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Stem xylem traits and wood formation affect sex-specific responses to drought and re-watering in *Populus cathayana*

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Head title: Sexual differences in drought and re-watering responses

Abstract The increased frequency and intensity of drought pose great threats to the survival of trees, especially in dioecious tree species, which usually display sex-related differences in mortality and biased sex ratios. The present study explored the roles of sex-specific differences in xylem anatomy and function and carbon metabolism in drought resistance and drought recovery in dioecious *Populus cathayana* Rehder. We showed that the sex-specific drought resistance and subsequent recovery were closely linked to the xylem anatomy and carbon metabolism. Females had a greater xylem vessel area per vessel, biomass and hydraulic efficiency (a higher theoretical hydraulic conductivity) under well-watered conditions. In contrast, males had a lower xylem lumen area, but greater vessel numbers, and a higher cell wall thickness and leaf photosynthesis, suggesting a conservative water-use strategy (a lower theoretical hydraulic conductivity) and drought resistance under drought stress. The recovery of photosynthetic ability and leaf water potential after drought in males was largely dependent on the recovery of xylem function and the regulation of the xylem carbohydrate metabolism. Additionally, numbers of up-regulated genes related to xylem cell wall biogenesis and cell turgor maintenance were greater in males relative to females under drought stress and subsequent re-watering, which facilitated drought resistance and xylem function restoration in males. These results suggested that sex-specific drought resistance and restoration were related to xylem anatomy and function, carbohydrate metabolism and cell turgor maintenance.

Key words: dioecy, drought, re-watering, xylem anatomy, hydraulic efficiency, hydraulic safety

Introduction

Increasingly frequent and intensive drought events have been linked with plant mortality. For instance, drought impacts growth and distribution of tree populations not only during the drought period, but there may be lasting effects afterwards even for years (Barigah et al., 2018; Kannenberg and Phillips, 2020). Therefore, it is critical to elucidate the physiological and molecular mechanism causing the drought-induced plant damage and recovery after drought.

As previously discovered, drought-induced tree mortality is caused by hydraulic failure and carbon starvation (Rodriguez-Zaccaro and Groover, 2019). Leaf photosynthesis is widely considered as the major carbon source of plants in response to drought. Maintaining the continued water supply for leaf photosynthesis is critical for plant survival and growth in dry environment (Sapes et al., 2019). In woody plants, leaf photosynthesis is closely associated with the plant's hydraulic system, which can supply water to leaves (Martorell et al., 2014). In woody plants, the drought-induced hydraulic conductivity of xylem coincides with stomatal closure and the reduced leaf photosynthetic capacity (Martorell et al., 2014). The recovery of leaf photosynthesis largely depends on the restoration of xylem structure and functions (Martorell et al., 2014). Moreover, the non-structural carbohydrate (NSC) pools in the wood limit the increasing xylem tension and maintain the hydraulic integrity of xylem (De Baerdemaeker et al., 2017; De Roo et al., 2020). The ability and extent to refill

67 embolized vessels after re-watering plants are also associated with NSC pools (Brunetti

68 et al., 2020; Kannenberg and Phillips, 2020). However, the regulation of the wood

69 xylem structure and NSC dynamics in response to drought and subsequent re-watering

70 is unclear, especially in dioecious plant species.

71

72 Drought increases xylem tension in woody plants. Stomatal closure reduces water loss

73 and xylem tension, which cannot prevent xylem embolism during extended drought and

74 consequent plant mortality (Kannenberg and Phillips, 2020). Variation in the

75 morphology and arrangement of xylem elements shares a basic structural principle for

76 the reduction of xylem embolism and adaptation to drought (Fonti et al., 2010; Torres-

77 Ruiz et al., 2017). It has been suggested thatthe anatomical traits of xylem could

78 successfully predict hydraulic function (Hacke et al., 2015). According to Hagen-

79 Poiseuillle's law, the rate of water flow is proportional to the fourth power of the

80 tracheid lumen diameter (Hacke and Sperry, 2001). The hydraulic efficiency in plants

81 with higher lumen diameter is more sensitive to xylem embolism under drought (Olano

82 et al., 2017). In contrast, the increased wall thickness relative to the lumen area tends

83 to indicate a more conservative strategy, with less conductivity and correspondingly

84 lower vulnerability to embolism, especially under drought stress (Rungwattana and

85 Hietz, 2018).

86

87 The anatomical traits of xylem are suggested to be developmentally plastic, which

88 reflects great morphological diversity across plant lineages and the adaptation of

89 hydraulic traits to different environmental conditions (Martorell et al., 2014; Hultine et

90 al., 2016). Moreover, plants can utilize NSC for osmotic regulation to maintain cell

91 turgor and/or refill embolized xylem vessels under drought and/or re-watering after

92 drought (Secchi and Zwieniecki, 2012). Despite the established association between the

93 physiological plasticity of xylem and water flow, very little is known about the

94 molecular mechanism underlying xylem traits under drought and re-watering,

95 especially in dioecious plants.

96

97 Wood growth is considered as an ideal system for studying xylem anatomy and function

98 and for providing implications for the carbon cycling under drought and re-watering.

99 Wood stores immense amounts of carbon with a slow carbon turnover, and the growth

100 is closely correlated with the net primary productivity (Anderegg et al., 2015). The

101 regulation of xylem traits and xylem NSC under drought and recoveryafter drought

102 varies among plant species and genotypes, as well as depending on the severity and

103 duration of drought (Olano et al., 2017; Falchi et al., 2020). Tree species exhibit major

104 interspecific and intraspecific variation in hydraulic conductivity along an

105 environmental gradient, especially under drought (Burton et al., 2017; Islam et al.,

106 2018).

107 Variation in hydraulic conductivity may show opposite patterns in males and females

108 of dioecious species if variation is tightly associated with sex (Olano et al., 2017).

109 Dioecious plants exhibit sexual dimorphism in response to abiotic stress (Liu et al.,

110 2020; Liu et al., 2021a). Female plants usually have a higher reproductive effort than

males, since females produce flowers, seeds and fruits, and they require contrasting water-use strategies compared to males (Hultine et al., 2016; Liu et al., 2021b). Indeed, the female trunk exhibits such anatomical traits of xylem that relate to a larger hydraulic efficiency, while male anatomy shows a more conservative strategy, especially under drought conditions, for instance, in the dioecious conifer *Juniperus thurifera* (Olano et al., 2017). However, much is still unknown.

In this study, we used *Populus cathayana* females and males as a model species to elucidate the sex-specific tolerance and responses to drought and subsequent re-watering based on the structure and function of wood xylem. We aimed to answer three main questions: (1) do wood xylem traits differ between sexes under drought and re-watering; (2) how is the trade-off between traits related to hydraulic efficiency in males and females; (3) how is the xylem structure of males and females regulated by drought and re-watering after drought stress at the molecular level.

Materials and Methods

Plant material and experimental design

P. cathayana cuttings were gathered from different female and male trees in riparian and valley flat habitats of the Qinghai Province, China (30°67′N, 104°06′E), as explained in detail by Liu et al. (2020). The cuttings were grown in a glasshouse with

natural light. After growing for 4 weeks, 32 healthy and uniform seedlings (about 30

cm) were transplanted into 10-l plastic pots with 10 kg of soil. The soil contained 1.75

g kg^{-1} total N, 122.56 mg kg^{-1} available P, 476.46 mg kg^{-1} available K, 106.33 mg kg^{-1}

NO_3^--N, 52.69 mg kg^{-1} NH_4^+-N, and 33.32 g kg^{-1} soil organic matter. After 6 weeks, 16

seedlings of each sex were completely randomized and divided into well-watered

condition, CK; drought, D; drought + re-watering 7 d, DW7; drought + re-watering 14

d, DW14. Firstly, all seedlings of each sex were randomly divided into two groups (CK,

D) and cultivated for 6 weeks. Subsequently, 8 plants (CK, D) of each sex were

harvested. The remaining plants were re-watered to 100% field capacity for 7 d and 14

d. In this study, we mainly explored sexual differences in responses to drought and re-

watering. For CK, the pots were weighed each day to maintain 100% field capacity. For

D, the pots were weighed each day to maintain 30% field capacity.

Gas exchange and leaf water potential measurements

Gas exchange parameters were measured on fully developed mature leaves between

09:00 and 11:00, and 14:00 and 16:30. Measurements were performed using a portable

photosynthesis measuring system (Ll-6400; Li-Cor Inc., Lincoln, NE, USA) equipped

with an integrated leaf chamber fluorometer (LI-6400-40). The conditions for

measuring leaf gas exchange parameters were as follows: relative humidity of 75%,

airflow rate of 500 μmol s^{-1}, CO_2 concentration in the cuvette (C_a) of 400 μmol mol^{-1}

and the saturating photon quantum flux density (PPFD) of 1200 μmol m^{-2} s^{-1} (with 90%

155   red light and 10% blue light). After measuring leaf gas exchange, sampled leaves were

156   cut for the analysis of leaf water potential early in the morning in a pressure chamber

157   (PMS Instruments, Albany, OR, USA).

158

159   *Microscopic observations and anatomical measurements*

160

161   After the determination of photosynthetic parameters, stems were cut into segments (1–

162   2 mm^2) and immediately immersed in FAA solutions. Afterwards, the samples were

163   rinsed three times with ultrapure water, and dehydrated with a series of ethanol

164   gradients. Samples were embedded in glycol methacrylate. Semi-thin cross-sections

165   were stained with safranin-fast green and viewed under a light microscope (Nikon

166   Corporation, Kyoto, Japan) with 20× magnification. The thickness, size and density of

167   leaf palisade and spongy tissues, and the sum of spongy and palisade tissue cell area

168   per transverse section were measured using light micrographs.

