism, can mitigate the oxidative damage induced by reactive oxygen species (ROS) under drought stress (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 of proteins, and disrupt the 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 15600 dioecious angiosperm species in 987 genera and 175 families (Renner, 2014), dioecious plant species are very important for the structure and function of terrestrial ecosystems. Compared to males, females have greater reproduction costs to produce flowers and seeds, resulting in a trade-off between reproduction and growth (Juvany and Munné-Bosch, 2015; Xia et al., 2020). Generally, compared to males, females associated with a higher reproduction investment perform worse 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, a dioecious tree species, grows in desert and arid regions worldwide. 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 environmental stresses (Polle and Chen, 2015; Sun et al., 2018; Yu et al., 2020; Guo et al., 2021). However, only few studies have investigated the differences between *P. euphratica* females and males, for example, sexual differences in bacterial species (Guo et al., 2021), root metabolic profiles 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 responds strongly to climate change (Chen et al., 2012) due to strong potential evapotranspiration aggravated by arid conditions (Su et al., 2018). More frequent and extreme droughts are expected to occur in the future (Chen et al., 2015; 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-dependent growth and physiological traits will change 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

The study was conducted in a nursery near the Tarim University, located on the northern edge of the Taklimakan Desert (elevation 1006 m above sea level, 40°56′ N, 81°25′ E) with a warm temperate and extremely arid continental climate. The annual sunshine duration is from 2750 to 3029 h, the 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, and it had a pH of 8.35 ± 0.03, soil organic matter content of 26.28 ± 0.72 mg g^{-1}, and total N content of 0.98 ± 0.06 mg g^{-1}. The experimental design was completely randomized with two sexes (female and male), two watering regimes (well-watered and drought), and two levels of salinity (no salt and added salt). Under salt stress, we added 150 mm sodium chloride solution (Wang et al., 2008) every other 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 moisture content of the well-watered group ranged from 27% to 32%, and in the drought group it ranged from 7% to 11%. We adjusted the watering regime according to this standard of the soil water content.

2.2. Determination of growth and leaf anatomical structure

At the end of the experiment, all plants were harvested and separated into leaves, stems and roots, then dried at 70 °C to a constant mass and weighed. The root/shoot ratio (R/S ratio) is the ratio between root biomass and aboveground biomass (the sum of leaf biomass and stem biomass). Five fresh leaves from each treatment were randomly chosen and fixed in a formalin-acetic acid-alcohol solution, 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 used the LI-COR 6400 portable photosynthesis measuring system (LI-COR, Lincoln, NE, USA) to measure the net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration (E) of the fourth fully expanded and intact leaf from five randomly chosen seedlings in each treatment. The conditions of measurements were as follows: leaf temperature 25 °C; air vapor pressure deficit 1.5 ± 0.5 kPa; relative humidity 50%; light intensity (PPFD) 1500 μmol m^{-2} s^{-1}; and CO_2 concentration 400 ± 5 μmol mol^{-1}. 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 from five randomly chosen seedlings in each treatment were used for Cl^- analyses according to the modified silver titration method (Chen et al., 2001).

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 calculated as the weight lost over the measurement interval at midday (Mitchell et al., 2013). Ψ_{soil} and Ψ_{leaf} were estimated from predawn (06:00-7:00) and midday (13:00-14:00) leaf water potential, respectively (Mitchell et al., 2013). The leaf water potential was measured in a pressure chamber (PMS Instruments, Albany, OR, USA).

2.5. Determination of reactive oxygen species and enzyme activities

Malondialdehyde (MDA) and superoxide radicals (O_2^-) were determined according to the method by Chen et al. (2011). Briefly, the leaf samples were ground with 10% trichloroacetic acid (TCA) and centrifuged at 12000g for 10 min. Then, MDA was measured by a spectrometer (UV-330, Unicam) at 450, 532, and 600 nm. O_2^- was measured by a spectrometer at 540 nm after adding 1 ml of 0.2% N-(1- naphthyl)-ethylenediamine and 1 ml of 1% sulfanilamide. Catalase (CAT) and glutathione reductase (GR) were measured according to 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. (2020). Briefly, 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 carried out 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, watering, salinity and their interactions. A principal component analysis (PCA) was used to test coordination among studied traits with Canoco 5.0 (Microcomputer Power, USA).

