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More Effective Protection Supports Male Better Than Female Siblings over Water Deficit in Artificially Bred Poplar Hybrids

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Abstract: Sexually dimorphic response to stress has been observed in assorted natural dioecious plants. Up to now, few studies have focused on the difference of stress responses between artificially bred siblings. To determine the sexual dimorphism between artificially bred sibling poplar trees, we conducted a study comparing the response to water deficit between male and female *Populus* × *euramericana* siblings. This pair of hybrids was analyzed in terms of growth, photosynthesis, membrane injury and repair systems, as well as gene regulation patterns. The female and male siblings presented distinct responses to water deficit, with greater inhibition in females' growth and photosynthesis. The results also displayed that in females, relative electrolyte leakage and malondialdehyde content were higher than those in males under water deficit conditions. On the other hand, water deficit caused a greater increase in both SOD activity and POD activity in males than those in females. Consistent with these physiological differences, the expression of several stress-related genes, including *SOD*, *GST*, *bHLH35*, and *PsbX1*, was regulated differently between female and male hybrids by water deficit stress. Higher expression of *SOD* in moderate-water-deficit-treated females and higher *GST*, *bHLH35* expression in both moderate- and severe-water-deficit-treated females suggest that the female sib is more sensitive, whilst higher expression of *SOD* in severe-water-deficit-treated males and higher *PsbX1* expression in water-deficit-treated males testify that males protect cells better. To achieve an integrated view, all these variables were analyzed through the use of a principal component analysis and a total discrepancy between the sexes in their response to water deficit was demonstrated. The results indicate that, compared with male poplar sibs, females are more sensitive, but deploy a weaker protective apparatus to deal with water deficit.

Keywords: sexual dimorphism; siblings; water deficit; gene regulation; ROS scavenging; *Populus* × *euramericana*

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1. Introduction

There are 15,600 dioecious plant species which have been identified to date, accounting for 5%–6% of the angiosperm plants on Earth [1]. Under environmental selection, dioecious plants separate females from male individuals, which has been hypothesized to increase outcrossing for facilitating species evolution and resolving intralocus sexual conflicts over the allocation of resources [2–5]. Despite their autosomal genetic similarity, females and males of various dioecious plants are different in morphological, physiological, and ecological features, including possessing different responses to environmental stresses [6,7]. In dioecious plants, sexual-related stress resistance bias is selected and evolves naturally along with sexual selection on both autosomes and sexual chromosomes over several decades to million years [2,8]. Artificially bred female and male siblings are generated as hybrids from a specific pair of parents, and are produced through carefully controlled fertilization, precluding paternal input except from the selected male parent. These hybrids are quite similar in autosomes, and this is reflected in similarities in morphological, physiological,

and ecological features. However, these similarities do not include the similarity in responses to environmental stresses [9,10]. The distinct physiological responses to stress between artificially bred female and male siblings may be linked to gene expression or sex determination biases [3].

Water is indispensable to plants in retaining the balance of cell turgor, osmotic potential, photosynthesis, respiration, etc. [11–13]. Drought has induced huge pecuniary losses in global crop production in the last decade and will increasingly be a misfortune for agriculture, humanity, and livestock alike [14]. Water deficit, another meaning for drought for plants, greatly restricts plant distribution, growth, development, and productivity [15]. To perceive, avoid, and compensate for drought-induced harm, plants have evolved various defense strategies. The perception of stress by plants is through initiating several complex signaling networks, such as phytohormone level change, kinase/phosphatase signaling cascade regulation, stress-related gene expression, and reactive oxygen species (ROS) production [15,16]. However, the excess ROS induced by water deficit is harmful to cellular integrity and biomacromolecules. To survive, plants have to detoxify excess ROS toxicity through enzymatic and/or nonenzymatic mechanisms [17–19]. It can be considered that the capability of detoxification of ROS might be correlated with the ability of plants to resist stress. Under water deficit stress, resistant and sensitive plants respond differently, spanning from morphology and physiology to biochemistry and gene regulation. Typically, the sensitive plant responds quickly to water deficit but is exposed to more harm due to a weaker protective strategy for water deficiency than a tolerant plant at either cell, tissue, or overall plant level [14,20,21]. This theory is supported in many native dioecious species, whereby it appears that the female is the sensitive sex and is harmed more severely by stress compared to male plants [22–28]. Few research studies have focused on whether and how the sexual-specific response to water deficit between sexes is manifest between cultured female and male sibs [9,10].

Several gene expression mechanisms, especially stress response and resistance-related-gene regulation, endue plants with different responses and resistance to stress [14,15,29,30]. The response- and resistance-related genes' products, for sensing and resisting drought, are classified into functional and regulatory groups [30]. In the first group, there are water channels and transporters, detoxification enzymes, protection factors, and osmolyte biosynthesis enzymes and proteases, and in the regulatory groups, there are transcription factors, such as DREBs, AREB, MYC, MYB, bZIP, bHLH, and NAC, and protein kinases and phosphatases, phospholipid metabolism and ABA biosynthetic pathway components [30]. Superoxide dismutase (SOD) is a crucial antioxidant enzyme responsible for ROS scavenging and its expression was higher in tolerant plants compared with sensitive ones [31–33]. Glutathione S-transferase (GST) reduces hydroperoxides produced during oxidative stress and was upregulated in drought-tolerance-enhanced transgenetic poplar [34,35]. Basic helix–loop–helix (bHLH) transcription factors are involved in plant growth and development, secondary metabolite biosynthesis, photomorphogenesis, signal transduction, and stress response. *bHLH*-gene-overexpressed poplar show higher resistance to drought [36–38]. Photosystem II subunit X (PsbX) protein maintains efficient electron transport in PS II and safeguards PSII integrity for photosynthesis [39–41]. It is sensitive to stresses and greater PsbX expression, the greater integrity of PSII, and higher photosynthesis capacity, even facing stress [42].

Populus, a dioecious tree with a fully sequenced genome, is a typical model for surveying both physiological and genetic sexual-specific response to stress in woody plants [43–45]. Previous studies have revealed different responses between female and male plants in many native poplars to various stresses [28,46–51]. Nevertheless, these poplars investigated in these studies were natural populations or cuttings (clones) from either male or female plants. The different responses between these native sexes developed under sex-specific evolutionary selection from several decades to million years, which means that the genomes between native female and male poplars are greatly different in both sex chromosomes and autosomes. However, the female and male poplar sibs, which are hybrids from the

same pair of parents, have been selected in only several years and are much more similar between females and males in morphological, physiological, and biochemical traits and gene expression, except for the sexes [9,10]. Here, to determine whether and what sexual dimorphism is present in artificial hybrids, the sibs of *P.* × *euramericana*, the female line 'Nanlin-895', and the male line 'Nanlin-1388', were chosen for a direct or integrated study of morphological variability, alteration of physiological and biochemical parameters, gene expression analysis, etc., under water deficit conditions.

2. Materials and Methods

2.1. Plant Materials and Water Deficit Treatment

The stalks of brother and sister sibling *P.* × *euramericana* were collected from the clones 'Nanlin-895' (*P.* × *euramericana* cv. 'Nanlin-895') and 'Nanlin-1388' (*P.* × *euramericana* cv. 'Nanlin-1388'), respectively, which are brother–sister sibs of the maternal clone 'I-69' (*P. deltoides* Bartr. cv. 'Lux') and the paternal clone 'I-45' (*P.* × *euramericana* (Dode) Guineir cv. 'I-45/51'). Stalks were planted in 5 L plastic pots filled with 3 kg of homogenized soil and 4 g of slow-release fertilizer (N:P:K = 13:10:14). Following two months of growth, eighteen male and eighteen female seedlings, similar in both stature and height, were chosen for the water deficit study. The seedlings were grown in a greenhouse with a glass shelter at Anhui Normal University in Wuhu, China. The experimental design was completely randomized with two factors: sex and water deficit stress. Water deficit treatment was set at three levels: control (70%–80% of soil water holding capacity (SWHC)), moderate water deficit stress (50%–60% of SWHC), and severe water deficit stress (30%–40% of SWHC). Each treatment involved six male and six female seedlings (three biological replicates per sex with two cuttings per biological replicate). To maintain the soil water content, each of the seedlings was watered with an adjustable volume water into the pots according to Li et al. [52]. The treatment was ongoing for three months from the 1 June to the 1 September 2019.