169

170   *Analysis of wood composition by fourier transform infrared spectroscopy (FTIR)*

171

172   The developing xylem was obtained according to Teichmann et al. (2008). Briefly, stem

173   segments were sampled at about two-thirds above the stem base. Only developing

174   xylem was harvested (mature wood increases toward the tree base). Developing xylem

175   was sampled by scraping the surface of the xylem with a razor after the tree bark was

176   removed. Afterwards, the scraped developing xylem was immediately put into liquid

177 nitrogen. Subsequently, the samples were divided into two parts. One part of the

178 samples was dried with a vacuum freeze dryer for 100 h. The freeze-dried sample

179 powder was pressed against the diamond crystal of an attenuated total reflectance

180 device and the infra-red spectra were determined with a FTIR spectrometer Nicolet iS5.

181 The scanning range was 400-4000 cm^{-1} wavenumber. The remaining samples were kept

182 at -80 ℃ until the subsequent transcriptome sequencing.

183

184 *RNA extraction and sequencing*

185

186 Frozen tissues of developing xylem samples (about 100 mg) were ground with a ball

187 mill in liquid nitrogen. Subsequently, total RNA was extracted with a RNA extraction

188 kit (TaKaRa MiniBEST Plant RNA Extraction Kit, TaKaRa, Otsu, Japan), and genomic

189 DNA was digested using DNase I according to the manufacturer's instructions (Takara,

190 Otsu, Japan). RNA yield and purity were assessed with the NanoDrop

191 spectrophotometer 2000 (NanoDrop Technologies Inc.). The concentration of RNA was

192 assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies Inc.). The synthesis

193 of cDNA was performed with random primers and reverse transcriptase. Double-strand

194 cDNA was synthesized using a SuperScript double-stranded cDNA synthesis kit

195 (Invitrogen, CA) according to the manufacturer's protocols, and the sequencing

196 libraries were synthesized with the TruSeqTM RNA sample preparation kit (Illumina,

197 USA). Subsequently, the cDNA products were purified and amplified with Phusion

198 DNA polymerase (NEB). The libraries were sequenced with the Illumina HiSeq 4000

Systems, each with three biological replicates. Sequencing reads were purified by removing adaptors, regions with more than 5% unknown N bases and low-quality reads using the SOAPnuke software (BGI, Shenzhen, China). Clean reads were aligned with the reference genome of *P. trichocarpa* (http://plants.ensembl.org/Populus_trichocarpa/Info/Index). The mapping reads were constructed with StringTie software (v1.04).

Data processing and analysis

We identified and calculated differentially expressed genes with transcripts per kilobase of the exon model per million mapped reads (TPM). The abundances of genes were quantified with RSEM (http://deweylab.biostat.wisc.edu/rsem/). The coding sequence of *P. trichocarpa* was blasted against the closest Arabidopsis homolog (AGI identification) with PoplarGene (http://bioinformatics.caf.ac.cn/PoplarGene/gene) and annotated using the Arabidopsis Information Resource genome (https://www.arabidopsis.org/). The analysis of differential expression was performed with eight groups (FCK, FD, FDW7, FDW14, MCK, MD, MDW7, MDW14). The thresholds (Padj-value $\leq$ 0.05 and |log2 fold-change| $\geq$1) were set to determine significant differences in gene expression. The sequencing data were submitted to NCBI (BioProject accession number: SUB9706978).

Quantitative PCR analyses

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About 100 mg frozen tissues of developing xylem samples were used to extract total

RNA with a RNA extraction kit (TaKaRa MiniBEST Plant RNA Extraction Kit,

TaKaRa, Otsu, Japan). RNA yield and purity were assessed with the NanoDrop

spectrophotometer 2000 (NanoDrop Technologies Inc.). The first cDNA strand was

synthesized using PrimeScript reverse transcription (RT) reagent kits (Takara)

following the manufacturer's protocols. Quantitative reverse transcription polymerase

chain reactions (qRT-PCR) were conducted in a 25 μl reaction volume with pairs of

gene-specific primers (Table S1) using a SYBR®Premix EX Taq kit (TliRNaseH Plus)

(TaKaRa) following the manufacturer's instructions. The relative expression levels

were calculated according to $2^{-\Delta\Delta Ct}$ (Liu et al., 2017). *TUB4.1* was chosen as the

housekeeping gene (He et al., 2013).

Statistical analyses

Differences among means within treatments were revealed using the SPSS software

(version 22.0). Before analyses of variance (ANOVAs), the data were checked for

normality. Differences between means were analyzed by Duncan's tests at a

significance level of $P < 0.05$

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Results

Sexual differences in plant growth and tolerance to drought and re-watering

The dry weight of leaves, stems and roots decreased by 78%, 76% and 60%, respectively, in females, and by 60%, 72% and 50%, respectively, in males under drought stress relative to controls (Table 1). The dry weight of leaves, stems and roots in females increased by 20%, 13% and 42%, respectively, after 7 d re-watering (DW7), and increased by 85%, 21% and 57%, respectively, after 14 d of re-watering after drought stress (DW14), when compared to the drought treatment (Table 1). In males, the dry weigh of leaves, stems and roots increased by 46%, 21% and 82%, respectively,

264   at 7 d re-watering, and by 119%, 72% and 204%, respectively, at re-watering 14 d after

265   drought stress when compared to drought (Table 1).

266

267   Both the photosynthesis rate and leaf gas conductance decreased significantly in

268   females compared to males under drought conditions (Fig. 1a, b). The leaf

269   photosynthesis and gas conductance recovered in females and males at 7 d and 14 d re-

270   watering after drought stress. In males, the leaf photosynthesis completely recovered to

271   the control level (without drought stress) at 7 d re-watering after drought stress (Fig.

272   1a). The leaf gas conductance in males recovered to 87% of control at 7 d re-watering

273   and 100% of control at 14 d re-watering after drought stress. In contrast, the leaf

274   photosynthesis of females did not completely recover to the control level at 7 d or even

275   at 14 d re-watering after drought stress. The gas conductance of females was lower at 7

276   d of re-watering than in controls but recovered to the control level after 14 d of re-

277   watering (Fig. 1b). Leaf water potential significantly decreased by drought stress in

278   both sexes, and the decrease was greater in females than in males (Fig. 1c). Leaf water

279   potential reached 229% of the control level in drought-stressed females and 127% in

280   drought-stressed males after 7 d of re-watering. Leaf water potential was fully

281   recovered to the control level after 14 d of re-watering in drought-stressed females and

282   males.

283

284   The proportions of xylem length relative to the radius of wood in the cross-section were

285   higher in males than in females under all treatments (Fig. 1d). Drought stress did not

affect the proportion of xylem length relative to the radius of wood in the cross-section of females, while drought stress lowered the xylem proportion in males relative to controls. The proportions of xylem length relative to the radius of wood in the cross-section increased in drought-stressed males after 7 d of re-watering, but not in drought-stressed females (Fig. 1d). The proportions of xylem length relative to the radius of wood were higher in both sexes at day 14 of re-watering after drought stress compared to drought stress.

Sexual differences in anatomical traits of wood xylem

The vessel number of both sexes was higher under drought stress, and at 7 d and 14 d of re-watering after drought compared to well-watered controls (Fig. 2a). The decreased vessel numbers in females observed by 7 d and 14 d re-watering after drought were similar. In males. the decrease in the number of vessels induced by re-watering after drought was greater at 14 d than at 7 d. The lumen area per vessel was higher in females than in males under all treatments (Fig. 2b). The lumen area per vessel decreased by drought stress, but increased by 7 d and 14 d of re-watering after drought in both sexes when compared to controls. Re-watering after drought increased the lumen area per vessel more at 14 d than that at 7 d in females. In males, the lumen area per vessel increased similarly by 7 d re-watering and 14 d re-watering after drought when compared to drought (Fig. 2b). When compared to drought, 7 d re-watering did not affect the cell wall thickness in either sex, whereas the cell wall thickness decreased at

308 14 d re-watering after drought in both sexes (Fig. 2c). The predicted hydraulic

309 conductivity in males decreased by drought stress, but completely recovered to control

310 levels at 7 d and 14 d re-watering after drought (Fig. 2d). In females, the predicted

311 hydraulic conductivity was not affected by drought stress, but it decreased similarly at

312 7 d or 14 d re-watering after drought (Fig. 2d).

313

314 *Sexual differences in wood NSC dynamics*

315

316 Overall, the levels of total sugar, sucrose and NSC were higher in male wood compared

317 to females under drought and subsequent re-watering. Drought increased the levels of

318 total sugar, sucrose and NSC in leaves of both sexes, which gradually decreased by re-

319 watering at 7 d and 14 d after drought in both sexes (Fig. 3a, b, d). Females had higher

320 wood starch levels than males under drought and re-watering at 7 d and 14 d after

321 drought (Fig. 3c). Drought increased wood starch concentrations in both sexes,

322 whereaswood starch concentrations decreased similarly at 7 d and 14 d re-watering after

323 drought in females and males (Table 1).

324

325 *Fourier transform infrared spectroscopy spectra of xylem*

326

327 The fourier transform infrared spectroscopy spectra were used to obtain the chemical

328 fingerprint of the molecular wood composition in the newly formed xylem of *P.*

329 *cathayana* females and males under drought and re-watering. The drought and re-

330   watering differently affected the wood xylem composition of both sexes (Fig. 4a, b;

331   Table S2). PCA analysis was performed to study the original absorbance data. The PC1

332   and PC2 accounted for 48% and 47% of the variation, respectively. Peaks at 1505, 1034,

333   1596 and 1462 cm^{-1} were key factors contributing to PC1, whereas 1508, 1157, 1110,

334   1425 and 1375 cm^{-1} were key factors contributing to PC2. The spectra showed a

335   pronounced difference in major wood compounds, such as lignin, cellulose, lignin and

336   carbohydrate, of the developing xylem between drought and control conditions in both

337   sexes. Additionally, the spectra showed significant differences in the developing xylem

338   in both sexes and pronounced changes in the xylem structure induced by drought and

339   re-watering 7 d after drought stress, including hemicellulose (peak 1), pection, cellulose,

340   lignin and carbohydrates, as well as the deformation in cellulose and hemicellulose

341   (peak 5). At 14 d re-watering after drought stress, the PCA did not distinguish

342   differences in the xylem structure between females and males.