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). When compared to the controls, drought, salinity and the combined stresses significantly increased the stem and root to shoot ratio (R/S ratio) in both *P. euphratica* females and males. In addition, stem, root and total biomass were significantly affected by sex × drought × salinity interactions (Table 1).

Under control conditions, there were no significant differences in palisade tissue thickness, spongy tissue thickness and total thickness between *P. euphratica* females and males. However, males had significantly higher palisade tissue thickness, spongy tissue thickness and total thickness than females under stress conditions, especially under the combined stress (Fig. 1). Additionally, the palisade tissue thickness/spongy tissue thickness ratio (PSR) increased under drought stress and salinity stress, especially under the combined stress. Furthermore, leaf thickness and palisade tissue thickness were significantly affected by the interaction of sex × drought and sex × salinity (Table 2).

3.2. Sexual differences in gas exchange and Cl⁻ content

When compared to the control treatment, drought, salinity and the combined stress 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 differences between the sexes under control conditions (Fig. 2). Drought, salinity and the combined stress 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, the P_n, E, and leaf Cl^- contents were significantly affected by the interaction of sex × salinity and drought × salinity (Table 2).

3.3. Sexual differences in water and nitrogen use

Drought, salinity and the combined stress significantly decreased PNUE and the whole plant hydraulic conductivity (Wp), but increased WUE in both sexes compared to control conditions. 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, PNUE, Wp and WUE were significantly affected by the interaction of sex × drought × salinity (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 all 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, O_2^-, proline and FAA were significantly affected by the interaction of sex × drought × salinity (Table 2).

3.5. Relationships among all traits under drought and salinity stress

The principal component analysis (PCA) with first two axes explained 78.7% of the total variation in the studied traits in *P. euphratica* females and males across 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 results implied that males can survive better and maintain higher growth under stress conditions, which is consistent with previous studies (Xu et al., 2008; Chen et al., 2010, 2014; Juvany and Munné-Bosch, 2015; Liu et al., 2020b). In addition, the total biomass was significantly affected by sex × drought × salinity interactions indicating that, under salinity, the total biomass of *P. euphratica* females decreased 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 et al., 1997; Dong et al., 2001). In the present 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 drought, salinity and the combined stress (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

Previous studies have reported sexual differences in gas exchange parameters of dioecious plants (Xu et al., 2008; Chen et al., 2010, 2014; Yu et al., 2018). For example, females have showed a lower photosynthetic capacity compared to males under stress conditions. In accordance with these studies, our results showed 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 were well separated along the second PCA axis under stress conditions. There were positive associations among leaf, stem, root and total 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). 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 consistent 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 an important functional trait and closely related to the leaf economics strategy (Niinemets, 1999; 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* could 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 interaction of sex × drought × salinity on Wp.

Earlier studies have reported that the exclusion of Cl^- plays a key role for salt tolerance in woody species, 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 the present 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 consistent with previous studies (Chen et al., 2010; Li et al., 2016). Additionally, the leaf Cl^- content was significantly affected by the interaction of sex × salinity, indicating that the leaf Cl^- content showed sexual dependence, and this may result in sex-specific differences in salt tolerance (Chen et al., 2010).

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 the production of reactive oxygen species (ROS) and oxidative damage on plant cells (Chen et al., 2010; 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 under drought, salinity and especially under the combined stress. Antioxidants, such as CAT and GR, play a key role in scavenging ROS and act as an important tolerance mechanism to abiotic stress (Petrov et al., 2015). In the present study, males had higher CAT and GR than females under drought, salinity and especially under the combined stress (Fig. 4), implying that males may have a better capacity for oxygen-scavenging and stress tolerance (Li et al., 2011; 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 osmotic adjustment 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 osmotic adjustment and to the maintenance of the osmotic potential in cells under stressful conditions.