2.2. Morphology and Photosynthesis Assay

Along with the treatments, the seedlings' heights were measured every two days, and the shoot basal diameters (diameters of the boles at the soil surface) were measured every ten days. The growth curves for both height and basal diameter were generated using the binomial regression method in the SPSS 23.0 package (Chicago, IL, USA). At the end of the treatments, photographs of the plants were taken. Four cuttings from each treatment and sex were randomly selected for gas exchange rate assays. Light response curves were generated on the third or fourth fully expanded leaf. The parameters measured included the net photosynthesis rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration rate (E), primary photochemical efficiency of PSII (Fv/Fm), sum of the quantum yields of PSII photochemistry (Φ_{PSII}), photochemical quenching (qP), and non-photochemical quenching (NPQ). These measurements were obtained using the Li-COR 6400 system (LI-COR, Inc., Lincoln, NE, USA) under the following conditions: leaf temperature of 25 ± 2 °C, photosynthetic photon flux (PPF) of 1400 μmol m^{-2} s^{-1}, relative air humidity of 70%, and ambient CO_2 concentration of 400 μmol mol^{-1}. Light response curves were generated using PPF values of 0, 100, 250, 500, 750, 1000, 1200, 1300, 1400, 1500, 1600, and 1800 μmol m^{-2} s^{-1}, at an ambient CO_2 concentration of 400 μmol mol^{-1}, leaf temperature of 25 ± 2 °C, and relative air humidity 70%. Gas exchange parameters and light response were measured between 10:00 a.m. and 1:00 p.m. The modified rectangular hyperbola model was used to generate a regression analysis for light response data, and the corresponding formula is as follows [53,54]:

$$A = \phi PAR \frac{1 - \beta\phi}{1 + \gamma\phi} - R_d$$

where φ represents the initial apparent quantum efficiency, PAR refers to photosynthetic photon flux density, R_d stands for the rate of dark respiration, and β and γ represent the

corresponding coefficients. The maximum net photosynthetic rate (A_{max}) can be calculated using the following formula:

$$A_{max} = \phi\left(\frac{\sqrt{\beta+\gamma}-\sqrt{\beta}}{\gamma}\right) - R_d$$

Nonlinear regression analysis was performed using the SPSS version 23.0 (Chicago, IL, USA) software to generate the light response curve regression.

2.3. Chlorophyll Pigment Content Assay

To determine the chlorophyll pigment content, leaves from cuttings of each gender and treatment were cut into approximately 0.2 cm strips. About 0.1 g of each sample was then ground using a mortar and pestle with 5 mL of 80% acetone, and the mixture was transferred to a 50 mL Falcon tube, with the mortar and pestle being washed several times using 80% acetone. A total of 25 mL of mixture was obtained by adding 80% acetone. The mixture was incubated in darkness at room temperature for 12 h, with gentle stirring every 4–5 h. After filtration with cheese cloth, the absorbance of the extractive was measured at 645 and 663 nm with a spectrophotometer (SMA5000, Merinton, StellarNet, Inc., Tampa, FL, USA). The pigment contents were calculated using the follows [55]:

$$\begin{aligned} Chla &= 12.25 * A_{663} - 2.79 * A_{645} \\ Chlb &= 21.50 * A_{645} - 5.10 * A_{663} \\ TC &= 7.15 * A_{663} + 18.71 * A_{645} \end{aligned}$$

where *Chl a*, *Chl b*, and *TC* represent chlorophyll a, chlorophyll b, and total chlorophyll contents, respectively, and A_{645}, A_{663} are the absorbance values of the extracted solution at 645 and 663 nm, respectively. The unit of chlorophyll is expressed in mg mL^{-1}.

2.4. Relative Electrolyte Leakage Assay

The relative electrolyte leakage (REL) was measured according to the method described by Liao et al. [56]. Leaves from four cuttings of each sex and treatment were selected and washed with deionized water several times. Leaf discs (0.3 g) were prepared by avoiding the main veins and then incubated at room temperature for 30 min with gentle shaking every several minutes. The electrical conductivity (C1) of the bathing solution was measured using a conductivity detector (DDB-303A, INESA Analytical Instrument Co., Ltd., Shanghai, China). The glass tubes containing the bathing solution and leaf discs were then boiled for 10 min and allowed to cool to room temperature. The electrical conductivity (C2) of the boiled solution was measured, and the relative electrolyte leakage was calculated as follows:

$$\mathrm{REL}(\%) = (\mathrm{C1}/\mathrm{C2}) * 100$$

where C1 is the electrical conductivity of the bathing solution before boiling, and C2 is the electrical conductivity of the boiled solution. The REL is expressed as a percentage.

2.5. ROS Scavenging Enzyme Activity Assay

Four fully expanded leaves were randomly chosen from each replicate to conduct the ROS scavenging enzyme activity assay. The extraction of superoxide dismutase (SOD: EC 1.15.1.1) and peroxidase (POD: EC 1.11.1.7) was carried out according to a previously published method, and the activity was measured [57]. To extract the enzymes, 0.5 g of fresh leaves was ground in 5 mL of iced 50 μM phosphate buffer (pH 7.8) containing 1% *w/v* polyvinyl pyrrolidone (PVP) and then centrifuged at 12,000× *g*, 4 °C for 15 min. After that, 10 μL of the supernatant was mixed with 4 mL of reaction system (50 μM phosphate buffer (pH 7.8), 77.12 μM nitroblue tetrazolium chloride (NBT), 13.37 mM methionine, 0.1 mM ethylene diamine tetraacetic acid (EDTA), and 80.2 μM riboflavin). The reaction was initiated by illuminating the mixture with a white fluorescent lamp

(4000 Lux). After 20 min of illumination, the absorbance at 560 nm was measured using a UV spectrophotometer (SMA5000, Merinton). A negative control system without enzymes was used for baseline measurements. One unit activity of SOD (U) was defined as the amount of enzyme necessary to inhibit 50% of NBT reduction [58]. The POD activity was initiated by adding 20 µL of the supernatant to the POD reaction system which contained 50 µM of phosphate buffer (pH 6.0), 50 µM of guaiacol, and 50 µM of H_2O_2, and mixing by inverting and righting the tube three times. The rate of absorbance change at 470 nm was monitored immediately with a UV spectrophotometer (SMA5000, Merinton) for three minutes. POD activity was defined as the ability to convert guaiacol to tetraguaiacol and was evaluated from the change in absorbance value per minute [59].

2.6. Malondialdehyde Content Assay

To assess the level of membrane lipid peroxidation, the malondialdehyde (MDA) content was determined following a previously established method [23]. For each treatment, four fully expanded leaves were randomly selected, and 0.5 g of fresh leaves was homogenized in an ice bath using 5 mL of phosphate buffer (pH 7.8). The homogenate was then centrifuged at 12,000× g for 20 min at 4 °C. Next, 1 mL of the supernatant was mixed with 2 mL of the reaction mixture containing 0.6% (w/v) TBA and 10% (v/v) TCA; then, the mixture was incubated in boiling water for 15 min and quickly cooled in an ice bath. Subsequently, the mixture was centrifuged at 12,000× g for 10 min and the absorbance of the supernatant was measured at 450, 532, and 600 nm using a UV spectrophotometer (SMA5000, Merinton). Finally, the MDA content was calculated:

$$C(\mu M) = 6.45 * (A_{532} - A_{600}) - 0.56 * A_{450}$$

where A_{450}, A_{532}, and A_{600} denote the absorption of the supernatant at the wavelengths of 450 nm, 532 nm, and 600 nm respectively.

2.7. RNA Extraction and qRT-PCR Assay

Poplar leaves were subjected to total RNA extraction using the Trizol Total RNA Extractor Kit (Sangon Biotech, Shanghai, China) following the manufacturer's instructions. Subsequently, complementary DNA (cDNA) was synthesized using the Monscript™ RTIII Super Mix Kit (Monad Biotech Co., Ltd., WuHan, China) according to the manufacturer's guidelines. The primers for *SOD*, *GST*, *bHLH35*, and *PsbX1* transcript were developed using SnapGene software 2.3.2 and are listed in Supplementary Table S4. PCR was performed with the MonAmp™ ChemoHS qPCR Mix Kit (Monad Biotech Co., Ltd. WuHan, China) on Roche LightCycler™ 96, using SYBRGreen as the fluorescent detection dye. The internal control used was UBQ, and the $2^{-\Delta\Delta CT}$ method was employed for determining the relative expression of each gene [60]. The entire experiment was conducted in triplicate to ensure the accuracy of the results.

2.8. Statistical Analysis

To test the different responses, data of growth, gas exchange, chlorophyll pigment content, chlorophyll fluorescence, REL, MDA content, ROS enzyme activities, and gene expression were compared between the sexes and among the treatments using generalized linear models in IBM SPSS 23.0 package (Chicago, IL, USA). Two-way analysis of variance (ANOVA) with a post hoc Duncan multiple comparison was used for statistically significantly differing means at a $p < 0.05$ level. All data were tested for, and validated to be, both normally distributed and with a homogeneity of variance before comparisons were performed.

To assess and compare the composite different response between female and male siblings, the principal component analysis (PCA) was carried out based on the growth, photosynthetic and biochemical parameters, and gene expression of *P.* × *euramericana* siblings. Prior to PCA, the correlation between the traits was examined and is documented

in Supplemental Table S5. The PCA analysis was conducted using SIMCA 13.0 software (Umetrics AB, Umea, Sweden).

