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Ammonium and nitrate affect sexually different responses to salt stress in *Populus cathayana*

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Abstract

Nitrogen (N) fertilization is a promising approach to improve salt tolerance. However, it is poorly known how plant sex and inorganic N alter salt stress-induced Na^+ uptake, distribution and tolerance. This study employed *Populus cathayana* Rehder females and males to examine sex-related mechanisms of salt tolerance under nitrate (NO_3^-) and ammonium (NH_4^+) nutrition. Males had a higher root Na^+ efflux, lower root-to-shoot translocation of Na^+ and higher K^+/Na^+, which enhanced salt tolerance under both N forms compared to females. On the other hand, decreased root Na^+ efflux and K^+ retention, and an increased ratio of Na^+ in leaves relative to shoots in females caused greater salt sensitivity. Females receiving NH_4^+ rather than NO_3^- had greater net root Na^+ uptake, K^+ efflux and translocation to the shoots, especially in leaves. In contrast, males receiving NO_3^- rather than NH_4^+ had increased Na^+ translocation to the shoots, especially in the bark, which may narrow the difference in leaf damage by salt stress between N forms despite a higher shoot Na^+ accumulation and lower root Na^+ efflux. Genes related to cell wall synthesis, K^+ and Na^+ transporters and denaturized protein scavenging in the barks showed differential expression between females and males in response to salt stress under both N forms. These results suggested that the regulation of N forms in salt stress tolerance was sex-dependent, which was related to the maintenance of the K^+/Na^+ ratio in tissues, the ability of Na^+ translocation to the shoots, and the transcriptional regulation of bark cell wall and proteolysis profiles.

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***Key words*:** salt stress, sexual differences, nitrogen forms, bark transcriptome.

1. Introduction

A high concentration of salt in soil is an important environmental stress factor, which affects sex ratios, spatial segregation and competitive relations of males and females (Varga and Kytöviita, 2012; Che-Castaldo et al., 2015; Li et al., 2016). Therefore, it is extremely important to discover sexually different response mechanisms to salt stress. Plants have evolved a series of mechanisms to enhance salt tolerance, such as the regulation of osmotic stress, maintenance of K^+/Na^+ and enhancement of antioxidation (Chakraborty et al., 2016; Yang and Guo, 2018). Among these, the maintenance of the K^+/Na^+ ratio is a critical protective mechanism against salt stress and a good criterion for evaluating salt tolerance in plants (de Souza-Miranda et al., 2016; Khan et al., 2016). The maintenance of K^+/Na^+ homeostasis is achieved by limiting Na^+ accumulation and promoting K^+ retention, especially in roots (Chakraborty et al., 2016; Khan et al., 2016). Improving root Na^+ efflux is known to be an effective approach to improve salt tolerance among different plant species and plants with different genotypic backgrounds (Wu et al., 2018; El Mahi et al., 2019). Therefore, it is reasonable to speculate that the maintenance of the root K^+/Na^+ ratio via the promotion of root Na^+ efflux and K^+ retention probably plays a critical role in determining sexually different tolerances to salt stress in dioecious plants.

Na^+ and K^+ absorbed by roots are then transported to shoots, which further deliver them to sink tissues (new shoots and roots) via the bark phloem (Møller et al., 2009; Suzuki et al., 2016; Ishikawa and Shabala, 2019). The accumulation of Na^+ in the leaves damages the leaf photosynthetic apparatus, consequently reducing plant biomass (Kotula et al., 2019). Therefore, a decreased shoot Na^+ level is important for the overall salt tolerance of many plant species. It has been shown that the recirculation of Na^+ from shoots to roots via phloem in the bark is an efficient approach to protect the mesophyll cells of leaves from the Na^+ toxicity, thus enhancing salt tolerance due to the limited vacuolar Na^+ sequestration in most salt-sensitive plants (Kong et al., 2012; Maathuis et al., 2014). Nevertheless, the physiological, biochemical and transcriptomic mechanisms underlying salt tolerance remain unknown for the bark of dioecious poplars. In particular, little is known about the effects of salt stress on

the Na^+ accumulation and reallocation in the bark of poplar females and males receiving different inorganic N forms, NH_4^+ vs. NO_3^-.