5. Conclusions

In the present study, we 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. 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.

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

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

Sex	Treatment	Leaf biomass	Stem biomass	Root biomass	Total biomass	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.

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.

	P value						
Parameters	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.

Figure legends

Figure 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.

Figure 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 Figure 1.

Figure 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 Figure 1.

Figure 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 Figure 1.

Figure 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 Figure 1.

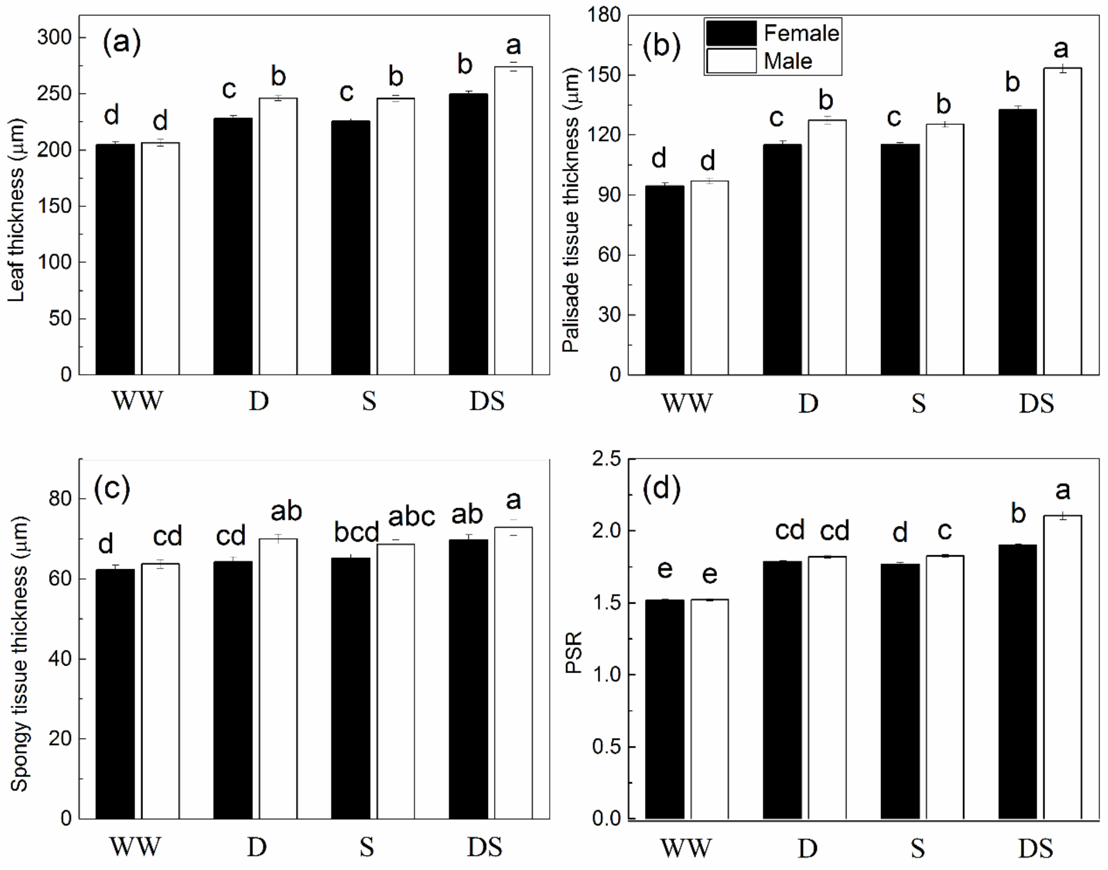

(a)
Leaf thickness (μm)
0
50
100
150
200
250
300
d
d
c
b
c
b
b
a
WW
D
S
DS
(b)
Palisade tissue thickness (μm)
0
30
60
90
120
150
180
Female
Male
d
d
c
b
c
b
b
a
WW
D
S
DS
(c)
Spongy tissue thickness (μm)
0
20
40
60
80
d
cd
cd
ab
bcd
abc
ab
a
WW
D
S
DS
(d)
PSR
0.0
0.5
1.0
1.5
2.0
2.5
e
e
cd
cd
d
c
b
a
WW
D
S
DS