3. Results

3.1. Water Deficit Had Different Effects on Growth, Net Photosynthesis, and Chlorophyll Fluorescence in Female and Male Poplar Sibs

Water deficit induced more serious leaf curling and chlorosis in females than in males (Figure 1A). The height and basal diameter increments were significantly reduced in both sexes by water deficit, and the reduction was more severe in females' basal diameter than that in males under severe water deficit (Figure 1B,D, Table S1). Furthermore, water deficit resulted in a greater depression in female height and basal diameter growth than in males (Figure 1C,E, Table S1). Water deficit decreased *A* in both female and male sibs and this decrease was statistically significantly greater in females (Table 1). However, other gas exchange parameters such as *gs*, *Ci*, and *E* were not greatly affected by water deficit and showed no significant difference between sexes of poplar cuttings (Table S2). Moreover, *Fv/Fm*, Φ_{PSII}, and *qP* were all statistically significantly lower in female sibs than in male sibs under water deficit conditions (Table 1).

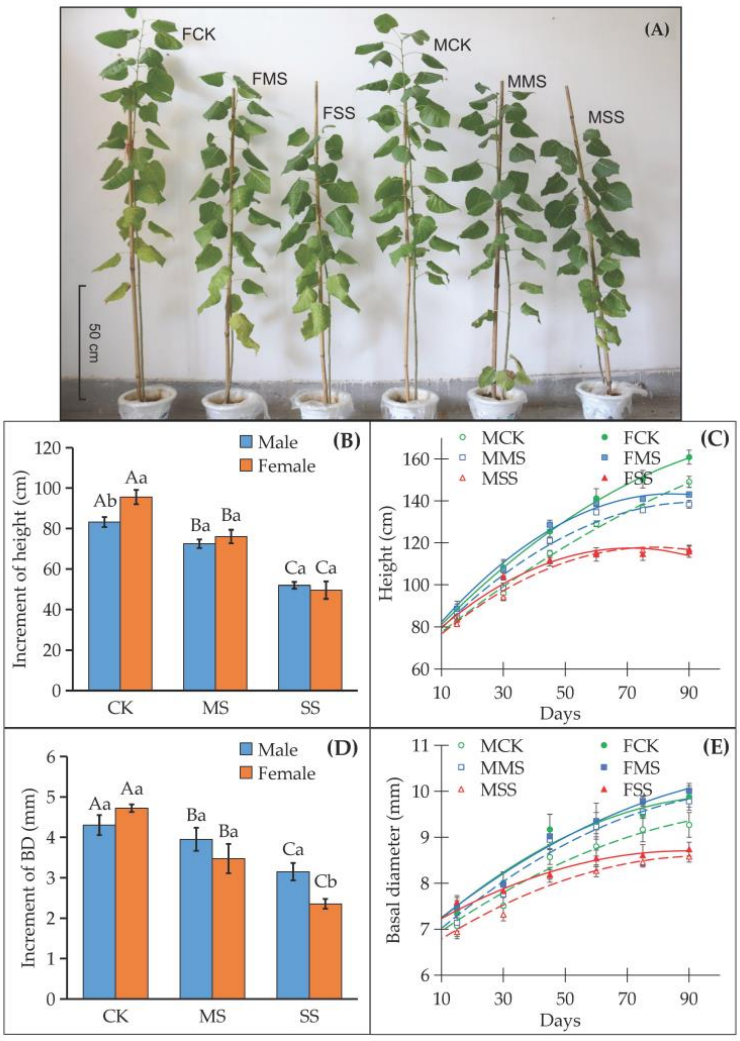

Figure 1. Phenotypic symptom and growth of female and male sibs of *P.* × *euramericana* under different irrigation conditions. (**A**) Phenotypic differences between sib lines; (**B**) increment of height growth; (**C**) height growth curves; (**D**) increment of basal diameter; (**E**) basal diameter growth curves.

MCK and FCK, male and female lines under control treatment (70%–80% of SWHC); MMS and FMS, male and female lines under moderate water deficit stress (50%–60% of SWHC); MSS and FSS, male and female lines under severe water deficit stress (30%–40% of SWHC). Different uppercase letters above the bars denote significant differences among the control and water-deficit-treated female and male lines separately, and different lowercase letters denote significant differences between the sexes of each treatment at the level of $p \leq 0.05$ according to Duncan post hoc tests. BD, basal diameter. Values are means ± SE ($n \geq 3$).

Table 1. Photosynthesis and chlorophyll fluorescence parameters of female and male *P.* × *euramericana* sibs under different irrigation conditions.

Parameters		*A* (μmol m^{-2} s^{-1})	*Fv/Fm*	Φ_{PSII}	*qP*
Male	CK	9.976 ± 0.208 Aa	0.824 ± 0.002 Aa	0.057 ± 0.003 Aa	0.116 ± 0.005 Aa
	MS	9.135 ± 0.391 ABa	0.823 ± 0.003 Aa	0.047 ± 0.001 Ba	0.096 ± 0.001 Ba
	SS	8.008 ± 0.752 Ba	0.808 ± 0.003 Ba	0.036 ± 0.001 Ca	0.075 ± 0.002 Ca
Female	CK	8.367 ± 0.562 Ab	0.825 ± 0.002 Aa	0.053 ± 0.001 Aa	0.116 ± 0.004 Aa
	MS	6.945 ± 0.494 ABb	0.817 ± 0.002 Ba	0.039 ± 0.001 Bb	0.083 ± 0.003 Bb
	SS	5.897 ± 0.676 Bb	0.799 ± 0.002 Cb	0.026 ± 0.001 Cb	0.050 ± 0.004 Cb
P-level	*P*stress	0.001	0.000	0.000	0.000
	*P*sex	0.000	0.018	0.000	0.000

Note: *A*, net photosynthesis rate; *Fv*/*Fm*, maximal PSII quantum yield; Φ_{PSII}, quantum yield in PSII; *qP*, photochemical quenching parameter. CK, control treatment (70%–80% of SWHC); MS, moderate water deficit stress (50%–60% of SWHC); SS, severe water deficit stress (30%–40% of SWHC). Within a column, different uppercase letters following values denote significant differences among the control and water-deficit-treated female and male lines separately, and different lowercase letters denote significant differences between the sexes of each treatment according to Duncan posthoc tests. P_{stress}, water deficit treatment effect; P_{sex}, sex effect. Values are means ± SE ($n \geq 3$).

3.2. Female and Male Poplar Sibs Responded Differently to Light and the Chlorophyll Content Was Reduced Differently by Water Deficit

Female and male hybrids showed different light response curve, especially under water deficit conditions (Figure 2). Regression analysis using the modified rectangular hyperbola model showed that the regressed *A* in males was higher than in females when the photosynthetic photon flux increased to above 500 μmol m^{-2} s^{-1}, and this difference was more pronounced under water deficit conditions. The coefficients of the fitted curves are presented in Table S3. Furthermore, *Amax*, regressed by the modified rectangular hyperbola model, was reduced in both males and females under water deficit stress, with a more noticeable reduction in females than that in males under severe water deficit (Table 2). Additionally, the chlorophyll a, b, and total chlorophyll content decreased in both female and male sibs under water deficit. The chlorophyll content was lower in females than males under both control and water deficit conditions (Table 2).

Table 2. The maximum net photosynthetic rate and chlorophyll pigments content of female and male *P.* × *euramericana* under different irrigation conditions.

Parameters		*Amax* (μmol m^{-2} s^{-1})	*Chl a* (mg mL^{-1})	*Chl b* (mg mL^{-1})	*TC* (mg mL^{-1})
Male	CK	11.045 ± 0.501 Aa	26.086 ± 0.194 Aa	47.599 ± 0.358 Aa	73.686 ± 0.552 Aa
	MS	9.473 ± 0.195 Ba	24.721 ± 0.176 Ba	45.086 ± 0.328 Ba	69.806 ± 0.504 Ba
	SS	7.444 ± 0.635 Ca	21.290 ± 0.139 Ca	38.787 ± 0.255 Ca	60.077 ± 0.394 Ca
Female	CK	10.684 ± 0.400 Aa	23.134 ± 0.261 Ab	42.155 ± 0.478 Ab	65.289 ± 0.738 Ab
	MS	8.522 ± 0.371 Ba	18.008 ± 0.601 Bb	32.802 ± 1.096 Bb	50.810 ± 1.697 Bb
	SS	6.059 ± 0.084 Cb	12.382 ± 0.399 Cb	22.569 ± 0.706 Cb	34.951 ± 1.105 Cb
P-level	*P*stress	0.019	0.000	0.000	0.000
	*P*sex	0.000	0.000	0.000	0.000

Note: A_{max}, maximum net photosynthetic rate; *Chl a*, chlorophyll a content; *Chl b*, chlorophyll b content; and *TC*, total chlorophyll content. CK, control treatment (70%–80% of SWHC); MS, moderate water deficit stress (50%–60% of SWHC); SS, severe water deficit stress (30%–40% of SWHC). Within a column, different uppercase letters following values denote significant differences among the control and water-deficit-treated female and male lines separately, and different lowercase letters denote significant differences between the sexes of each treatment according to Duncan post hoc tests. The *p* values for water deficit, sex, and their combined effects are denoted. P_{stress}, water deficit treatment effect; P_{sex}, sex effect. Values are means ± SE ($n \geq 3$).