343

344   *Transcriptome analysis of developing xylem*

345

346   Transcriptomic changes in the developing xylem were analyzed to study the response

347   mechanism in xylem development to drought and re-watering in both sexes. The

348   sequencing data coincided well with the data obtained by qRT-PCR (Fig. S1). The

349   numbers of differentially expressed genes (DEGs) equaled 5262 (2632 down-regulated

350   and 2630 up-regulated) between FW (female, well-watered) and MW (male, well-

351   watered), 9210 (4145 down-regulated and 5065 up-regulated) between FD (female,

352   drought) and MD (male, drought), 6339 (3210 down-regulated and 3129 up-regulated)

353   between FDR7 and MDR7, and 5999 (2704 down-regulated and 3295 up-regulated)

354   between FDR14 and MDR1 (Fig. 5a, c). Specifically, there were 3283 common DEGs

355   between "FW versus MW" and "FD versus MD", 3509 common DEGs between "FD

356   versus MD" and "FDR7 versus MDR7", and 3283 common DEGs between "FD versus

357   MD" and "FDR14 versus MDR14" (Fig. 5a, c).

358

359   In the total analysis, the responses of FW versus MW were not significant for up-

360   regulated and down-regulated genes (Fig. 5b). Significantly up-regulated and down-

361   regulated genes existed between "FD versus MD". Furthermore, up-regulated genes

362   were found between "FD versus MD", mainly those related to metabolism, and cell

363   wall-related and cell cycle processes (Fig. 5c, d). Up-regulated genes between "FD

364   versus MD" were mainly involved in the cell wall biosynthesis, cell wall polysaccharide

365   biosynthesis and metabolism, xylan and lignin biosynthesis and metabolism, and

366   carbohydrate metabolism (Fig. 5c, d). In contrast, down-regulated genes enriched

367   between "FD versus MD" were mainly involved in responses to stress, transport,

368   metabolism and signaling processes (Fig. S2). These down-regulated genes between

369   "FD versus MD" were mainly involved in abiotic stress responses, such as heat, osmotic

370   and salt stresses, and protein modification processes (Fig. S2). Up-regulated genes

371   between "FDW7 versus MDW7" were enriched in cell cycle, cellular organization,

372   signaling and transport, such as mitotic cell cycle processes and regulation, regulation

373   of microtubule cytoskeleton organization, organic acid transport and protein

374 phosphorylation. Down-regulated genes between "FDW7 versus MDW7" were

375 enriched in development, catabolism, metabolism, proteostasis and stress responses,

376 such as phloem development, aminoglycan catabolic process, the regulation of

377 peptidase activity and responses to fatty acid (Fig. 5d). Up-regulated genes between

378 "FDW14 versus MDW14" were mainly involved in organic acid transport, protein

379 modification and regulation of the cellular biosynthetic process. Down-regulated genes

380 between "FDW14 versus MDW14" were mainly enriched in protein phosphorylation,

381 defense, sugar metabolism, regulation of mitotic cell cycle and microtubule-based

382 movement (Fig. S2).

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386

387 **Discussion**

388

389 *Sex-specific anatomical traits of xylem in response to drought*

390

391 *Populus* females and males exhibit sex-specific differences in responses to abiotic stress,

392 probably associated with sex-specific demands of nutrients and water (Liu et al., 2021a;

393 Liu et al., 2021b). Some studies have suggested that females have higher reproductive

394 costs than males in some dioecious plants due to the demand for carbon and water

395 during fruiting and seed set (Olano et al., 2017; Liu et al., 2021b). Therefore, females

396   tend to improve photosynthetic carbon gain and water uptake via higher hydraulic

397   conductivity and stomatal conductance under high-resource conditions (Hultine et al.,

398   2016; Olano et al., 2017). Such a better ability for resource acquisition (including water)

399   in females is easily subjected to carbon starvation and hydraulic failure under drought

400   stress (Hultine et al., 2016). Indeed, female poplars have a lower leaf water potential,

401   photosynthetic rate and leaf water use efficiency, and thus they suffer more damage

402   from drought stress than males (Han et al., 2013; Yu et al., 2018). In contrast, males of

403   some dioecious plants show superior performance, which is associated with a more

404   conservative water use strategy to maximize drought stress tolerance (Olano et al.,

405   2017). Interestingly, the sex-specific water acquisition and utilization might disappear

406   under well-watered conditions in *P. cathayana* (Chen et al., 2014), further suggesting

407   that sexual dimorphism in dioecious poplars is environment-dependent.

408

409   Drought-induced tree mortality is mainly attributable to carbon starvation or hydraulic

410   failure or both (Mitchell et al., 2013; Choat et al., 2018). Moreover, the photosynthetic

411   carbon gain of leaves is associated with the hydraulic system in woody plants (Deans

412   et al., 2020; Qi et al., 2021). The hydraulic system of plants has been reported to

413   determine the ability of plants to cope with drought among plants with similar or

414   different genetic backgrounds (Blackman et al., 2017; Kannenberg et al., 2019). It has

415   been suggested that the tradeoff between hydraulic efficiency and safety affects plants'

416   tolerance to drought stress (Olano et al., 2017; Yao et al., 2021). Plant species with a

417   higher hydraulic conductance generally exhibit greater vulnerabilities to embolism

418 (Scoffoni et al., 2017). In contrast, plants with a hydraulic safety strategy emphasize

419 the ability to maintain xylem conductivity with decreasing water potential levels (Olano

420 et al., 2017; Scoffoni et al., 2017). Indeed, females with a greater theoretical hydraulic

421 conductance are more sensitive to drought stress than males (Fig. 2). We found that

422 females have a lower leaf water potential and photosynthesis but a greater theoretical

423 hydraulic conductance further demonstrating the tradeoff between hydraulic efficiency

424 and safety (Figs 1-2).

425

426 Adjustments in the hydraulic system can be assessed based on the xylem anatomy and

427 function (Fonti et al., 2010). In this study, we found that water conditions modified

428 xylem anatomic traits of stem, affected the tradeoff between hydraulic efficiency and

429 safety, and caused sex-specific responses to water conditions (Fig. 2). In a previous

430 study on *P. cathayana*, the secondary sexual dimorphisms in physiological characters

431 possibly disappeared under well-watered conditions (Chen et al., 2014). Here, we found

432 that such disappearance of secondary sexual dimorphism under well-watered

433 conditions appeared to be associated with the anatomic traits of stem xylem (Fig. 2).

434

435 Under well-watered conditions, females had a greater lumen area per vessel and

436 predicted hydraulic conductivity. The proportion of xylem was greater in males than in

437 females, although they had a lower lumen area per vessel and predicted hydraulic

438 conductivity in males (Figs 1-2). The greater lumen area per vessel in males than

439 females appeared to increase the total amount of water transported into shoots, thus

440 compensating for their lower hydraulic conductivity. Under well-watered conditions,

441 there was no significant difference in leaf potential between the sexes (Fig. 1), further

442 demonstrating the lack of significant secondary sexual dimorphism in *P. cathayana* in

443 no-stress conditions. However, under drought stress, females still tended to adopt a

444 greater hydraulic efficiency (a higher theoretical hydraulic conductivity) to maximize

445 water transport. The increased lumen area per vessel in females elevates the risks of

446 cavitation and air embolism, thus easily resulting in hydraulic failure under drought, as

447 exhibited by the lower photosynthesis and leaf water potential compared to males. In

448 contrast, males preferred to adopt a more conservative water use strategy (a lower

449 theoretical hydraulic conductivity) with a lower lumen area per vessel to maximize the

450 stress tolerance under drought stress.

451

452 *Sex-specific responses to drought and subsequent re-watering*

453

454 It has been suggested that the time for completing the recovery of physiological

455 functioning is essential for tree survival. As proposed by Rehschuh et al. (2020), the

456 recovery ability and time in woody tree species after re-watering largely depends on

457 whether drought impairs the critical physiological processes, such as carbon uptake and

458 water transport. Here, males quickly recovered from drought after re-watering. The

459 extent of recovery in the leaf photosynthesis, leaf water potential and gas conductance

460 were greater in males than in females after 7 d with a favorable water status (Fig. 1).

461 Importantly, the treatment of 14 d re-watering after drought fully recovered leaf

photosynthesis and gas conductance in males, but did not fully recover those in females (Fig. 1). As suggested by Brodribb and Cochard (2009), no hysteresis is expected when the recovery of gas exchange is restricted by leaf water potential. In females, the leaf water potential was fully restored within 14 d, but leaf photosynthesis did not fully recover. The occurrence of hysteresis in females within 14 d recovery after drought suggested that other non-hydraulic limitations probably continued to limit the leaf photosynthesis after complete re-watering, and this needs to be explored in future studies.

As we discussed above, the recovery time largely depended on the extent of problems in key physiological processes, such as carbon uptake and water transport. The carbon uptake may be assessed by the leaf photosynthesis and gas conductance. Males with a faster rate of recovery from the drought stress may be associated with less impairment in key physiological processes, such as the leaf photosynthesis and gas conductance, when compared to females (Fig. 1). Recent studies have revealed that the xylem anatomical traits and function-associated hydraulic failure play an important role in lowering survival during drought and subsequent recovery (Creek et al., 2018). Additionally, the restoration of xylem function may be associated with modifications in anatomical traits (Secchi et al., 2017). The lumen area per vessel and predicted hydraulic conductivity of wood recovered to the control level in females at 7 d and 14 d re-watering after drought (Fig. 2). Interestingly, the vessel number was significantly higher in both sexes under 7 d or 14 d re-watering after drought. This phenomenould

be associated with the legacy effects of drought. Some studies have suggested that drought has long-lasting legacy effects on the ecosystem and plant growth (Arnone et al., 2008; Kaisermann et al., 2017). However, the mechanism underlying such effects on the xylem vessel number remains to be elucidated in future studies.