Appropriate levels of nutrients play a critical role in improving plant survival under salt stress (Fayez and Bazaid, 2014; Naeem et al., 2017). Particularly, N is known to be a critical factor among mineral nutrients for strengthening plant tolerance to salt stress through the alleviation of ion toxicity, osmotic adjustment, antioxidative enzymatic activation and ion balance maintenance (Esmaili et al., 2008; Nasab et al., 2014; Iqbal et al., 2015; Khan et al., 2016). NO_3^- and NH_4^+ are the main inorganic N sources for plants (Ashraf et al., 2018). The responses of plants to N supply under salt stress mainly depend on whether NO_3^- and/or NH_4^+ are applied in the growth medium (Crawford and Forde, 2002; Ashraf et al., 2018). There may be a lower sensitivity to salt stress in NO_3^--fed plants, or higher sensitivity under NH_4^+ supply (de Souza-Miranda et al., 2016; Ashraf et al., 2018). Some studies suggested that the NO_3^- uptake in roots promotes Na^+ efflux outside of cells and functions in the plant's osmotic adjustment, thus salt tolerance (Marschner, 2011; Hessini et al., 2019). In contrast, other studies have argued that NH_4^+ supplementation improves salt tolerance by enhancing the antioxidation capacity and maintaining a high K^+/Na^+ ratio (Fernández-Crespo et al., 2012; Hessini et al., 2013; de Souza-Miranda et al., 2016; Arghavani et al., 2017). The plant responses to salt stress that differ depending on the N source in high-salt environments are probably based on the differences in NO_3^- and NH_4^+ metabolism and absorption in plants, plant species, and salt composition and concentration (Arghavani et al., 2017; Ashraf et al., 2018). Therefore, understanding the tolerance mechanism of salt stress in different species, and even among genotypes within the same species, is critically important to be able to develop a method to reduce salt toxicity.

Populus species display sexual dimorphisms (Juvany and Munne-Bosch, 2015; Yu et al., 2018; Liu et al., 2021a). Many studies have suggested that dioecious plants exhibit sex-related morphological, physiological and molecular differences in responses to environmental factors, including salt stress (Olano et al., 2017; Liu et al., 2021b). Moreover, sexual differences under abiotic stresses can further be affected by the N status and forms (Liu et al., 2021). Moreover, females and males exhibited N preference in response to sufficient N supply (Chen et al. 2014). Therefore, we expect that sexually different responses to salt stress are probably

associated with N forms. In this study, we employed *P. cathayana* females and males to examine sex-specific adaptive strategies to salt stress under NO_3^- and NH_4^+ supplies. Our questions were following: (1) Is the sexual difference in salt stress tolerance associated with dynamic changes in root Na^+ and K^+ fluxes? (2) Are changes in the physiological and transcriptomic reprogramming of bark responsible for the sexual differences in the salt tolerance of poplar? (3) How do N forms affect the K^+/Na^+ homeostasis and transcriptional changes in the bark? This study will help us to develop a better understanding of the sexually differential response mechanisms to salt stress and N forms.

2. Materials and Methods

2.1 Plant material and experimental design

Cuttings of *P. cathayana* females and males were collected and treated as described previously by Liu et al. (2020). Briefly, *P. cathayana* cuttings were obtained from 45 different trees of each sex, sampled from 15 populations of each sex (three adult trees per population of each sex) in the riparian and valley floor habitats of the Qinghai Province, China (36°31′N, 102°28′E, 3160 m Alt). Annual solar radiation, mean annual rainfall and annual temperature in this area are 4500 MJ m^{-2}, 335 mm and 6.9°C (maximum 38°C, minimum -20°C), respectively (Zhao et al., 2009). Each cutting was obtained from a different individual tree to ensure the independence and randomness of experimental treatments. After sprouting for 5 weeks, healthy and even-sized cuttings were selected and randomly planted into 10-l plastic pots with a 10 kg mixture of sand, vermiculite and perlite (1:1:1) (one cutting per pot). All saplings were grown in a greenhouse under ambient conditions at the Hangzhou Normal University (China, 30.03′N, 120.12′E) with a daytime temperature of 21-25°C, nighttime temperature of 15-18°C, photoperiod of 12-14 h and relative humidity of 76-81% throughout the growth period.

The experiment consisted of eight treatments, four replications for each treatment. The treatments included two sexes (females and males) × two salt regimes (0, 50 mM NaCl) × two N regimes (3.75 mM NO_3^- and 3.75 mM NH_4^+) (Fig. S1; Table S1). The female receiving NO_3^- was designed as the controls. About 100 ml nutrient solution (pH = 6.0) was added to each pot every 2 days containing 3.75 mM NO_3^- or 3.75 NH_4^+ as the N source (Table S1 of

Supplementary data). After the seedlings had grown for further 30 d, uniform cuttings were selected and treated with or without 200 mM NaCl until the final concentration of NaCl reached 50 mM kg^{-1} growth substrate. The control cuttings received the same amount of distilled water.