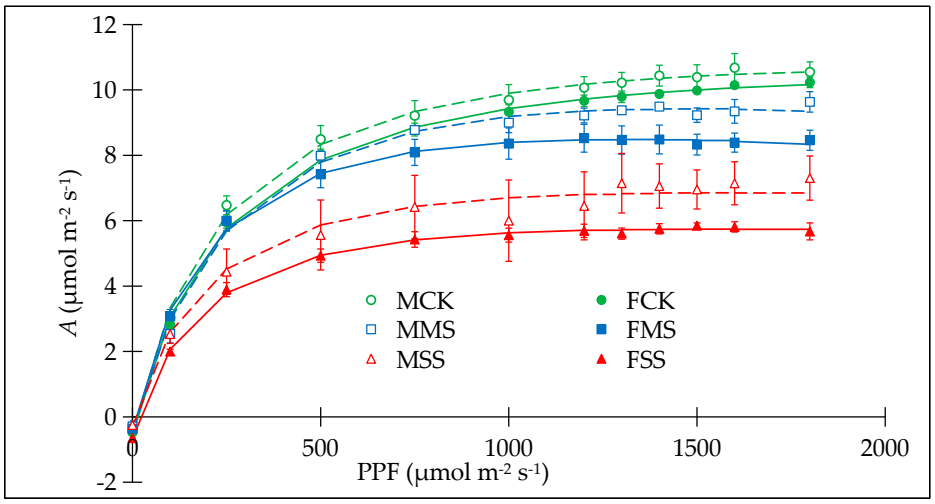

Figure 2. Light response curves of female and male *P.* × *euramericana* hybrids under different irrigation conditions. MCK and FCK, male and female lines under control treatment (70%–80% of SWHC); MMS and FMS, male and female lines under moderate water deficit stress (50%–60% of SWHC); MSS and FSS, male and female lines under severe water deficit stress (30%–40% of SWHC). Values are means ± SE (n = 3).

3.3. Water Deficit Damaged Cell Membrane Differently and Induced MDA Contents Differently between Female and Male Sibs

The parameter REL is used to measure plant membrane damage, and its analysis was conducted on both female and male *P.* × *euramericana* under different irrigation conditions. It was observed that the water deficit resulted in an increase in REL in both males and females. Additionally, when subjected to severe water deficit conditions, the REL levels were significantly higher in females than in males (Figure 3A). Another key observation was related to MDA, which is the final product of lipid oxidation caused by ROS and is known to be harmful to other macromolecules within the cell. The content of MDA was examined in female and male *P.* × *euramericana* under different irrigation conditions. It was found that after water deficit treatments, MDA contents increased in both sexes (Figure 3B). Notably, under severe water deficit conditions, the MDA content in females was significantly higher than that in males (as shown in Figure 3B).

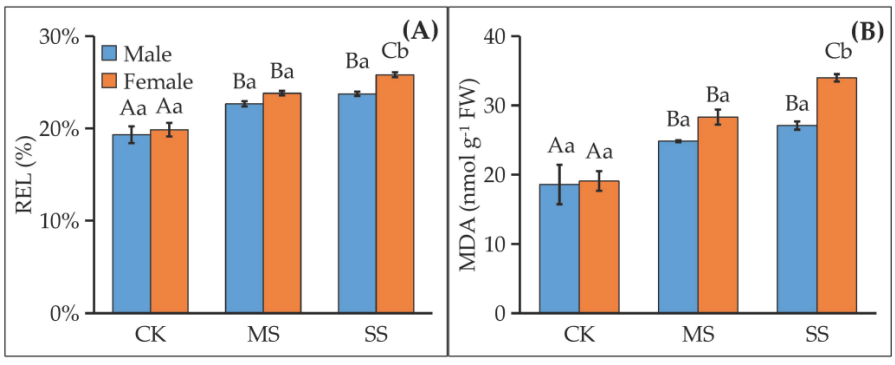

Figure 3. Relative electrolyte leakage (**A**) and MDA content (**B**) of female and male *P.* × *euramericana* lines under different irrigation conditions. CK, control treatment (70%–80% of SWHC); MS, moderate water deficit stress (50%–60% of SWHC); SS, severe water deficit stress (30%–40% of SWHC). Different uppercase letters above the bars denote significant differences among the control and water-deficit-treated female and male lines separately, and different lowercase letters denote significant differences between the sexes of each treatments according to at the level of $p \leq 0.05$ according to Duncan post hoc tests. Values are means ± SE ($n \geq 3$).

3.4. Water Deficit Induced Different Activities of ROS Scavenging Enzymes

It is widely recognized that a plant's ability to scavenge ROS is positively correlated with its resistance to stress. Therefore, in this study, the activities of ROS scavenging enzymes, SOD and POD, were measured in female and male cuttings subjected to different irrigation treatments. As anticipated, the enzyme activities in control females and males were quite similar. However, the response to water deficit treatment was distinct between the two genders. Specifically, the activities of SOD (Figure 4A) and POD (Figure 4B) were significantly higher in males following exposure to water deficit.

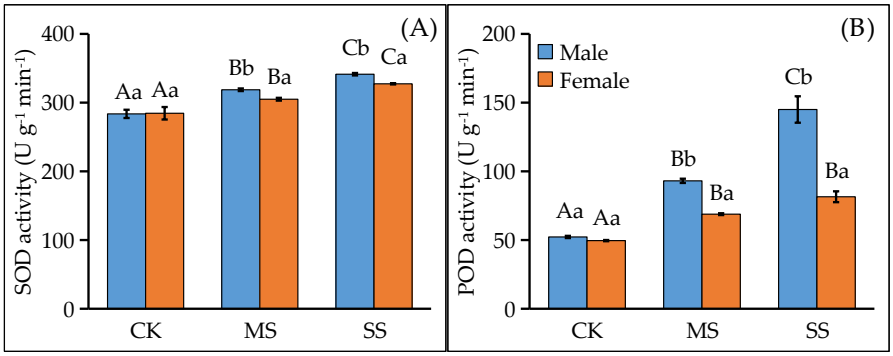

Figure 4. Activities of SOD (**A**) and POD (**B**) of female and male *P.* × *euramericana* lines under different irrigation conditions. CK, control treatment (70%–80% of SWHC); MS, moderate water deficit stress (50%–60% of SWHC); SS, severe water deficit stress (30%–40% of SWHC). Different uppercase letters above the bars denote significant differences among the control and water-deficit-treated female and male lines separately, and different lowercase letters denote significant differences between the sexes of each treatments according to at the level of $p \leq 0.05$ according to Duncan post hoc tests. Values are means ± SE (n = 3).

3.5. Water Deficit Regulated Stress-Related Gene Expression Differently in Male and Female Poplar Hybrids

In order to clarify the different regulation patterns in response to water deficit, we investigated the expression of four stress-related genes in female and male *P.* × *euramericana* sibs. It was found that under moderate water deficit conditions, the expression of *SOD* and *GST* was significantly higher in females than in males (Figure 5A,B). However, under severe water deficit, *SOD* expression was higher in male sibs (Figure 5A). The gene *bHLH35* was upregulated in females under water deficit stress but downregulated in males, and the expression level in females was higher than that in males under both moderate and severe water deficit (Figure 5C). In contrast, *PsbX1* gene was downregulated in females but upregulated in males in response to water deficit (Figure 5D).

3.6. PCA Displayed Comprehensively Different Responses between Male and Female Sibs to Water Deficit

To gain a comprehensive understanding of the differential responses to water deficit between female and male sibs of *P.* × *euramericana*, we performed a principal component analysis (PCA) based on growth, physiological and biochemical parameters, and gene expression data of stress-related genes after different irrigation treatments (Figure 6, Table S6). The first principal component (PC1), which accounted for 58.6% of the total variation, was identified as the stress axis, effectively separating the control group from the water deficit treatment groups, except for the moderate-water-deficit-treated males in the control group. The second principal component (PC2), accounting for 19.4% of the total variation, was identified as the sex axis. PC2 separated the male and female sibs into upper and lower regions, except for MCK3, which was located in the lower region (Figure 6A). The weights of the variables' contribution to PCA revealed that the distribution of REL, MDA content, and NPQ aligned with the direction of severe-water-deficit-treated females, whereas the distribution of SOD activity and gene expression of *GST* and *bHLH35* (in Figure 6B) aligned

with the direction of severe-water-deficit-treated males (Figure 6). Other variables, such as gas exchange, chlorophyll content, and growth, contributed to PCA in distinguishing control from water-deficit-treated samples (Figure 6B).