It was noteworthy that the leaf water potential did not fully recover to the control levels in either sex at 7 d re-watering after drought despite the full recovery of the predicted hydraulic conductivity by this time (Figs, 1-2). This could be associated with the carbohydrate metabolism during the embolism repair. According to the current active embolism removal models, the starch hydrolysis-induced osmotica accumulation in the apoplast facilitated the refilling of the vessels and, thus, becoming functional (Secchi and Zwieniecki, 2012; Pagliarani et al., 2019). In this study, we found that the NSC, including sugar and sucrose, was higher in males than in females (Fig. 3; Table 1). The increased sugar contents in the male wood probably strengthened the ability of trees to repair xylem functionality after drought stress. Importantly, the total sugar, sugar and NSC decreased at 7 d or 14 d re-watering after drought, but the contents were higher in males than in females. Actually, the concentration of starch in the wood was lower in males than females under all treatments (Fig. 3; Table 1). The lower starch concentration in the male wood compared to females was probably attributable to starch hydrolysis into sugars to regulate the apoplastic water potential and the recovery of empty vessel function. Indeed, males had higher sugar concentrations, and higher gene expression levels related to starch degradation in the wood compared to females (Fig.

506 3; Table 1). These results suggested that the xylem for drought stress recovery was

507 probably associated with sugar metabolism in the wood.

508

509 *Transcriptome regulation of developing xylem underlying the molecular mechanism to*

510 *salt stress and re-watering*

511

512 Xylem anatomy and functions, as well as carbohydrate metabolism play critical roles

513 in drought tolerance and hydraulic recovery (Olano et al., 2017; Brunetti et al., 2020).

514 The sex-specific transcriptional regulation in response to drought further suggested that

515 females and males adopt different strategies to cope with drought (Fig. 5). The vessel

516 development is initiated from the vessel differentiation and then continued through the

517 production of thick and lignified cell walls (Rodriguez-Zaccaro and Groover, 2019).

518 The increased expression of auxin transporter, such as *AUX1*, *PIN1* and *PIN5,* in males

519 under drought stress can facilitate the differentiation of vessel elements and enhance

520 vessel production (Fig. 6). Expression levels of genes related to the xylem cell fate,

521 such as *VND1*, *TED6*, *LBD15*, members of the *NAC* transcription factor family, and

522 *MYB* transcription factors, were higher in males than in females under drought, in spite

523 of their reduced expression by drought stress relative to controls and re-watering at

524 either 7 d or 14 d (Fig. 6; Fig. S3). Such down-regulated genes induced by drought

525 stress in both sexes are probably associated with the lower proportion of xylem in the

526 cross-sections of stem (Fig. 1).

527

In addition, the vessel thickness affected the hydraulic conductance of stem (Olano et al., 2017; Scoffoni et al., 2017). Plants with a smaller vessel diameter usually exhibit a more conservative hydraulic strategy and stronger tolerance to drought stress, and the smaller vessel diameter is usually associated with secondary cell wall deposition in plants (Olano et al., 2017). Expression levels of genes related to secondary cell formation, such as *MYB* transcription factors, *NST1* (secondary wall thickening factors), cellulose synthase family *IRX1*, *IRX6* and *IRX9* (Endo et al., 2015; Taylor-Teeples et al., 2015), were higher in males than females under drought stress, in spite of the reduced expression relative to controls (Fig. 6). Such gene expression modes under drought were consistent with the greater cell wall thickness and lower lumen area in males compared to females (Fig. 2). It was noted that there appears to be inconsistency in the expression patterns of genes related to cell wall synthesis and cell thickness in both sexes under drought stress. Such inconsistency may be explained by the large demand for cell wall materials needed for cell expansion (Rodriguez-Zaccaro and Groover, 2019). The expansion from 5 to 50 μm for the tracheary element needs 10 times more cell wall material to maintain a constant cell wall thickness (Rodriguez-Zaccaro and Groover, 2019). As shown in Fig. 2, the lumen area per vessel significantly decreased by drought in both sexes when compared to the controls. The reduced expression of genes related to cell wall synthesis and metabolism in both sexes under drought stress relative to the controls was probably associated with the reduced demand for cell wall material to cell expansion.

550   To recover growth after drought, plant cells must regain their ability to loosen their

551   walls and, over a longer period, incorporate new polymers to avoid wall failure (Rui

552   and Dinneny, 2020). In this study, re-watering after drought differentially recovered the

553   expression of genes related to cell wall biosynthesis in both sexes (Fig. 6). The cell wall

554   and the microtubule cytoskeleton are also tightly linked through a mechanical feedback

555   loop to achieve growth and morphogenesis in plants (Eng and Sampathkumar, 2018).

556   The up-regulation of genes related to xylan, actin cytoskeleton organization, lignin

557   synthesis and metabolism, secondary cell wall biogenesis and metabolism, and

558   cytoskeleton organization in both sexes at 7 d and 14 d re-watering promoted the cell

559   wall function recovery and probably turgor restoration (Fig. 5). The up-regulation of

560   genes related to xyloglucan metabolisms, such as expansins (EXPs), xyloglucan endo-

561   β-transglucosylase/hydrolases (XETs/XTHs) and endo-1,4-β-glucanases (EGases),

562   promote the cell wall extensibility (Sasidharan et al., 2011). The higher expression level

563   of genes related to the cell wall and the microtubule cytoskeleton induced by drought

564   in both sexes suggested that males had a better ability to recover cell wall expansion-

565   driving cell enlargement and optimal plant growth. Interestingly, the sexual difference

566   in DEGs enriched in cell wall metabolism and cytoskeleton organization at 7 d re-

567   watering disappeared at 14 d re-watering after drought, further demonstrating that

568   sexual dimorphism largely depends on abiotic stress factors (Chen et al., 2014; Liu et

569   al., 2020).

570

571 Drought reduced the plant growth by causing various physiological changes, including

572 loss of turgor (Le Gall et al., 2015). Ions and solutes absorbed by plants could lower

573 cells' osmotic potential to maintain turgor and withstand drought (Patakas et al., 2002).

574 Increased transcript levels of some ABC transporters induced by drought in males might

575 contribute to the accumulation of ions and solutes, and to the maintenance of osmotic

576 potential. In addition, the up-regulation of some ion transporters induced by drought in

577 males relative to the controls, such as sulfate transporter, potassium transporter *AKT2*,

578 *ADT4* and calcium transporter *PHO1,* facilitate the maintenance of turgor and drought

579 resistance (Patakas et al., 2002). Plasma membrane ATPases are primary active

580 transporters of cations that maintain steep concentration gradients of ions (Morth et al.,

581 2011). The up-regulation of genes related to ATP synthesis and activities facilitated the

582 proton-dependent transport, which was responsible for metabolites and other ion

583 transport, and drought stress tolerance in males (Fig. S3).

584

585 In addition, the increased NSC storage plays a critical role in osmotic regulation to

586 maintain turgor and/or refill the embolized xylem under drought and re-watering after

587 drought (Pagliarani et al., 2019). Under drought stress, the xylem sugar accumulation

588 is derived from either starch degradation in parenchyma cells or translocation from

589 phloem to parenchyma rays (Pagliarani et al., 2019). The pathway of starch degradation

590 is regulated by phosphoglucan water dikinase, phosphoglucan phosphatases starch

591 excess 4 (SEX4), disproportionating enzyme (DPE1/2) (Chia et al., 2004), starch

592 debranching enzymes (LSA3, LDA) and β-amylases (BAM1/3) (Fulton et al., 2008).

593   Additionally, maltose excess protein (MEX1) transports glucose and maltose to the

594   cytosol (Cho et al., 2011; Fan et al., 2018). In our study, the up-regulation of *GWD1*,

595   *PWD1*, SEX4, *DPE1*, *DPE2* and *MEX1* genes induced by drought stress and subsequent

596   re-watering in males relative to females was consistent with their lower starch content

597   and higher sugar levels compared to females. The up-regulation of starch degradation

598   and sucrose transport in males under drought and re-watering lowered cell osmotic

599   potential to maintain turgor and hydraulic refill, thus enhancing drought tolerance.

600   Interestingly, we found that only the *BAM1* gene was up-regulated under drought in

601   females but not under subsequent re-watering relative to the controls, further suggesting

602   that females are more sensitive to drought and have greater difficulties to recover from

603   drought relative to males.

604

605   In addition, the up-regulation of membrane sugar transporters, *SUT4*, in males under

606   drought and subsequent re-watering promoted the apoplastic sugar accumulation and

607   aquaporin-mediated water entry into the empty vessels immediately when re-watering

608   after drought. Indeed, we found that most genes encoding the aquaporins, such as

609   *PIP1;4*, *PIP2* and *PIP3,* were up-regulated in males but not in females at 7 d re-

610   watering after drought stress. Interestingly, we found that the expression of genes

611   related to aquaporins, such as *PIP1;3*, *PIP1B*, *TIP1;3*, *SIP1;2* and *NIP1;2,* was higher

612   in females than in males under well-watered and drought stress conditions (higher than

613   in controls). The expression modes of these genes related to aquaporins further

614   demonstrated that females have a greater hydraulic efficiency, both under drought and

615 well-watered conditions, which easily makes females more sensitive to drought. These

616 results suggested that males have a better ability to cope with drought and recover better

617 and faster from drought stress compared to females, which is largely dependent on the

618 regulation of the xylem structure and function, as well as on the osmotic maintenance

619 at the molecular level.

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625 **Conclusions**

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627 The present study showed that males have a greater tolerance to drought and a better

628 ability of recover from drought compared to females, which was associated with sex-

629 specific anatomical traits of xylem and carbohydrate dynamics. Males showed xylem

630 anatomical traits related to a conservative water-use strategy (a lower theoretical

631 hydraulic conductivity) and higher leaf photosynthesis. In contrast, females had a

632 greater hydraulic efficiency over safety (thinner tracheid walls and a larger lumen area

633 per vessel), more sensitivity to drought and a slower recovery of leaf photosynthesis.