Figure 1

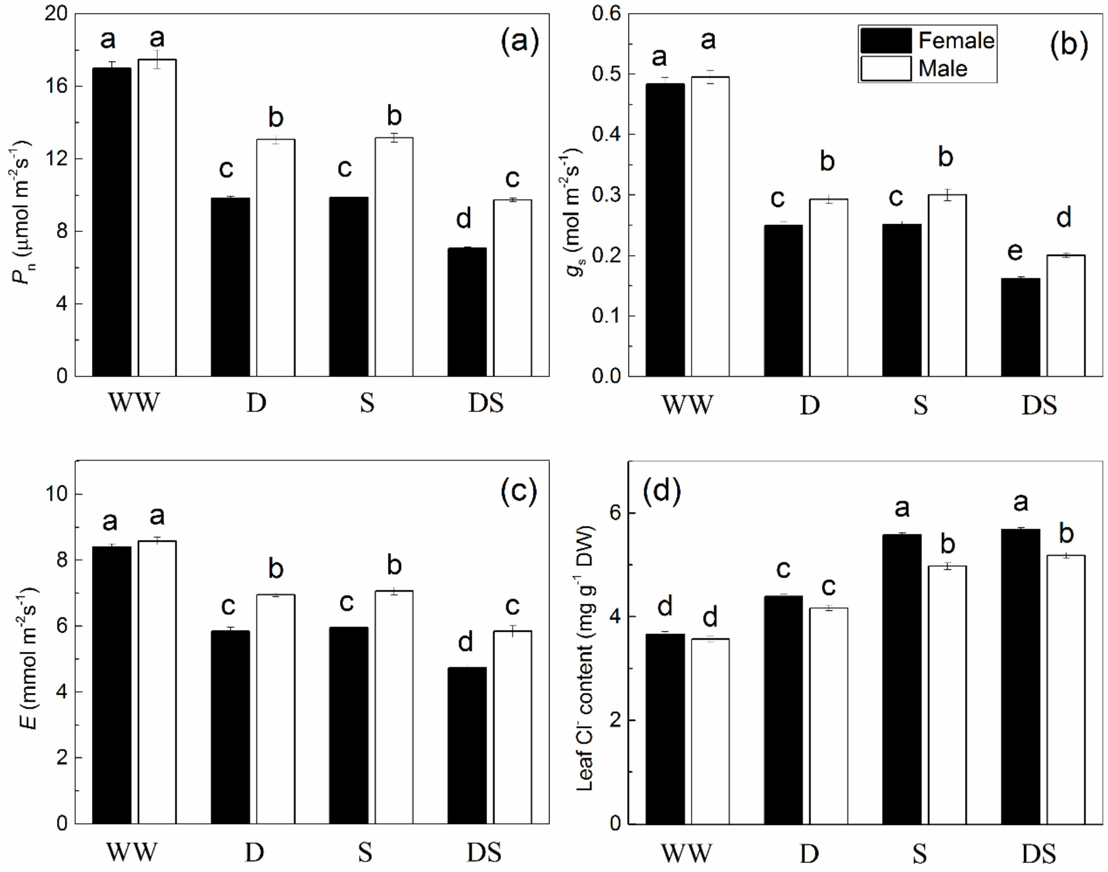

(a)
Pn (μmol m-2s-1)
0
4
8
12
16
20
a
a
c
b
c
b
d
c
WW
D
S
DS
(b)
Female
Male
gs (mol m-2s-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
a
a
c
b
c
b
e
d
WW
D
S
DS
(c)
E (mmol m-2s-1)
0
2
4
6
8
10
a
a
c
b
c
b
d
c
WW
D
S
DS
(d)
Leaf Cl- content (mg g-1 DW)
0
2
4
6
d
d
c
c
a
b
a
b
WW
D
S
DS