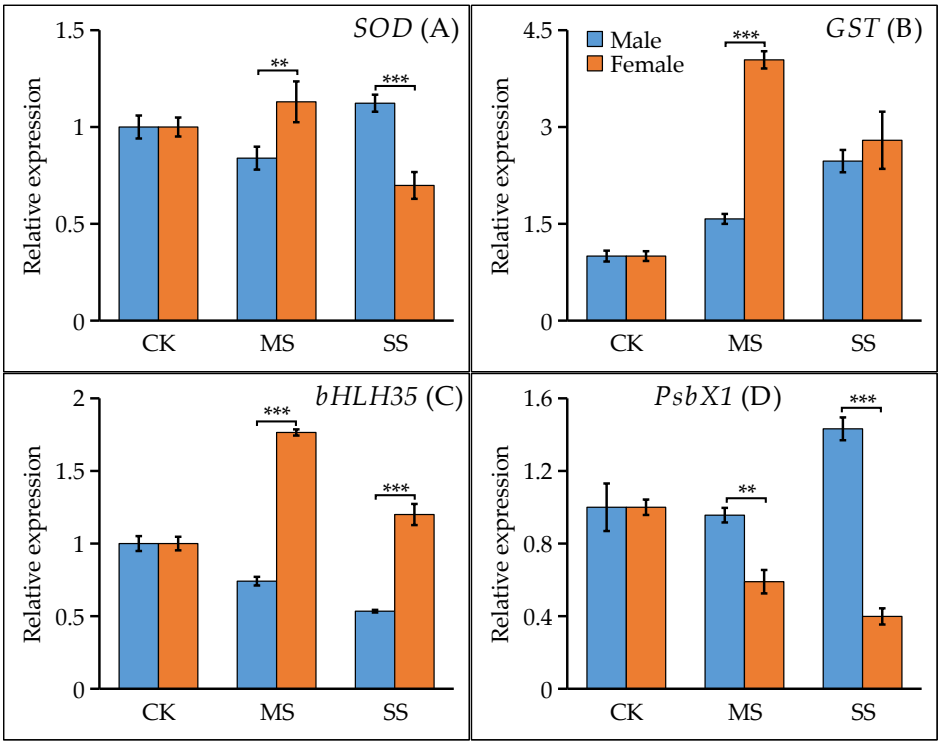

Figure 5. Expression of stress-related genes in female and male *P.* × *euramericana* sibs under different irrigation conditions. (**A**) *SOD*, (**B**) *GST*, (**C**) *bHLH35*, (**D**) *PsbX1*. CK, control treatment (70%–80% of SWHC); MS, moderate water deficit stress (50%–60% of SWHC); SS, severe water deficit stress (30%–40% of SWHC). Duncan post hoc tests at the following levels, **: $0.001 < p < 0.01$; ***: $p < 0.001$. Values are means ± SE ($n \geq 3$).

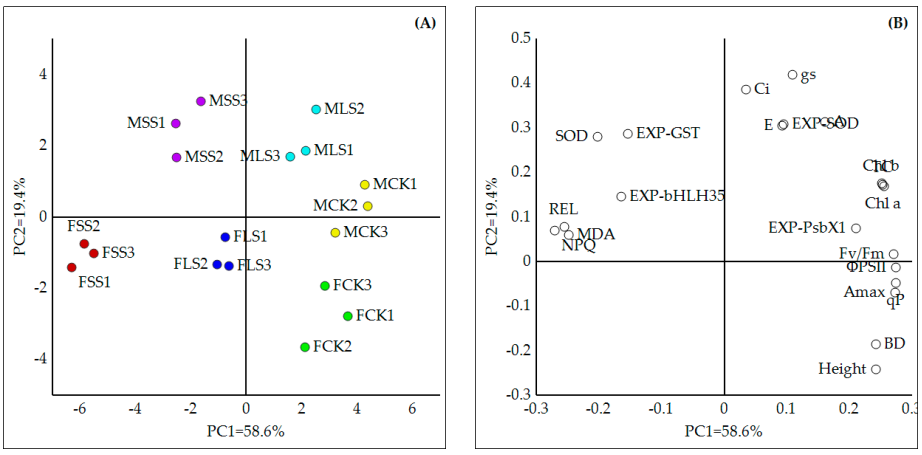

Figure 6. PCA of physiological, biochemical, and the gene expression parameters response to water deficit of female and male *P.* × *euramericana* lines under different irrigation conditions. (**A**) Score scatter plot and (**B**) loading scatter plot of all parameters. MCK and FCK, male and female lines under control treatment (70%–80% of SWHC); MMS and FMS, male and female lines under moderate water deficit stress (50%–60% of SWHC); MSS and FSS, male and female lines under severe water deficit stress (30%–40% of SWHC); EXP with hyphen in front of each gene in (**B**) indicates gene expression value.

4. Discussion

We present evidence of sexual dimorphism in the response to water deficit in artificially bred female and male poplar siblings. Our study investigated the physiological, biochemical, and gene regulation responses of female and male poplar lines to water deficit, and the results indicated that artificially bred male poplar siblings were stronger in resisting water deficit due to more effective ROS scavenging over female siblings.

4.1. Females Were More Vulnerable to Water Deficit in Growth and Photosynthesis Than Males

Water deficit causes plant yield loss through stunted plant growth or even mortality [14,61,62]. Plants that are more resistant to drought are typically able to maintain better growth under stress conditions [11]. Studies have shown that female plants tend to suffer more in terms of growth than males under suboptimal environmental conditions [10,56,63,64]. This sexual bias in growth response may be the result of millions of years of natural and sexual selection [65]. However, dioecious populations that have been artificially bred do not have the benefit of millions of years of selection and are, therefore, more similar in their secondary sexual traits [9,10]. Despite this, we observed sexually dimorphic responses to water deficit in the growth of female and male hybrid poplars in our study.

One mechanism that may contribute to growth depression under water deficit is the reduction in photosynthesis. Stress conditions are known to inhibit photosynthesis by decreasing the net photosynthesis rate and the capacity for photosynthesis, as well as reducing light use efficiency [12,66]. Our results show that water deficit significantly reduced the photosynthetic capacity of *P.* × *euramericana* sibs, and this inhibition was more pronounced in females under severe water deficit stress. This result is consistent with previous findings in natural dioecious plants but has never been found before in artificially bred sibling plants [27,49]. Chlorophyll fluorescence parameters have been used to examine photosynthetic performance under stress conditions [67–70]. A previous study found that freezing reduced Fv/Fm, Φ_{PSII}, and qP in *P. euphratica*, but these parameters were higher in transgenic lines with higher tolerance compared to wild lines under freezing conditions [71]. Our results showed lower Fv/Fm, Φ_{PSII}, and qP in female sib *P.* × *euramericana* compared to males, suggesting that the maximal and actual quantum yield of PSII in females was lower than in males during water deficit. This result is consistent with the transgenic study and natural population observations, indicating that males can acquire efficient photosynthesis ability even under water stress conditions [71,72].

4.2. Female Was Damaged More Severely by Water Deficit Due to Its Weaker ROS Scavenging Enzyme Activities

The cell membrane is highly susceptible to stress, and its integrity and stability are key indicators of a plant's ability to tolerate stress [73,74]. Two important markers of damage caused by stress in plants are REL and MDA content, which are products of membrane lipid peroxidation [74,75]. Higher membrane permeability, as measured by REL, is positively correlated with cell membrane injury induced by drought [74,76]. In the present study, REL was found to be elevated in both sexes of *P.* × *euramericana* under water deficit, with higher levels observed in females. Similarly, higher levels of MDA were observed in female poplar lines than in males under severe water deficit. This suggests that the cell membrane is more intact in artificially bred male poplar lines during water deficit, in line with previous studies of natural dioecious plants [48,56,77]. Excessive production of ROS under stress can harm cellular macromolecules, raise REL, and lead to excess MDA accumulation, which ultimately result in plant death [13,17,19]. To combat this, plants possess enzymatic systems, including superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and glutathione reductase, that scavenge ROS and provide protection against oxidative stress [19,78]. SODs, the first defensive line against ROS, convert superoxide into stable H_2O_2, whereas PODs detoxify H_2O_2 to H_2O [13,17,78]. Previous studies have found that drought led to a rapid increase in ROS in plant cells and a subsequent rise in SOD and

POD activity [19,72,79,80]. In the present study, SOD and POD activity increased under water deficit conditions in both sexes of poplar, indicating activation of the enzymatic system to avert ROS damage. Notably, the activities of SOD and POD were significantly higher in males than in females under severe water deficit, suggesting that males are more efficient at converting superoxide into H_2O_2 and then detoxifying them through POD.

4.3. Water Deficit Regulated Genes Encoding Stress-Related Proteins Differently in Female and Male Lines of *P.* × *euramericana*

Plants regulate gene expression to respond to and resist stresses. Different regulatory patterns of genes which encode stress-related proteins endow plants with different levels of stress resistance. For example, in poplar, the *SOD* gene, which affects the balance of ROS, is upregulated by salt and drought stress [34]. In tetraploid *Poncirus trifoliata*, the tolerant genotype has higher levels of *SOD* gene expression than its diploid progenitors under drought stress [32]. In our research, the *SOD* gene was expressed higher in male poplar than that in females under water deficit conditions, and its transcript levels correlated with higher SOD activity in males. These results suggest that male poplar may possess more effective ROS scavenging abilities than their female siblings. *GST* genes confer plant tolerance by increasing the activities of enzymes that scavenge ROS to maintain ROS homeostasis and decrease cellular damage [81–83]. Previous research has demonstrated that improved GST activity enhances drought tolerance in *P.* × *euramericana* by eliminating excess ROS [34]. Similarly, in female *P.* × *euramericana* under moderate water deficit conditions, *GST* expression was significantly higher than in male siblings, indicating females have higher sensitivity to stress. Despite the sensitive response to water deficit, the final SOD and POD activity levels were lower in females, indicating weaker ROS scavenging capacities. bHLH genes participate in various biological processes, including plant stress responses [36,84]. *bHLH* genes promote plant tolerance to drought by regulating photosynthesis, ROS scavenging, and growth [37,85]. Similar to the *GST* expression pattern, in male *P.* × *euramericana*, the expression of *bHLH35* was lower than in females under water deficit conditions. We attribute these results to the fact that females showed a more sensitive response to water deficit, whereas males exhibited higher levels of photosynthesis, growth, and ROS scavenging, thus indicating that males are more tolerant to water deficit than females. PsbX, a low-molecular-weight protein in PSII, is a key component affecting the integrity of PSII and subsequently regulating photosynthesis [39–41]. In both female and male cuttings of poplar siblings, the expression of *PsbX1* increased significantly under water deficit conditions. Importantly, the degree of upregulation in males was significantly higher than females. These results are consistent with the physiological results of higher *A* and *Fv*/*Fm* ratio in water-deficit-treated males. Physiological and photosynthesis gene expression results indicate that male poplar siblings possess a higher photosynthesis capacity than their female siblings under water deficit conditions, likely due to higher PSII integrity and greater protection in males under water deficit conditions.