634 The transcription analysis conducted for the developing xylem further demonstrated

635 that the tolerance of drought and quickly recovery in males was associated with the

636 processes of cell wall biogenesis, starch metabolism and ion transport.

637

Author contributions Miao Liu had the main responsibility for data collection,

analysis and writing, Yang Zhao and Yuting Wang contributed to data collection, Helena

Korpelainen contributed to the interpretation of data and manuscript preparation, and

Chunyang Li (the corresponding author) had the overall responsibility for experimental

design and project management.

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

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References

648

Anderegg WR, Schwalm C, Biondi F, Camarero JJ, Koch G, Litvak M, Ogle K, Shaw

JD, Shevliakova E, Williams AP, Wolf A (2015) Pervasive drought legacies in forest

ecosystems and their implications for carbon cycle models. Science 349:528-532.

Arnone Iii JA, Verburg PS, Johnson DW, Larsen JD, Jasoni RL, Lucchesi AJ, Batts CM,

von Nagy C, Coulombe WG, Schorran DE, Buck PE (2008) Prolonged suppression of

ecosystem carbon dioxide uptake after an anomalously warm year. Nature 455:383-386.

Barigah TS, Charrier O, Douris M, Bonhomme M, Herbette S, Améglio T, Fichot R,

Choat B, Brodribb TJ, Brodersen CR, Duursma RA, Lopez R, Medlyn BE (2018)

659   Triggers of tree mortality under drought. Nature 558:531-559.

660

661   Blackman CJ, Aspinwall MJ, Tissue DT, Rymer PD (2017) Genetic adaptation and

662   phenotypic plasticity contribute to greater leaf hydraulic tolerance in response to

663   drought in warmer climates. Tree Physiology 37:583-592.

664

665   Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of

666   death in water-stressed conifers. Plant Physiology 149:575-584.

667

668   Brunetti C, Savi T, Nardini A, Loreto F, Gori A, Centritto M (2020) Changes in abscisic

669   acid content during and after drought are related to carbohydrate mobilization and

670   hydraulic recovery in poplar stems. Tree Physiology 40:1043-1057.

671

672   Burton JI, Perakis SS, McKenzie SC, Lawrence CE, Puettmann KJ (2017) Intraspecific

673   variability and reaction norms of forest understorey plant species traits. Functional

674   Ecology 31:1881-1893.

675

676   Chen J, Duan B, Wang M, Korpelainen H, Li C (2014) Intra-and inter-sexual

677   competition of *Populus cathayana* under different watering regimes. Functional

678   Ecology 28:124-136.

679

680   Chia T, Thorneycroft D, Chapple A, Messerli G, Chen J, Zeeman SC, Smith SM, Smith

681 AM (2004) A cytosolic glucosyltransferase is required for conversion of starch to

682 sucrose in Arabidopsis leaves at night. The Plant Journal 37:853-863.

683

684 Cho JI, Kim HB, Kim CY, Hahn TR, Jeon JS (2011) Identification and characterization

685 of the duplicate rice sucrose synthase genes *OsSUS5* and *OsSUS7* which are associated

686 with the plasma membrane. Molecules and Cells 31:553-561.

687

688 Choat B, Brodribb TJ, Brodersen CR, Duursma RA, Lopez R, Medlyn BE (2018)

689 Triggers of tree mortality under drought. Nature 558:531-539.

690

691 Creek D, Blackman CJ, Brodribb TJ, Choat B, Tissue DT (2018) Coordination between

692 leaf, stem, and root hydraulics and gas exchange in three arid-zone angiosperms during

693 severe drought and recovery. Plant, Cell & Environment 41:2869-2881.

694

695 De Baerdemaeker NJ, Salomón RL, De Roo L, Steppe K (2017) Sugars from woody

696 tissue photosynthesis reduce xylem vulnerability to cavitation. New Phytologist 216:

697 720-727.

698

699 De Roo L, Salomón RL, Oleksyn J, Steppe K (2020) Woody tissue photosynthesis

700 delays drought stress in *Populus tremula* trees and maintains starch reserves in branch

701 xylem tissues. New Phytologist 228:70-81.

702

703 Deans RM, Brodribb TJ, Busch FA, Farquhar GD (2020) Optimization can provide the

704 fundamental link between leaf photosynthesis, gas exchange and water relations.

705 Nature Plants 6:1116-1125.

706

707 Endo H, Yamaguchi M, Tamura T, Nakano Y, Nishikubo N, Yoneda A, Kato K, Kubo

708 M, Kajita S, Katayama Y, Ohtani M (2015) Multiple classes of transcription factors

709 regulate the expression of VASCULAR-RELATED NAC-DOMAIN7, a master switch

710 of xylem vessel differentiation. Plant and Cell Physiology 56:242-254.

711

712 Eng RC, Sampathkumar A (2018) Getting into shape: the mechanics behind plant

713 morphogenesis. Current Opinion in Plant Biology 46:25-31.

714

715 Falchi R, Petrussa E, Braidot E, Sivilotti P, Boscutti F, Vuerich M, Calligaro C, Filippi

716 A, Herrera JC, Sabbatini P, Zancani M (2020) Analysis of non-structural carbohydrates

717 and xylem anatomy of leaf petioles offers new insights in the drought response of two

718 grapevine cultivars. International Journal of Molecular Sciences 21:1457.

719

720 Fan ZQ, Ba LJ, Shan W, Xiao YY, Lu WJ, Kuang JF, Chen JY (2018) A banana R2R3-

721 MYB transcription factor MaMYB3 is involved in fruit ripening through modulation

722 of starch degradation by repressing starch degradation-related genes and MabHLH6.

723 The Plant Journal 96:1191-205.

724

725 Fonti P, von Arx G, García-González I, Eilmann B, Sass-Klaassen U, Gärtner H,

726 Eckstein D (2010) Studying global change through investigation of the plastic

727 responses of xylem anatomy in tree rings. New Phytologist 185:42-53.

728

729 Fulton DC, Stettler M, Mettler T, Vaughan CK, Li J, Francisco P, Gil M, Reinhold H,

730 Eicke S, Messerli G, Dorken G (2008) β-AMYLASE4, a noncatalytic protein required

731 for starch breakdown, acts upstream of three active β-amylases in Arabidopsis

732 chloroplasts. The Plant Cell 20:1040-1058.

733

734 Hacke UG, Lachenbruch B, Pittermann J, Mayr S, Domec JC, Schulte PJ (2015) The

735 hydraulic architecture of conifers. In: Hacke UG (ed) Functional and ecological xylem

736 anatomy. Springer, New York, NY, pp 39–75.

737

738 Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. Perspectives

739 in Plant Ecology, Evolution and Systematics 4:97-115.

740

741 Han Y, Wang Y, Jiang H, Wang M, Korpelainen H, Li C (2013) Reciprocal grafting

742 separates the roles of the root and shoot in sex‐related drought responses in *Populus*

743 *cathayana* males and females. Plant, Cell & Environment 36:356-364.

744

745 He J, Li H, Luo J, Ma C, Li S, Qu L, Gai Y, Jiang X, Janz D, Polle A, Tyree M (2013)

746 A transcriptomic network underlies microstructural and physiological responses to

747   cadmium in *Populus × canescens*. Plant Physiology 162:424-439.

748

749   Hultine KR, Grady KC, Wood TE, Shuster SM, Stella JC, Whitham TG (2016) Climate

750   change perils for dioecious plant species. Nature Plants 2:1-8.

751

752   Islam M, Rahman M, Bräuning A (2018) Long-term hydraulic adjustment of three

753   tropical moist forest tree species to changing climate. Frontiers in Plant Science 9:1761.

754

755   Kaisermann A, de Vries FT, Griffiths RI, Bardgett RD (2017) Legacy effects of drought

756   on plant–soil feedbacks and plant–plant interactions. New Phytologist 215:1413-1424.

757

758   Kannenberg SA, Novick KA, Phillips RP (2019) Anisohydric behavior linked to

759   persistent hydraulic damage and delayed drought recovery across seven North

760   American tree species. New Phytologist 222:1862-1872.

761

762   Kannenberg SA, Phillips RP (2020) Non-structural carbohydrate pools not linked to

763   hydraulic strategies or carbon supply in tree saplings during severe drought and

764   subsequent recovery. Tree Physiology 40:259-271.

765

766   Le Gall H, Philippe F, Domon JM, Gillet F, Pelloux J, Rayon C (2015) Cell wall

767   metabolism in response to abiotic stress. Plants 4:112-166.

768

769 Liu M, Bi J, Liu X, Kang J, Korpelainen H, Niinemets Ü, Li C (2020) Microstructural

770 and physiological responses to cadmium stress under different nitrogen levels in

771 *Populus cathayana* females and males. Tree Physiology 40:30-45.

772

773 Liu M, Liu X, Du X, Korpelainen H, Niinemets Ü, Li C (2021a) Anatomical variation

774 of mesophyll conductance due to salt stress in *Populus cathayana* females and males

775 growing under different inorganic nitrogen sources. Tree Physiology.

776 https://doi.org/10.1093/treephys/tpab017.

777

778 Liu M, Liu XX, He XL, Liu LJ, Wu H, Tang CX, Zhang YS, Jin CW (2017) Ethylene

779 and nitric oxide interact to regulate the magnesium deficiency-induced root hair

780 development in Arabidopsis. New Phytologist 213:1242-1256.

781

782 Liu M, Korpelainen H, Li C (2021b) Sexual differences and sex ratios of dioecious

783 plants under stressful environments. Journal of Plant Ecology.

784 https://doi.org/10.1093/jpe/rtab038.

785

786 Martorell S, DIAZ-ESPEJO AN, Medrano H, Ball MC, Choat B (2014) Rapid hydraulic

787 recovery in *Eucalyptus pauciflora* after drought: linkages between stem hydraulics and

788 leaf gas exchange. Plant, Cell & Environment 37:617-626.

789

790 Mitchell PJ, O'Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA (2013)

Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytologist 197:862-872.