Figure 2

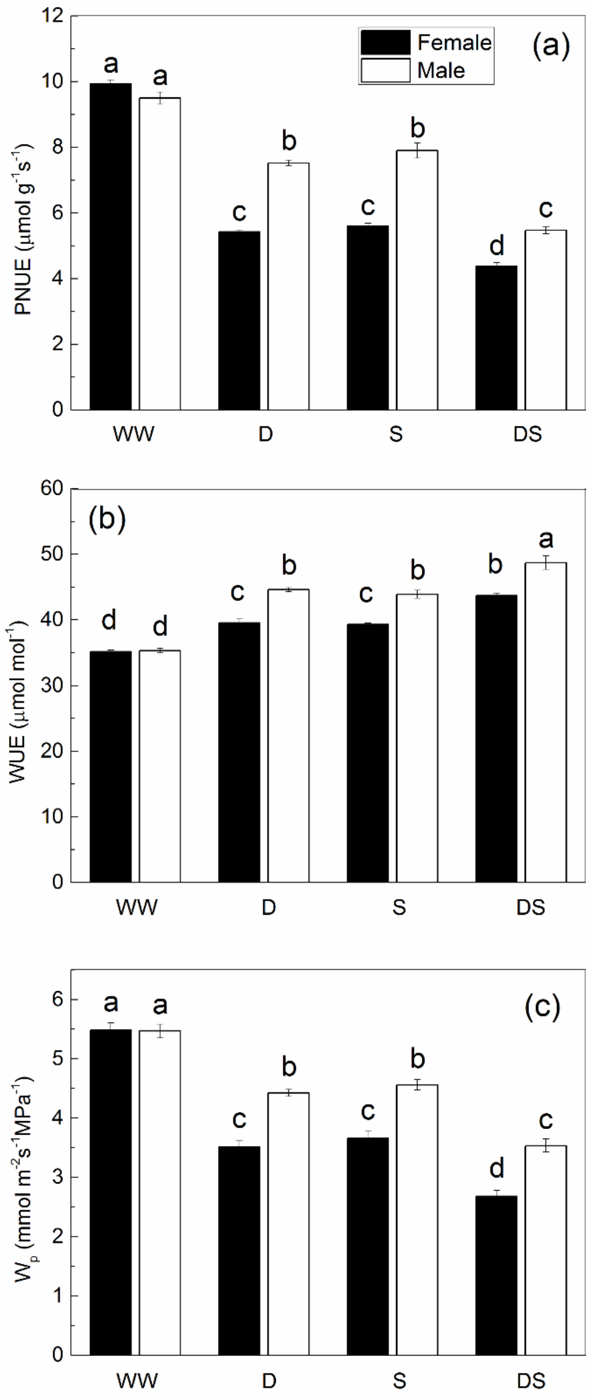
(a)
Female
Male
PNUE (μmol g-1s-1)
12
10
8
6
4
2
0
WW
D
S
DS
a
a
c
b
c
b
d
c
(b)
WUE (μmol mol-1)
60
50
40
30
20
10
0
d
d
c
b
c
b
b
a
(c)
Wp (mmol m-2s-1MPa-1)
6
5
4
3
2
1
0
a
a
c
b
c
b
d
c