4.4. PCA Showed an Overall Difference in Water Deficit Response between Female and Male Poplar Sibs

PCA is a powerful tool for analyzing large datasets as it depicts the relationships among variables and observations. It enables us to identify which variables contribute unique or similar information to the model [86]. In our study, PCA revealed that males and females responded differently to water deficit. Principal component 1 (PC1) clearly separated control and water-deficit-treated lines, except for the moderate-water-deficit-treated males in the control samples, suggesting that females were more sensitive to water deficit than males. The second component, PC2 separated males from females under both control and water deficit conditions. However, the separation between water-deficit-treated males and females was more pronounced than that of the control-treated lines. These results confirmed that female and male poplar siblings showed striking differences in physiological processes and gene regulation under stress conditions. The loading plot demonstrated the variables' contribution to the PCA of observations. The distribution

of REL, MDA content, and *NPQ* was in line with the distribution of severe water deficit females in the score plot. In contrast, gas exchange parameters grouped moderate-water-deficit-treated males into control-treated poplar. These results align with previous studies showing native females' higher sensitivity to stress than males [49,57,87].

5. Conclusions

Our findings indicate that male and female *P.* × *euramericana* siblings show different responses to water deficit, which is especially evident in artificially cultivated female and male pairs. Females exhibit greater inhibition of growth and photosynthesis, with higher REL and MDA levels indicating more severe damage caused by water deficit. In contrast, males show higher levels of SOD and POD activity, as well as greater expression of the *SOD* and *PsbX1* genes, suggesting that they are better equipped to handle water deficit by more effectively scavenging ROS. These results confirm that sexually dimorphic response to stress is present in both natural and artificially cultivated dioecious plants. Based on our findings and the work of others, we suggest examining the correlation between reduced growth and survival of stress in sibling plants. While our research shows that males may maintain better growth during drought stress for various reasons, it is possible that female plants may be less susceptible to death in the long run due to their ability to reduce their growth and conserve energy in times of stress. It is important to reconsider the consequences of response to different degrees of stresses and the short-term/long-term consequences of stress resistance in plants. Furthermore, more research should be conducted to investigate whether female plants are hedging their long-term survival by responding poorly to drought stress and limiting their growth. This may lead to a paradigm shift in scientists' understanding of plant adaptation to stress and open the door for further investigation into the survival strategies of dioecious plants under stress. Moreover, identifying and characterizing genes that influence this sex-specific response to water deficit stress would be an invaluable pursuit for future studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14050995/s1, Table S1: Formulas of height and basal diameter growth fitting curves; Table S2: Gas exchange parameters; Table S3: Coefficients of light response curves; Table S4: Primers used in fluorescence quantitative PCR; Table S5: Correlation matrix of parameters in all samples for PCA. Table S6: Variables used in PCA.

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References

1. Renner, S.S. The relative and absolute frequencies of angiosperm sexual systems: Dioecy, monoecy, gynodioecy, and an updated online database. *Am. J. Bot.* **2014**, *101*, 1588–1596. [CrossRef] [PubMed]
2. Bachtrog, D.; Mank, J.E.; Peichel, C.L.; Kirkpatrick, M.; Otto, S.P.; Ashman, T.-L.; Hahn, M.W.; Kitano, J.; Mayrose, I.; Ming, R.; et al. Sex Determination: Why So Many Ways of Doing It? *PLoS Biol.* **2014**, *12*, e1001899. [CrossRef] [PubMed]
3. Zemp, N.; Tavares, R.; Muyle, A.; Charlesworth, D.; Marais, G.A.B.; Widmer, A. Evolution of sex-biased gene expression in a dioecious plant. *Nat. Plants* **2016**, *2*, 16168. [CrossRef] [PubMed]
4. Henry, I.M.; Akagi, T.; Tao, R.; Comai, L. One hundred ways to invent the sexes: Theoretical and observed paths to dioecy in plants. *Annu. Rev. Plant Biol.* **2018**, *69*, 553–575. [CrossRef]
5. Darwin, C. *The Different Forms of Flowers on Plants of the Same Species*; Murray: London, UK, 1877. [CrossRef]
6. Álvarez-Cansino, L.; Diaz Barradas, M.C.; Zunzunegui, M.; Paz Esquivias, M.; Dawson, T.E. Gender-specific variation in physiology in the dioecious shrub *Corema album* throughout its distributional range. *Funct. Plant Biol.* **2012**, *39*, 968–978. [CrossRef]
7. Liu, X.; Dong, T.; Zhang, S. Adaptation Responses of Dioecious and Hermaphroditic Tree Species to Abiotic Stress. *Forests* **2023**, *14*, 383. [CrossRef]
8. Barrett, S.C.; Hough, J. Sexual dimorphism in flowering plants. *J. Exp. Bot.* **2013**, *64*, 67–82. [CrossRef]
9. Sun, Z.; Shen, Y.; Niinemets, Ü. Responses of isoprene emission and photochemical efficiency to severe drought combined with prolonged hot weather in hybrid *Populus*. *J. Exp. Bot.* **2020**, *71*, 7364–7381. [CrossRef]
10. Lin, T.; Tang, J.; He, F.; Chen, G.; Shi, Y.; Wang, X.; Han, S.; Li, S.; Zhu, T.; Chen, L. Sexual differences in above- and belowground herbivore resistance between male and female poplars as affected by soil cadmium stress. *Sci. Total Environ.* **2022**, *803*, 150081. [CrossRef] [PubMed]
11. Choat, B.; Brodribb, T.J.; Brodersen, C.R.; Duursma, R.A.; López, R.; Medlyn, B.E. Triggers of tree mortality under drought. *Nature* **2018**, *558*, 531–539. [CrossRef]
12. Basu, S.; Ramegowda, V.; Kumar, A.; Pereira, A. Plant adaptation to drought stress. *F1000Research* **2016**, *5*, 1554. [CrossRef] [PubMed]
13. Miller, G.; Suzuki, N.; Ciftci-Yilmaz, S.; Mittler, R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.* **2010**, *33*, 453–467. [CrossRef] [PubMed]
14. Gupta, A.; Rico-Medina, A.; Caño-Delgado, A.I. The physiology of plant responses to drought. *Science* **2020**, *368*, 266–269. [CrossRef] [PubMed]
15. Zhang, H.; Zhu, J.; Gong, Z.; Zhu, J. Abiotic stress responses in plants. *Nat. Rev. Genet.* **2022**, *23*, 104–119. [CrossRef] [PubMed]
16. Devireddy, A.R.; Zandalinas, S.I.; Fichman, Y.; Mittler, R. Integration of reactive oxygen species and hormone signaling during abiotic stress. *Plant J.* **2021**, *105*, 459–476. [CrossRef]
17. Apel, K.; Hirt, H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **2004**, *55*, 373–399. [CrossRef]
18. Mittler, R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **2002**, *7*, 405–410. [CrossRef]
19. Sachdev, S.; Ansari, S.A.; Ansari, M.I.; Fujita, M.; Hasanuzzaman, M. Abiotic Stress and Reactive Oxygen Species: Generation, Signaling, and Defense Mechanisms. *Antioxidants* **2021**, *10*, 277. [CrossRef]
20. Mukarram, M.; Choudhary, S.; Kurjak, D.; Petek, A.; Khan, M.M.A. Drought: Sensing, signalling, effects and tolerance in higher plants. *Physiol. Plant.* **2021**, *172*, 1291–1300. [CrossRef]
21. Wahab, A.; Abdi, G.; Saleem, M.H.; Ali, B.; Ullah, S.; Shah, W.; Mumtaz, S.; Yasin, G.; Muresan, C.C.; Marc, R.A. Plants' Physio-Biochemical and Phyto-Hormonal Responses to Alleviate the Adverse Effects of Drought Stress: A Comprehensive Review. *Plants* **2022**, *11*, 1620. [CrossRef]
22. Wang, X.; Curtis, P.S. Gender-specific responses of *Populus tremuloides* to atmospheric CO_2 enrichment. *New Phytol.* **2001**, *150*, 675–684. [CrossRef]
23. Xu, X.; Yang, F.; Xiao, X.; Zhang, S.; Korpelainen, H.; Li, C. Sex-specific responses of *Populus cathayana* to drought and elevated temperatures. *Plant Cell Environ.* **2008**, *31*, 850–860. [CrossRef] [PubMed]
24. Chen, L.; Zhang, S.; Zhao, H.; Korpelainen, H.; Li, C. Sex-related adaptive responses to interaction of drought and salinity in *Populus yunnanensis*. *Plant Cell Environ.* **2010**, *33*, 1767–1778. [CrossRef] [PubMed]
25. Lei, Y.B.; Jiang, Y.L.; Chen, K.; Duan, B.; Zhang, S.; Korpelainen, H.; Niinemets, Ü.; Li, C. Reproductive investments driven by sex and altitude in sympatric *Populus* and *Salix* trees. *Tree Physiol.* **2017**, *37*, 1503–1514. [CrossRef]
26. Melnikova, N.V.; Borkhert, E.V.; Snezhkina, A.V.; Kudryavtseva, A.V.; Dmitriev, A.A. Sex-Specific Response to Stress in *Populus*. *Front. Plant Sci.* **2017**, *8*, 1827. [CrossRef]
27. Xia, Z.; He, Y.; Yu, L.; Lv, R.; Korpelainen, H.; Li, C. Sex-specific strategies of phosphorus (P) acquisition in *Populus cathayana* as affected by soil P availability and distribution. *New Phytol.* **2020**, *225*, 782–792. [CrossRef]
28. Yu, L.; Huang, Z.; Tang, S.; Korpelainen, H.; Li, C.J.E.; Botany, E. *Populus euphratica* males exhibit stronger drought and salt stress resistance than females. *Environ. Exp. Bot.* **2023**, *205*, 105114. [CrossRef]
29. Yamaguchi-Shinozaki, K.; Shinozaki, K. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* **2006**, *57*, 781–803. [CrossRef]