Morth JP, Pedersen BP, Buch-Pedersen MJ, Andersen JP, Vilsen B, Palmgren MG, Nissen P (2011) A structural overview of the plasma membrane Na^+, K^+-ATPase and H^+-ATPase ion pumps. Nature Reviews Molecular Cell Biology 12:60-70.

Olano JM, González-Muñoz N, Arzac A, Rozas V, von Arx G, Delzon S, García-Cervigón AI (2017) Sex determines xylem anatomy in a dioecious conifer: hydraulic consequences in a drier world. Tree Physiology 37:1493-1502.

Pagliarani C, Casolo V, Ashofteh Beiragi M, Cavalletto S, Siciliano I, Schubert A, Gullino ML, Zwieniecki MA, Secchi F (2019) Priming xylem for stress recovery depends on coordinated activity of sugar metabolic pathways and changes in xylem sap pH. Plant, Cell & Environment 42:1775-1787.

Patakas A, Nikolaou N, Zioziou E, Radoglou K, Noitsakis B (2002) The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. Plant Science 163:361-367.

Qi JH, Fan ZX, Fu PL, Zhang YJ, Sterck F (2021) Differential determinants of growth rates in subtropical evergreen and deciduous juvenile trees: carbon gain, hydraulics and

813 nutrient-use efficiencies. Tree Physiology 41:12-23.

814

815 Rehschuh R, Cecilia A, Zuber M, Faragó T, Baumbach T, Hartmann H, Jansen S, Mayr

816 S, Ruehr N (2020) Drought-induced xylem embolism limits the recovery of leaf gas

817 exchange in Scots pine. Plant Physiology 84:852-864.

818

819 Rodriguez-Zaccaro FD, Groover A (2019) Wood and water: How trees modify wood

820 development to cope with drought. Plants, People, Planet 1:346-355.

821

822 Rui Y, Dinneny JR (2020) A wall with integrity: surveillance and maintenance of the

823 plant cell wall under stress. New Phytologist 225:1428-1439.

824

825 Rungwattana K, Hietz P (2018) Radial variation of wood functional traits reflect size-

826 related adaptations of tree mechanics and hydraulics. Functional Ecology 32:260-272.

827

828 Sapes G, Roskilly B, Dobrowski S, Maneta M, Anderegg WR, Martinez-Vilalta J, Sala

829 A (2019) Plant water content integrates hydraulics and carbon depletion to predict

830 drought-induced seedling mortality. Tree Physiology 39:1300-1312.

831

832 Sasidharan R, Voesenek LA, Pierik R (2011) Cell wall modifying proteins mediate plant

833 acclimatization to biotic and abiotic stresses. Critical Reviews in Plant Sciences 30:548-

834 562.

835

836 Scoffoni C, Albuquerque C, Brodersen CR, Townes SV, John GP, Cochard H, Buckley

837 TN, McElrone AJ, Sack L (2017) Leaf vein xylem conduit diameter influences

838 susceptibility to embolism and hydraulic decline. New Phytologist 213:1076-1092.

839

840 Secchi F, Pagliarani C, Zwieniecki MA (2017) The functional role of xylem

841 parenchyma cells and aquaporins during recovery from severe water stress. Plant, Cell

842 & Environment 40:858-871.

843

844 Secchi F, Zwieniecki MA (2012) Analysis of xylem sap from functional (nonembolized)

845 and nonfunctional (embolized) vessels of *Populus nigra*: chemistry of refilling. Plant

846 Physiology 160:955-964.

847

848 Taylor-Teeples M, Lin L, De Lucas M, Turco G, Toal TW, Gaudinier A, Young NF,

849 Trabucco GM, Veling MT, Lamothe R, Handakumbura PP (2015) An Arabidopsis gene

850 regulatory network for secondary cell wall synthesis. Nature 517:571-575.

851

852 Teichmann T, Bolu-Arianto WH, Olbrich A, Langenfeld-Heyser R, Göbel C,

853 Grzeganek P, Feussner I, Hänsch R, Polle A (2008) GH3::GUS reflects cell-specific

854 developmental patterns and stress-induced changes in wood anatomy in the poplar stem.

855 Tree Physiology 28:1305-1315.

856

Torres-Ruiz JM, Cochard H, Fonseca E, Badel E, Gazarini L, Vaz M (2017) Differences in functional and xylem anatomical features allow *Cistus* species to co-occur and cope differently with drought in the Mediterranean region. Tree Physiology 37:755-766.

Yao GQ, Nie ZF, Turner NC, Li FM, Gao TP, Fang XW, Scoffoni C (2021) Combined high leaf hydraulic safety and efficiency provides drought tolerance in *Caragana* species adapted to low mean annual precipitation. New Phytologist 229:230-244.

Yu L, Han Y, Jiang Y, Dong T, Lei Y (2018) Sex-specific responses of bud burst and early development to nongrowing season warming and drought in *Populus cathayana*. Canadian Journal of Forest Research 48:68-76.

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Figure legends

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882

883 **Figure 1** The photosynthetic rate (a), gas conductance (b), leaf water potential (c) and

884 proportion of xylem (d) in *P. cathayana* females and males under well-watered (CK) or

885 drought stress (D) conditions, and 7 d (DW7) or 14 d re-watering (DW14) after drought

886 stress. Bars indicate means ± SD ($n = 4$). Different lower-case letters above bars indicate

887 significant differences at $P < 0.05$ based on ANOVA followed by Duncan's tests.

888

889 **Figure 2** The vessel number (a), lumen area per vessel (b), cell wall thickness (c) and

890 predicted conductivity (d) in the xylems of *P. cathayana* females and males under well-

891 watered (CK) or drought stress (D) conditions, and 7 d (DW7) or 14 d re-watering

892 after drought stress (DW14). Bars indicate means ± SD ($n = 4$). Different lower-case

893 letters above bars indicate significant differences at $P < 0.05$ based on ANOVA followed

894 by Duncan's tests.

895

896 **Figure 3** The levels of total sugar (a), sucrose (b), starch (c) and non-structural

897 carbohydrates (NSC) (d) in the xylems of *P. cathayana* females and males under well-

898 watered (CK) or drought stress (D) conditions, and 7 d (DW7) or 14 d re-watering after

899 drought stress (DW14). Bars indicate means ± SD ($n = 4$). Different lower-case letters

900 above bars indicate significant differences at $P < 0.05$ based on ANOVA followed by

901 Duncan's tests.

902

903 **Figure 4** FTIR spectra (a) and the corresponding principle component analysis (PCA)

904 (b) plot of the xylems of *P. cathayana* females and males under well-watered (CK) or

905 drought stress (D) conditions, and 7 d (DW7) or 14 d re-watering after drought stress

906 (DW14). The average spectrum of the developing xylem was plotted ($n = 4$). PCA was

907 conducted with the data of the selected peaks separately for leaves and roots (Table S2).

908 The spectral peaks and their corresponding molecular components were as follows: 1,

909 1738 cm^{-1} for vibration of C=O from esterified pectin; 2, 1650 cm^{-1} for C-N vibration

910 from protein; 3, 1596 cm for aromatic skeletal vibrations in lignin; 4, 1505 cm^{-1} for

911 C=O stretch and aromatic skeletal vibrations in lignin; 5, 1462 cm^{-1} C-H deformation,

912 lignin and carbohydrates; 6, 1425 cm^{-1} for vibration of COO- from pectin; 7, 1375 cm^{-1}

913 ^{1} for C-H deformation in hemicellulose and cellulose; 8, 1330 cm^{-1} for guaiacyl ring

914 condensed plus syringyl ring; 9, 1235 cm^{-1} for deformation of cellulose; 10, 1157 cm^{-1}

915 for vibration of C-C and C-O stretch from carbohydrates (such as soluble sugar,

916 cellulose and hemicellulose; 11, 1111 cm^{-1} for C-C or C-O vibration from pectin; 12,

917 1034 cm^{-1} for vibration of C-H or C-C and C-O from cellulose and hemicellulose.

918

919 **Figure 5** Comparative profiles of transcriptional responses to drought stress, or 7 d or

920 14 d re-watering after drought stress in the developing xylem of *P. cathayana* females

921 and males. (a) Venn diagrams of common and sex-specific gene ontology (GO)

922 categories under well-watered (W) or drought stress (D), 7 d (DW7) or 14 d (DW14)

re-watering after drought stress. (b) Circle diagram of GO terms enriched in up-regulated genes (above) and down-regulated genes (below) between females and males under well-watered or drought stress conditions, and 7 d or 14 d re-watering after drought stress. (c) Total number of differentially expressed genes. Blue bars mean up-regulated genes between treatments, and orange bars mean down-regulated genes between treatments. (d) Comparative transcriptional profiling showing the up-regulated genes between females and males. The X-axis indicates the GO term, and the Y-axis indicates the rich factor (the ratio of gene expression level enriched in males relative to fe males). The higher rich factor means the greater enrichment. The size of a circle indicates the number of genes in the GO term, and the color of a circle indicates the P-value range. By default, the enrichment result of top 20 is displayed on the premise of the P-value < 0.05. FD, female + drought stress; MD, male + drought stress; MW, male + well-watered; MDW7, male + re-watering 7 d after drought stress; MDW14, male + re-watering 7 d after drought stress; FW, female + well-watered; FDW7, female + re-watering 7 d after drought stress; FDW14, female + re-watering 14 d after drought stress.

Figure 6 Hierarchical clustering of key genes involved in starch auxin transport, tracheary element differentiation, secondary cell wall biogenesis (a), as well as in sucrose transport and starch metabolism (b) in the developing xylem of *P. cathayana* females and males. The columns show the samples, and the rows show the genes. The colors of cells represent the log10 (TPM) values in each sample. Red cells indicate that genes are highly expressed in the samples, and blue cells indicate that genes are lowly

945   expressed in the samples. FD, female + drought stress; MD, male + drought stress; MW,

946   male + well-watered; MDW7, male + re-watering 7 d after drought stress; MDW14,

947   male + re-watering 7 d after drought stress; FW, female + well-watered; FDW7, female

948   + re-watering 7 d after drought stress; FDW14, female + re-watering 14 d after drought

949   stress.