Figure 3

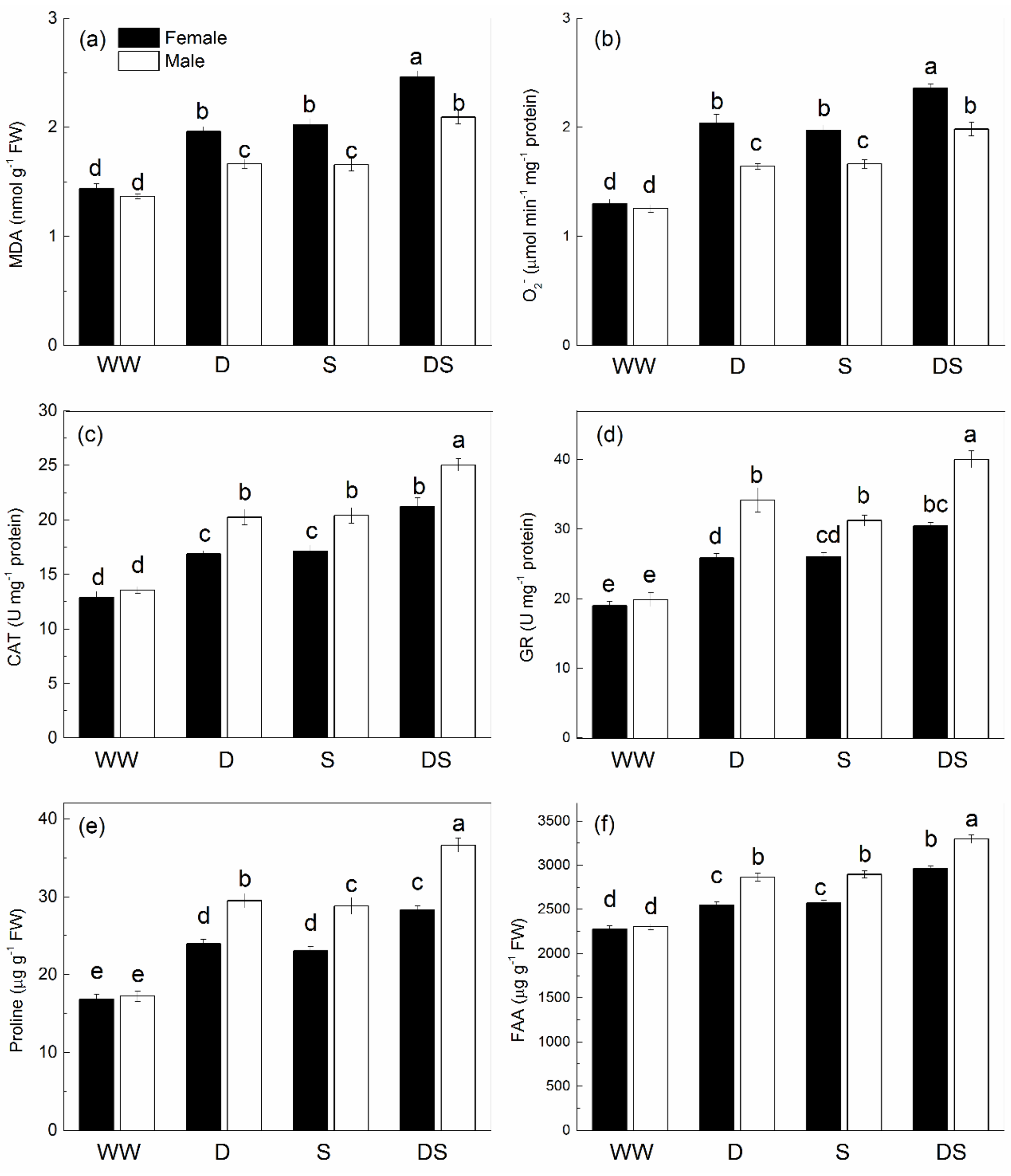
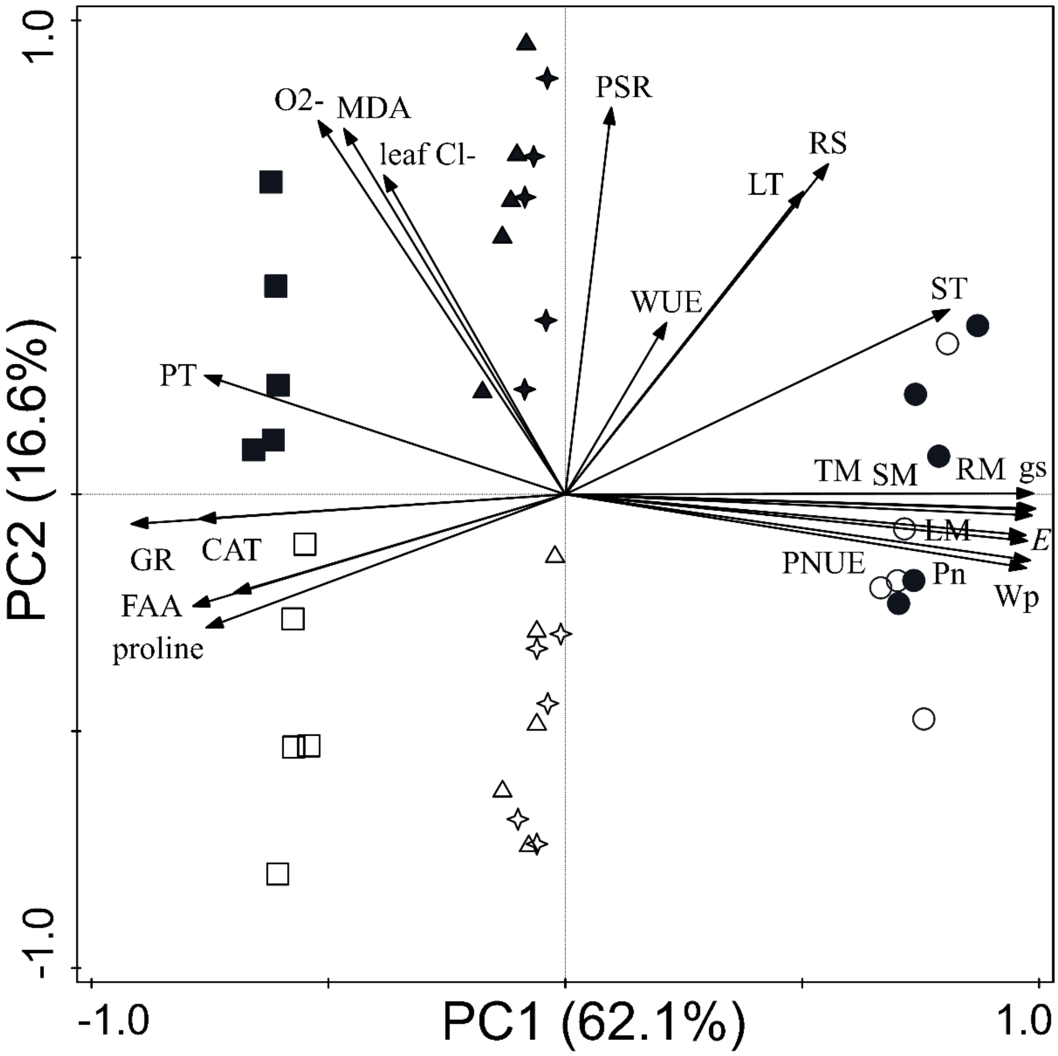

(a)
Female
Male
MDA (nmol g^{-1} FW)
0
1
2
3
d
d
b
c
b
c
a
b
WW
D
S
DS
(b)
O$_2^-$ (μmol min^{-1} mg^{-1} protein)
0
1
2
3
d
d
b
c
b
c
a
b
WW
D
S
DS
(c)
CAT (U mg^{-1} protein)
0
5
10
15
20
25
30
d
d
c
b
c
b
b
a
WW
D
S
DS
(d)
GR (U mg^{-1} protein)
0
10
20
30
40
e
e
d
b
cd
b
bc
a
WW
D
S
DS
(e)
Proline (μg g^{-1} FW)
0
10
20
30
40
e
e
d
b
d
c
c
a
WW
D
S
DS
(f)
FAA (μg g^{-1} FW)
0
500
1000
1500
2000
2500
3000
3500
d
d
c
b
c
b
b
a
WW
D
S
DS

Figure 4



PC2 (16.6%)
1.0
-1.0
PC1 (62.1%)
-1.0
1.0
O2-
MDA
leaf Cl-
PSR
RS
LT
WUE
ST
PT
TM
SM
RM
gs
LM
E
PNUE
Pn
Wp
GR
CAT
FAA
proline

Figure 5