30. Shinozaki, K.; Yamaguchi-Shinozaki, K. Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.* **2006**, *58*, 221–227. [CrossRef]
31. Choudhury, F.K.; Rivero, R.M.; Blumwald, E.; Mittler, R. Reactive oxygen species, abiotic stress and stress combination. *Plant J.* **2017**, *90*, 856–867. [CrossRef]
32. Wei, T.; Wang, Y.; Xie, Z.; Guo, D.; Chen, C.; Fan, Q.; Deng, X.; Liu, J. Enhanced ROS scavenging and sugar accumulation contribute to drought tolerance of naturally occurring autotetraploids in *Poncirus trifoliata*. *Plant Biotechnol. J.* **2019**, *17*, 1394–1407. [CrossRef] [PubMed]
33. Zhang, H.; Wang, Z.; Li, X.; Gao, X.; Dai, Z.; Cui, Y.; Zhi, Y.; Liu, Q.; Zhai, H.; Gao, S.; et al. The IbBBX24–IbTOE3–IbPRX17 module enhances abiotic stress tolerance by scavenging reactive oxygen species in sweet potato. *New Phytol.* **2022**, *233*, 1133–1152. [CrossRef] [PubMed]
34. Wei, H.; Movahedi, A.; Xu, C.; Sun, W.; Li, L.; Wang, P.; Li, D.; Zhuge, Q. Overexpression of *PtHMGR* enhances drought and salt tolerance of poplar. *Ann. Bot.* **2019**, *125*, 785–803. [CrossRef]
35. Edwards, R.; Dixon, D.P. Plant Glutathione Transferases. In *Methods Enzymology*; Sies, H., Packer, L., Eds.; Academic Press: Cambridge, MA, USA, 2005; Volume 401, pp. 169–186.
36. Gao, S.; Li, C.; Chen, X.; Li, S.; Liang, N.; Wang, H.; Zhan, Y.; Zeng, F. Basic helix-loop-helix transcription factor *PxbHLH02* enhances drought tolerance in *Populus* (*Populus simonii* × *P. nigra*). *Tree Physiol.* **2023**, *43*, 185–202. [CrossRef]
37. Liang, B.; Wan, S.; Ma, Q.; Yang, L.; Hu, W.; Kuang, L.; Xie, J.; Huang, Y.; Liu, D.; Liu, Y. A Novel bHLH Transcription Factor PtrbHLH66 from Trifoliate Orange Positively Regulates Plant Drought Tolerance by Mediating Root Growth and ROS Scavenging. *Int. J. Mol. Sci.* **2022**, *23*, 15053. [CrossRef] [PubMed]
38. Abe, H.; Urao, T.; Ito, T.; Seki, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell* **2003**, *15*, 63–78. [CrossRef] [PubMed]
39. García-Cerdán, J.G.; Sveshnikov, D.; Dewez, D.; Jansson, S.; Funk, C.; Schröder, W.P. Antisense Inhibition of the PsbX Protein Affects PSII Integrity in the Higher Plant *Arabidopsis thaliana*. *Plant Cell Physiol.* **2008**, *50*, 191–202. [CrossRef]
40. Xiao, L.; Liu, X.; Lu, W.; Chen, P.; Quan, M.; Si, J.; Du, Q.; Zhang, D. Genetic dissection of the gene coexpression network underlying photosynthesis in *Populus*. *Plant Biotechnol. J.* **2020**, *18*, 1015–1026. [CrossRef]
41. Biswas, S.; Eaton-Rye, J.J. PsbX maintains efficient electron transport in Photosystem II and reduces susceptibility to high light in Synechocystis sp. PCC 6803. *Biochim. Biophys. Acta (BBA)—Bioenerg.* **2022**, *1863*, 148519. [CrossRef]
42. Xiao, Y.; Huang, G.; You, X.; Zhu, Q.; Wang, W.; Kuang, T.; Han, G.; Sui, S.-F.; Shen, J.-R. Structural insights into cyanobacterial photosystem II intermediates associated with Psb28 and Tsl0063. *Nat. Plants* **2021**, *7*, 1132–1142. [CrossRef]
43. Jansson, S.; Douglas, C.J. *Populus*: A model system for plant biology. *Annu. Rev. Plant Biol.* **2007**, *58*, 435–458. [CrossRef]
44. Wullschleger, S.; Weston, D.; Davis, J. *Populus* responses to edaphic and climatic cues: Emerging evidence from systems biology research. *Crit. Rev. Plant Sci.* **2009**, *28*, 368–374. [CrossRef]
45. Wullschleger, S.D.; Jansson, S.; Taylor, G. Genomics and Forest Biology: *Populus* Emerges as the Perennial Favorite. *Plant Cell* **2002**, *14*, 2651–2655. [CrossRef] [PubMed]
46. Chen, F.; Shen, J.; Min, D.; Ke, L.; Tian, X.; Korpelainen, H.; Li, C. Male *Populus cathayana* than female shows higher photosynthesis and less cellular injury through ABA-induced manganese transporting inhibition under high manganese condition. *Trees* **2018**, *32*, 255–263. [CrossRef]
47. Song, H.; Cai, Z.; Liao, J.; Zhang, S. Phosphoproteomic and Metabolomic Analyses Reveal Sexually Differential Regulatory Mechanisms in Poplar to Nitrogen Deficiency. *J. Proteome Res.* **2020**, *19*, 1073–1084. [CrossRef] [PubMed]
48. Miao, L.F.; Yang, F.; Han, C.Y.; Pu, Y.J.; Ding, Y.; Zhang, J. Sex-specific responses to winter flooding, spring waterlogging and post-flooding recovery in *Populus deltoides*. *Sci. Rep.* **2017**, *7*, 2534. [CrossRef]
49. Zhang, R.; Liu, J.Y.; Liu, Q.S.; He, H.G.; Xu, X.; Dong, T.F. Sexual differences in growth and defence of *Populus yunnanensis* under drought stress. *Can. J. For. Res.* **2019**, *49*, 491–499. [CrossRef]
50. Zhang, Y.D.; Virjamo, V.; Sobuj, N.; Du, W.; Yin, Y.; Nybakken, L.; Guo, H.; Julkunen-Tiitto, R. Sex-related responses of European aspen (*Populus tremula* L.) to combined stress: TiO2 nanoparticles, elevated temperature and CO_2 concentration. *J. Hazard. Mater.* **2018**, *352*, 130–138. [CrossRef]
51. Stromme, C.B.; Julkunen-Tiitto, R.; Olsen, J.E.; Nybakken, L. The dioecious *Populus tremula* displays interactive effects of temperature and ultraviolet-B along a natural gradient. *Environ. Exp. Bot.* **2018**, *146*, 13–26. [CrossRef]
52. Li, C.; Ren, J.; Luo, J.; Lu, R. Sex-specific physiological and growth responses to water stress in *Hippophae rhamnoides* L. populations. *Acta Physiol. Plant.* **2004**, *26*, 123. [CrossRef]
53. Yang, Z.; Ma, L.; Jia, Z.; Sun, Y. Application on Spss Software in Photosynthetic Light-Response Curve of *Quercus Variabilis* Leaf. In *Recent Advances in Computer Science and Information Engineering*; Qian, Z., Cao, L., Su, W., Wang, T., Yang, H., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; Volume 3, pp. 567–573.
54. Ye, Z.; Suggett, D.J.; Robakowski, P.; Kang, H. A mechanistic model for the photosynthesis–light response based on the photosynthetic electron transport of photosystem II in C3 and C4 species. *New Phytol.* **2013**, *199*, 110–120. [CrossRef]
55. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods Enzymology*; Academic Press: Cambridge, MA, USA, 1987; Volume 148, pp. 350–382.
56. Liao, J.; Song, H.F.; Tang, D.T.; Zhang, S. Sexually differential tolerance to water deficiency of *Salix paraplesia*—A female-biased alpine willow. *Ecol. Evol.* **2019**, *9*, 8450–8464. [CrossRef]