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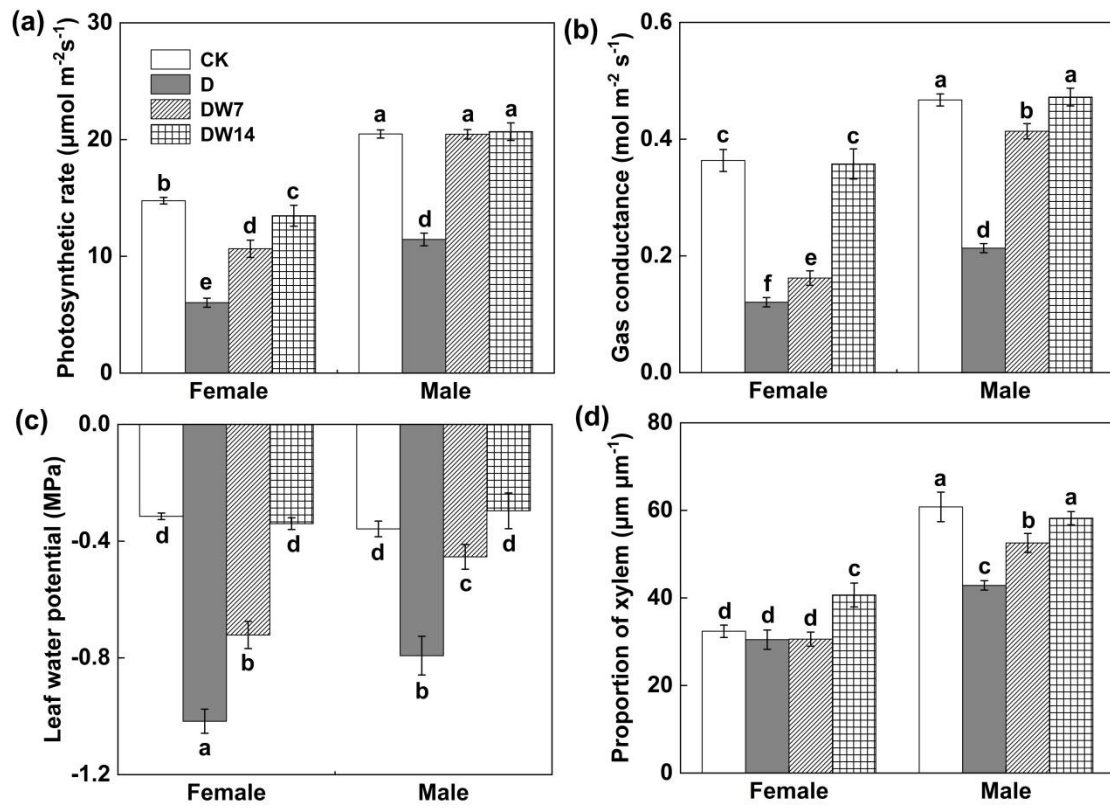
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Figure 1

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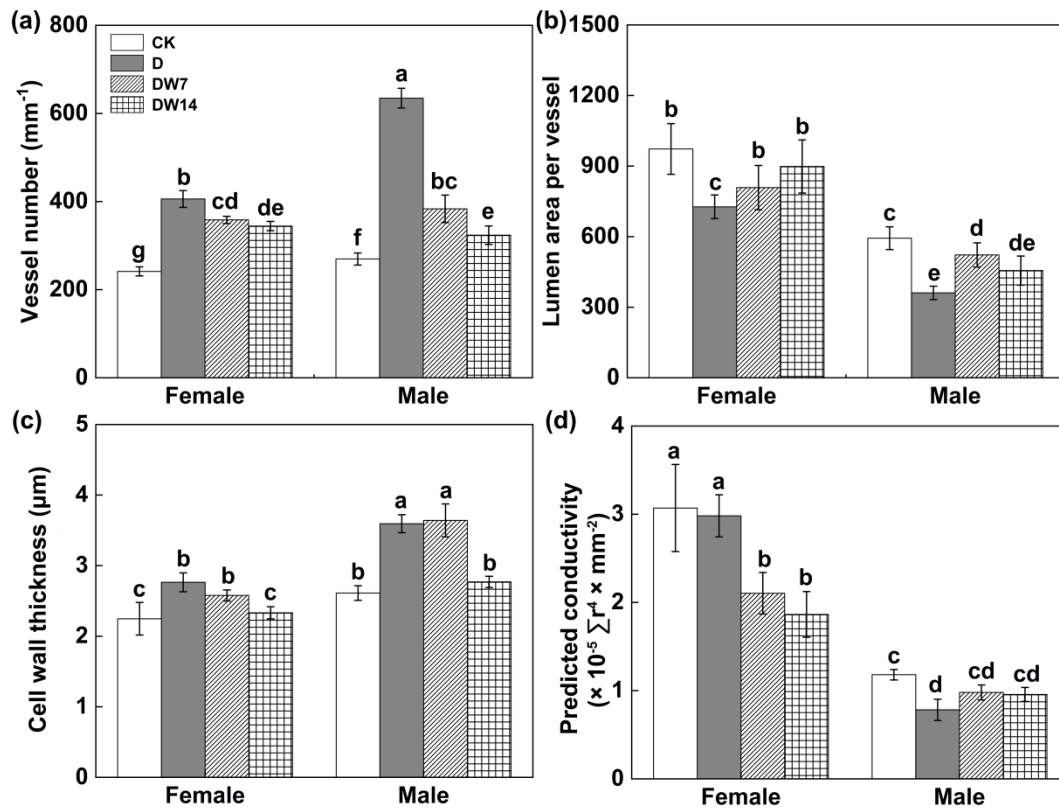
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Figure 2