2.2 Gas exchange measurements

At the end of the experiment, the fourth fully developed leaves from each treatment were used to determine the net photosynthesis rate and stomatal conductance with a portable photosynthesis measuring system (Ll-6400, Li-Cor, Inc.). The measurements were performed on the selected leaves of four replicates from each treatment between 09:00 and 16:30 h. The standard environmental conditions during the measurements of leaf photosynthetic characteristics were as follows: leaf temperature of 25°C, saturating photon quantum flux density (PPFD) of 1200 μmol m^{-2} s^{-1} (with 90% red light and 10% blue light), relative humidity of 75%, airflow rate of 500 μmol s^{-1}, and CO_2 concentration in the cuvette (C_a) of 400 μmol CO_2. The steady-state data were recorded when the steady-state gas exchange was reached. The leaf water potential (ψ) was measured by the pressure chamber (Corvallis).

2.3 Determination of MDA and $O_2^{\cdot-}$ levels

After harvesting, the fully expanded leaves of each treatment were used to measure malondialdehyde (MDA), superoxide radical ($O_2^{\cdot-}$) levels in leaves, roots and barks, as described by Bi et al. (2020) and Liu et al. (2020). For MDA measurements, 0.2 g samples were ground finely with liquid N and then extracted with 5% trichloroacetic acid (TCA). Thereafter, clear supernatant of 0.2 ml was finely mixed with 1 ml thiobarbituric acid (dissolved in 10% TCA). The mixture was heated in boiling water for 15 min and then cooled quickly on ice. The mixture was centrifuged at 12000 *g* for 10 min and then the clear supernatant was measured colorimetrically at 450, 320 and 600 nm. MDA was calculated as follows: c (nM) = 6.45 (OD_{532}–OD_{600}) –0.56×OD_{450}. For $O_2^{\cdot-}$, 0.2 g samples were ground with liquid N and extracted with the extraction mixture (50 mM Na_2HPO_4-NaH_2PO_4, pH 7.8) and then centrifuged at 12000 *g* for 10 min. The supernatant of 0.5 ml was reacted with 2 ml hydroxylamine hydrochloride for 30 min at 25°C. $O_2^{\cdot-}$ was measured colorimetrically at 540

nm by adding 1 ml of 1% sulfanilamide and 1 ml of 0.2% N-(1-naphthyl)-ethylenediamine.

2.4 Determination of Na^+ and K^+

Samples of leaves, roots, stem and bark were washed thoroughly with deionized water, followed by drying at 75°C until reaching a constant weight. Dry samples were ground and digested with 3:1 (v/v) of HNO_3 and $HClO_4$. The Na^+ and K^+ concentrations were determined using ICP-MS (inductively coupled plasma mass spectrometer Agilent 7500a, Agilent Technologies) followed by the method of Yang et al. (2019).

2.5 Measurements of net Na^+ and K^+ fluxes in roots

Net Na^+ and K^+ fluxes were measured with a non-invasive microelectrode ion flux measurement system (NMT) with an IPA-2 Ion/Polarographic amplifier (Applicable Electronics, Inc., USA) according to Zhao et al. (2018) and Sun et al. (2009). The measuring electrode and reference electrode were connected to an before the flux measurements, Na^+ and K^+ microelectrodes were respectively calibrated with Na^+ (Na^+: 0.1, 0.5 and 1.0 mM) and K^+ calculation series (K^+: 0.1, 0.5 and 1.0 mM). K^+ measuring solution (mM): NaCl (0.1), KCl (0.5), $CaCl_2$ (0.1) and $MgCl_2$ (0.1), pH, 6.0; Na^+ measuring solution (mM): NaCl (0.1), KCl (0.1), $CaCl_2$ (0.1) and $MgCl_2$ (0.1), pH, 6.0. Glass micropipettes were filled with a backfilling solution (Na^+, 100 mM NaCl; K^+, 100 mM KCl). The micropipettes were filled with selective liquid ion-exchange cocktails (LIXs). DRIREF-2 was used as the reference electrode (World Precision Instruments).