57. Chen, F.; Zhang, S.; Zhu, G.; Korpelainen, H.; Li, C. *Populus cathayana* males are less affected than females by excess manganese: Comparative proteomic and physiological analyses. *Proteomics* **2013**, *13*, 2424–2437. [CrossRef]
58. Maehly, A.C. The Assay of Catalases and Peroxidases. *Methods Biochem. Anal.* **1954**, *1*, 357–424.
59. Giannopolitis, C.N.; Ries, S.K. Superoxide Dismutases: I. Occurrence in Higher Plants. *Plant Physiol.* **1977**, *59*, 309–314. [CrossRef]
60. Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
61. Canning, C.M.; Mood, B.J.; Bonsal, B.; Howat, B.; Laroque, C.P. Comparison of tree-growth drought legacies of three shelterbelt species in the *Canadian prairies*. *Agric. For. Meteorol.* **2023**, *330*, 109317. [CrossRef]
62. Brodribb, T.J.; Powers, J.; Cochard, H.; Choat, B. Hanging by a thread? Forests and drought. *Science* **2020**, *368*, 261–266. [CrossRef]
63. Hultine, K.R.; Bush, S.E.; Ward, J.K.; Dawson, T.E. Does sexual dimorphism predispose dioecious riparian trees to sex ratio imbalances under climate change? *Oecologia* **2018**, *187*, 921–931. [CrossRef]
64. Morales, M.; Pinto-Marijuan, M.; Munne-Bosch, S. Seasonal, sex- and plant size-related effects on photoinhibition and photoprotection in the dioecious Mediterranean dwarf palm, *Chamaerops humilis*. *Front. Plant Sci.* **2016**, *7*, 1116. [CrossRef]
65. Korgiopoulou, C.; Bresta, P.; Nikolopoulos, D.; Karabourniotis, G. Sex-specific structural and functional leaf traits and sun-shade acclimation in the dioecious tree *Pistacia vera* (Anacardiaceae). *Funct. Plant Biol.* **2019**, *46*, 649–659. [CrossRef]
66. Hu, H.; He, B.; Ma, L.; Chen, X.; Han, P.; Luo, Y.; Liu, Y.; Fei, X.; Wei, A. Physiological and transcriptome analyses reveal the photosynthetic response to drought stress in drought-sensitive (Fengjiao) and drought-tolerant (Hanjiao) *Zanthoxylum bungeanum* cultivars. *Front. Plant Sci.* **2022**, *13*, 968714. [CrossRef] [PubMed]
67. Baker, N.R. Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* **2008**, *59*, 89–113. [CrossRef] [PubMed]
68. Helm, L.T.; Shi, H.; Lerdau, M.T.; Yang, X. Solar-induced chlorophyll fluorescence and short-term photosynthetic response to drought. *Ecol. Appl.* **2020**, *30*, e02101. [CrossRef] [PubMed]
69. Donaldson, L. Autofluorescence in Plants. *Molecules.* **2020**, *25*, 2393. [CrossRef]
70. Sherstneva, O.; Khlopkov, A.; Gromova, E.; Yudina, L.; Vetrova, Y.; Pecherina, A.; Kuznetsova, D.; Krutova, E.; Sukhov, V.; Vodeneev, V. Analysis of chlorophyll fluorescence parameters as predictors of biomass accumulation and tolerance to heat and drought stress of wheat *Triticum aestivum*. *Funct. Plant Biol.* **2022**, *49*, 155–169. [CrossRef]
71. He, F.; Li, H.; Wang, J.; Su, Y.; Wang, H.; Feng, C.; Yang, Y.; Niu, M.; Liu, C.; Yin, W.; et al. PeSTZ1, a C2H2-type zinc finger transcription factor from *Populus euphratica*, enhances freezing tolerance through modulation of ROS scavenging by directly regulating *PeAPX2*. *Plant Biotechnol. J.* **2019**, *17*, 2169–2183. [CrossRef]
72. Zhang, S.; Chen, L.; Duan, B.; Korpelainen, H.; Li, C. *Populus cathayana* males exhibit more efficient protective mechanisms than females under drought stress. *For. Ecol. Manag.* **2012**, *275*, 68–78. [CrossRef]
73. Bowler, C.; Van Montagu, M.; Inzé, D. Superoxide dismutase and stress tolerance. *Annu. Rev. Plant Biol.* **1992**, *43*, 83–116. [CrossRef]
74. Bajji, M.; Kinet, J.-M.; Lutts, S. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regul.* **2002**, *36*, 61–70. [CrossRef]
75. Hodges, D.M.; DeLong, J.M.; Forney, C.F.; Prange, R.K. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **1999**, *207*, 604–611. [CrossRef]
76. Whitlow, T.H.; Bassuk, N.L.; Ranney, T.G.; Reichert, D.L. An improved method for using electrolyte leakage to assess membrane competence in plant tissues. *Plant Physiol.* **1992**, *98*, 198–205. [CrossRef]
77. Qin, F.; Liu, G.; Huang, G.Q.; Dong, T.F.; Liao, Y.M.; Xu, X. Zinc application alleviates the adverse effects of lead stress more in female *Morus alba* than in males. *Environ. Exp. Bot.* **2018**, *146*, 68–76. [CrossRef]
78. Kumar, V.; Khare, T.; Sharma, M.; Wani, S.H. ROS-Induced Signaling and Gene Expression in Crops Under Salinity Stress. In *Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress*; Khan, M.I.R., Khan, N.A., Eds.; Springer: Singapore, 2017; pp. 159–184.
79. Melandri, G.; Abd Elgawad, H.; Floková, K.; Jamar, D.C.; Asard, H.; Beemster, G.T.S.; Ruyter-Spira, C.; Bouwmeester, H.J. Drought tolerance in selected aerobic and upland rice varieties is driven by different metabolic and antioxidative responses. *Planta* **2021**, *254*, 13. [CrossRef]
80. Li, C.; Wan, Y.; Shang, X.; Fang, S. Responses of Microstructure, Ultrastructure and Antioxidant Enzyme Activity to PEG-Induced Drought Stress in *Cyclocarya paliurus* Seedlings. *Forests* **2022**, *13*, 836. [CrossRef]
81. Hasanuzzaman, M.; Nahar, K.; Anee, T.I.; Fujita, M. Glutathione in plants: Biosynthesis and physiological role in environmental stress tolerance. *Physiol. Mol. Biol. Plants* **2017**, *23*, 249–268. [CrossRef]
82. Nianiou-Obeidat, I.; Madesis, P.; Kissoudis, C.; Voulgari, G.; Chronopoulou, E.; Tsaftaris, A.; Labrou, N.E. Plant glutathione transferase-mediated stress tolerance: Functions and biotechnological applications. *Plant Cell Rep.* **2017**, *36*, 791–805. [CrossRef]
83. Yang, G.; Wang, Y.; Xia, D.; Gao, C.; Wang, C.; Yang, C. Overexpression of a GST gene (*ThGSTZ1*) from *Tamarix hispida* improves drought and salinity tolerance by enhancing the ability to scavenge reactive oxygen species. *Plant Cell Tissue Org.* **2014**, *117*, 99–112. [CrossRef]
84. Toledo-Ortiz, G.; Huq, E.; Quail, P.H. The Arabidopsis basic/helix-loop-helix transcription factor family. *Plant Cell* **2003**, *15*, 1749–1770. [CrossRef]

85. Dong, Y.; Wang, C.; Han, X.; Tang, S.; Liu, S.; Xia, X.; Yin, W. A novel bHLH transcription factor PebHLH35 from *Populus euphratica* confers drought tolerance through regulating stomatal development, photosynthesis and growth in *Arabidopsis*. *Biochem. Biophys. Res. Commun.* **2014**, *450*, 453–458. [CrossRef]
86. Ringnér, M. What is principal component analysis? *Nat. Biotechnol.* **2008**, *26*, 303–304. [CrossRef] [PubMed]
87. Rozas, V.; Le Quesne, C.; Rojas-Badilla, M.; Gonzalez-Reyes, A.; Donoso, S.; Olano, J.M. Climatic cues for secondary growth and cone production are sex-dependent in the long-lived dioecious conifer Araucaria araucana. *Agric. For. Meteorol.* **2019**, *274*, 132–143. [CrossRef]