993   **Figure 3**
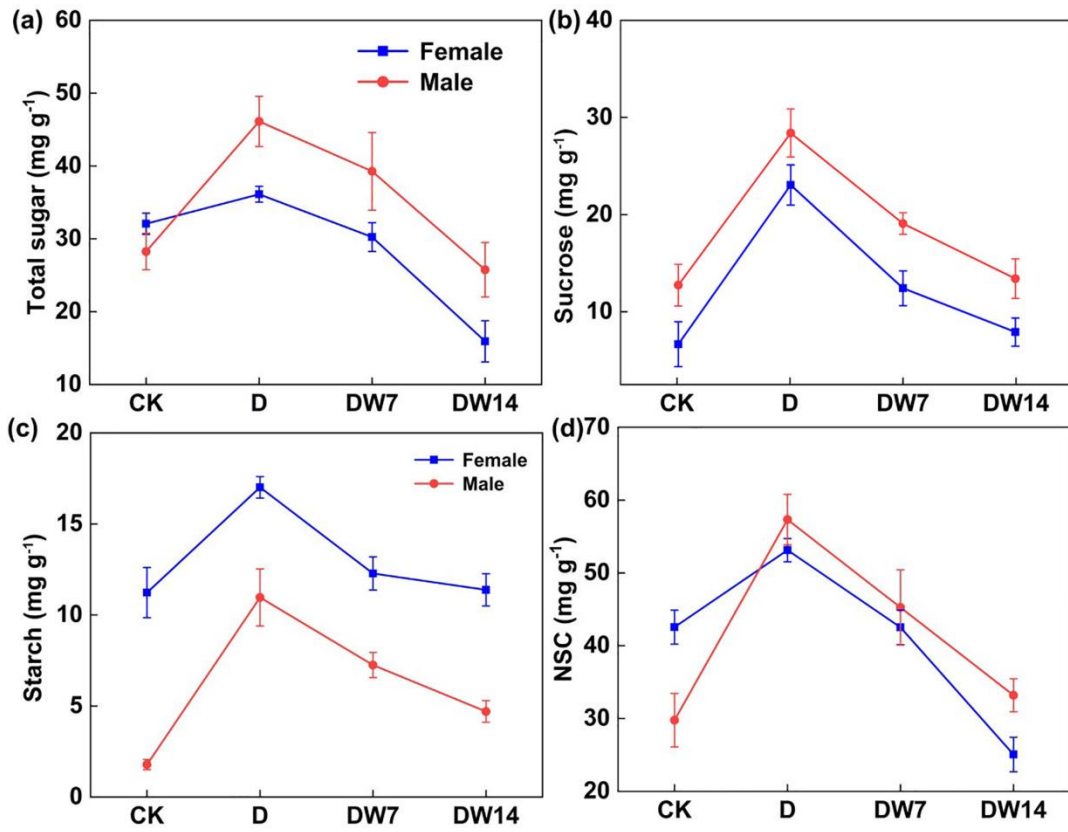
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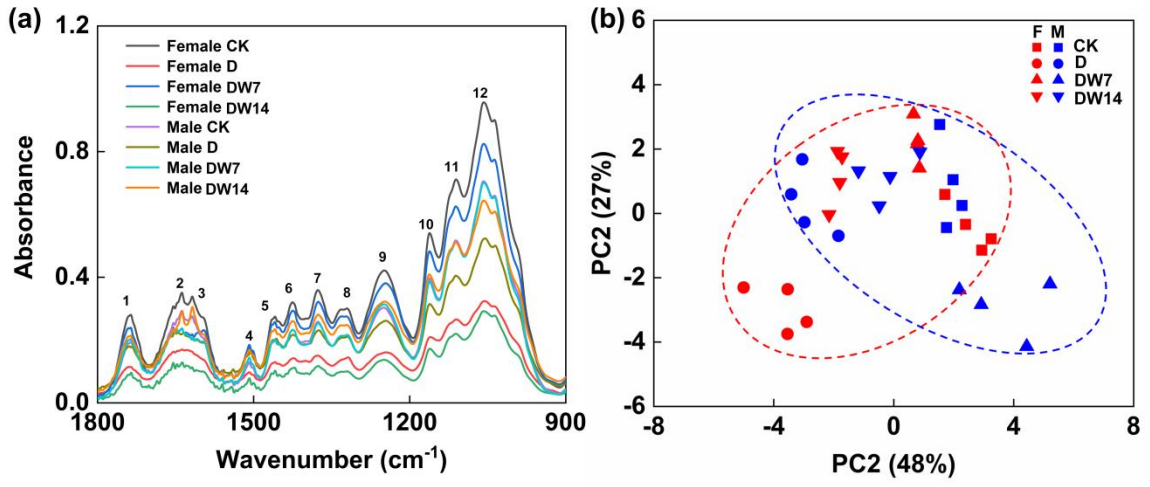
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1023   **Figure 5**
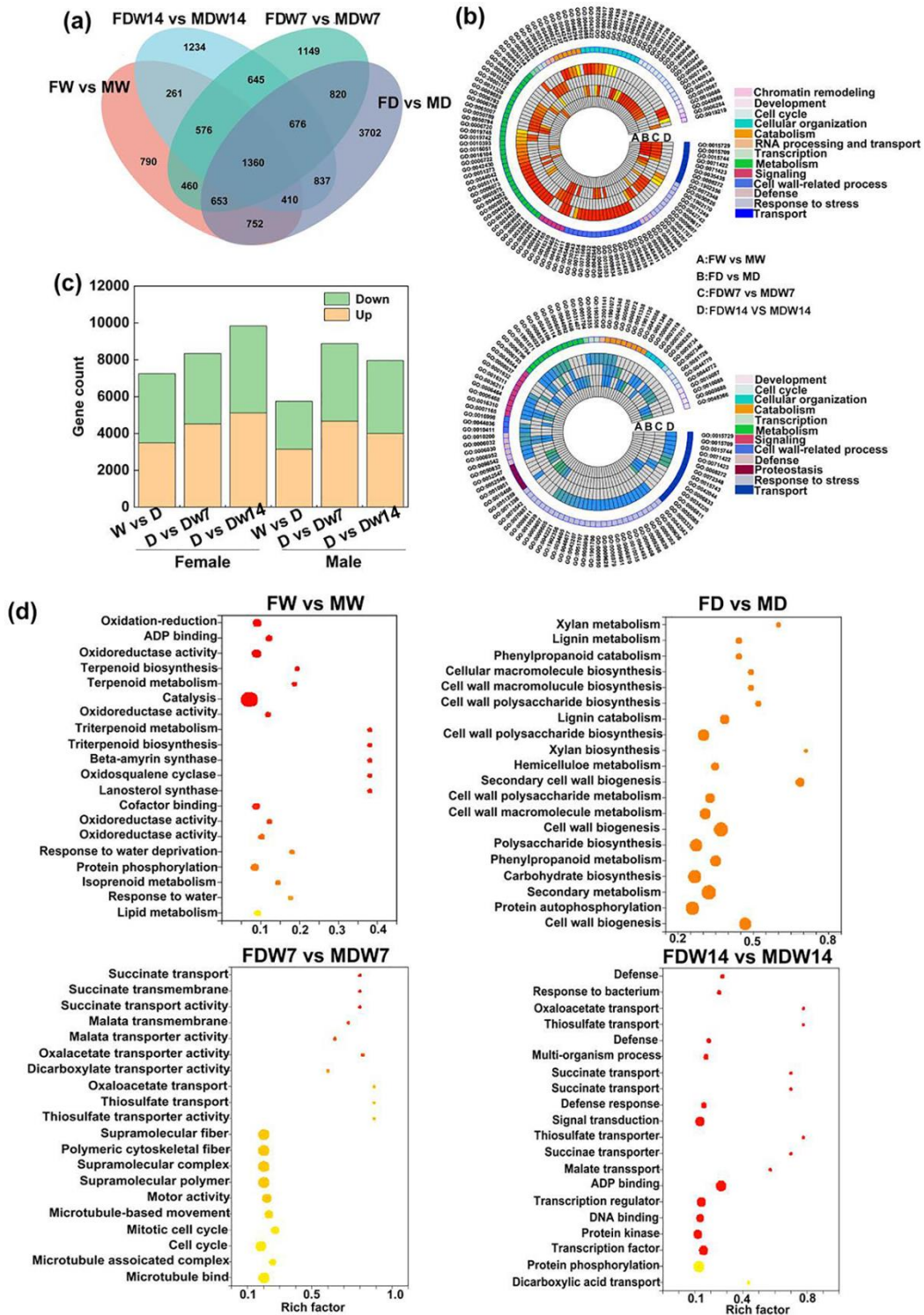
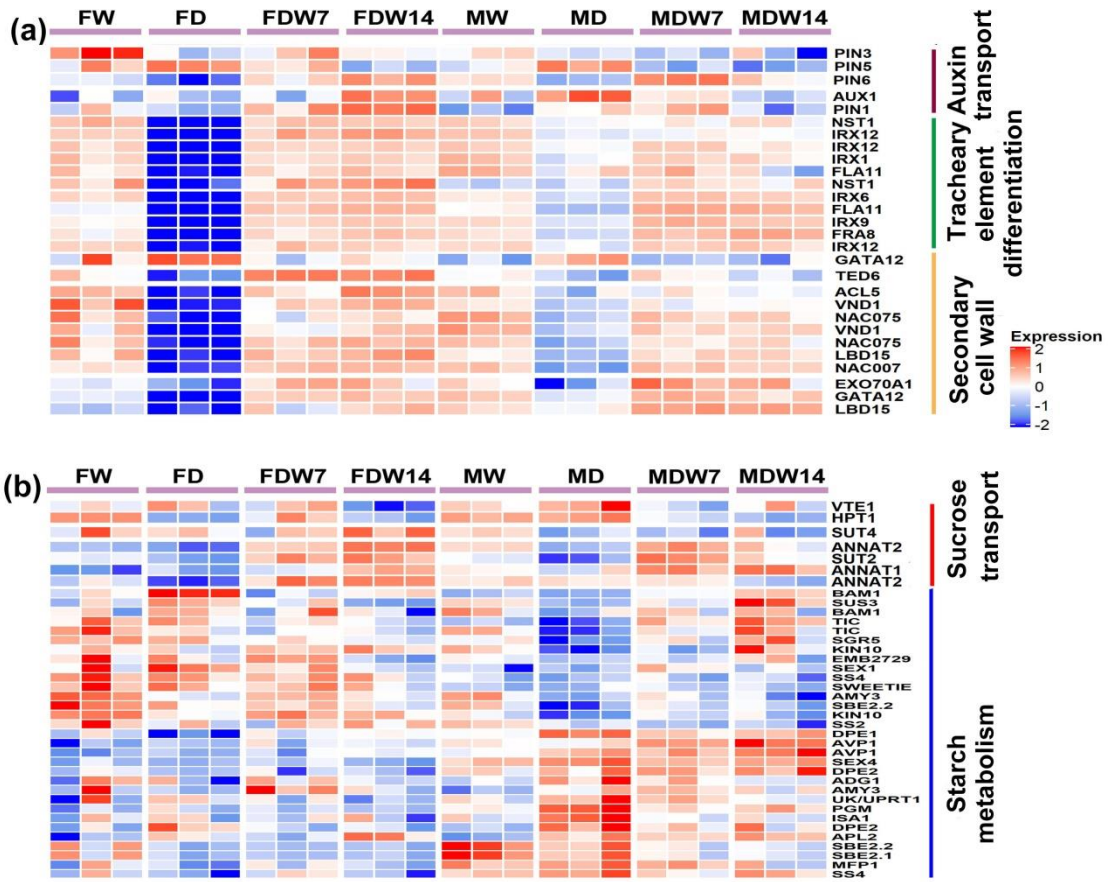1024

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1028　**Figure 6**

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Table 1 Leaf water potential (ψ), leaf dry weight (DW), stem DW, root DW, and wood total sugar, sucrose, starch and non-structural carbohydrates (NSC) in *P. cathayana* females (F) and males (M), as affected by well-watered (CK) or drought stress (D) conditions, and 7 d (DW7) and 14 re-watering (DW14) after drought stress. Different letters in the column indicate significant differences between treatments ($P < 0.05$, Duncan's test).

Values are expressed as means ± SE ($n = 4$).

Treatment	Sex	Leaf ψ (MPa)	Leaf DW (g)	Stem DW (g)	Root DW (g)	Wood			
						Total sugar (mg m^{-1})	Sucrose (mg g^{-1})	Starch (mg g^{-1})	NSC (mg g^{-1})
CK	F	-0.315±0.01d	20.54±0.58a	25.65±1.50a	5.67±0.59a	32.07±1.46cd	6.66±2.32e	11.23±1.38b	42.55±2.33b
D	F	-1.02±0.01a	4.61±0.60g	6.21±0.56d	2.27±0.36c	36.12±1.09bc	23.13±2.08b	17.01±0.59a	53.13±1.59a
DW7	F	-0.721±0.05b	5.77±0.80f	6.99±0.87d	3.22±0.41b	30.24±1.97de	12.45±1.80d	12.28±0.91b	42.52±2.37b
DW14	F	-0.34±0.02d	8.54±0.38d	8.79±0.80c	3.57±0.13b	15.93±2.83f	7.92±1.46e	11.38±0.89b	25.06±2.37d
CK	M	-0.358±0.03d	12.27±0.47b	21.41±1.72b	3.75±0.41b	28.25±2.50de	12.77±2.15d	1.78±0.28e	29.77±3.67c
D	M	-0.792±0.07b	4.85±0.47g	5.89±0.67d	1.86±0.67c	46.12±3.45a	28.50±2.49a	10.96±1.57b	57.33±3.46a
DW7	M	-0.454±0.04c	7.09±0.51e	7.10±0.59d	3.39±0.29b	39.27±5.33b	19.14±1.12c	7.25±0.69c	45.25±5.18b
DW14	M	-0.296±0.06d	10.61±0.78c	10.12±0.83c	5.66±0.50a	25.75±3.74e	13.44±2.05d	4.70±0.59d	33.20±2.27c