After that, root segments (2 cm from the root apex) of *P. cathayana* females and males were sampled from all eight treatments described above. The root samples were rinsed with deionized water and immediately equilibrated for 30 min in the following basic measuring solutions: (1) K^+ measuring solutions (mM): 0.1 mM NaCl, 0.1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.5 mM KCl, pH 6.0; (2) Na^+ measuring solutions (mM): 0.1 mM NaCl, 0.1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.1 mM KCl, pH 6.0. After equilibration, root samples were transferred to a measuring chamber with the fresh measuring solution and immobilized at their bases. The steady-state fluxes of K^+ and Na^+ were recorded individually with NMT selective electrodes by moving the electrode between two positions close to the root surface at 0.3-0.5 Hz

frequency. The K^+ and Na^+ fluxes of root apical (0-2 mm from root apex, measuring point per 300 μm, a total of 6 measuring positions) and root mature zones (about 10 mm from root apex, a total of 6 measuring positions) were continually recorded for 30 min. The K^+ and Na^+ fluxes of root apical and root mature zones were the average values of 6 measuring points. Each treatment was replicated four times and six root segments were sampled from each plant.

For transient Na^+ and K^+ recordings, the roots were rinsed with deionized water and then immediately exposed to the measuring solution for 30 min. After equilibration, root samples were transferred to a measuring chamber with a fresh measuring solution and immediately immobilized at their bases. The root Na^+ and K^+ fluxes were recorded individually with NMT selective electrodes for 5-6 min by moving the electrode between two positions close to the root surface at 0.3-0.5 Hz frequency. Thereafter, a salt shock (100 mM NaCl) was performed by adding NaCl stock (10 M, pH 6.0, adjusted with NaOH and HCl). The transient root Na^+ and K^+ kinetic recording was restarted and continued for 30 min. Each treatment was replicated four times and six root segments were sampled from each plant.

2.6 RNA sequencing

Fine powder of bark (approximately 50 mg) was used for RNA extraction, according to He et al. (2013). Total RNA was extracted using TRIzol reagent (Invitrogen), and genomic DNA was digested using DNase I according to the manufacturer's instructions (Takara). The quality of RNA was determined using the Agilent 2100 Bioanalyser (Agilent Technologies Inc.), and RNA was quantified by the NanoDrop spectrophotometer 2000 (NanoDrop Technologies Inc.). A total of 24 cDNA libraries were constructed with nanoball-based nanoarrays, and the stepwise sequencing was conducted with a combinational probe-anchor synthesis sequencing (BGI) (Yu et al., 2018). Briefly, the total RNA was used to capture poly (A) mRNA with poly-T oligo-attached magnetic beads (Thermo Fisher Scientific), and then cut into short sequences by heat and treatment with divalent cations. After that, cDNA was synthesized with random primers and reverse transcriptase using small mRNA fragments as templates. Double-strand cDNA was synthesized using a SuperScript double-stranded cDNA synthesis kit (Invitrogen) according to the manufacturer's protocol. Sequencing libraries were synthesized using the TruSeqTM RNA sample preparation kit (Illumina). The products were

then purified and amplified by PCR using Phusion DNA polymerase (NEB) for 15 PCR cycles to create the final cDNA libraries. The libraries were sequenced with the Illumina HiSeq 4000 Systems. The single-strand DNA circles were used to generate the DNA nanoballs by rolling circle replication. The DNA nanoballs were loaded into the nanoarrays and 100-bp pair-end reads were read with the BGISEQ-500 platform.

2.7 Sequencing, data processing and analysis

Sequencing reads were purified by removing adaptors, regions with more than 5% unknown N bases and low-quality reads using the SOAPnuke software (BGI). 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). Differentially expressed genes (DEGs) were identified and calculated based on the transcripts per kilobase of the exon model per million mapped reads (TPM). Gene abundances were quantified with RSEM (http://deweylab.biostat.wisc.edu/rsem/). The coding sequence of *P. trichocarpa* was blasted against the closest *Arabidopsis* homolog (Arabidopsis Genome Initiative identification) with PoplarGene (http://bioinformatics.caf.ac.cn/PoplarGene/gene) and annotated using the *Arabidopsis* Information Resource (https://www.arabidopsis.org/). The functional enrichment analysis was performed using Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do). According to the negative binomial distribution, the differential expression analysis was performed with eight groups (FA, MA, FAS, MAS, FN, MN, FNS, MNS, three biological replicates per group) by the DEseq 2 package (Love et al., 2014). The P_{adj}-value $\leq$ 0.05 and $|\log_2$ fold-change$| \geq 1$ were the thresholds to determine significant differences in gene expression. The sequencing data were submitted to NCBI (BioProject accession number: PRJNA629449).

In addition, we used a weighted gene co-expression network analysis (WGCNA) to identify the co-expression gene modules (Langfelder and Horvath, 2008, Marín-de la Rosa et al., 2019). The matrix of pairwise correlations across all treatments was constructed with a soft-thresholding power (β = 18). The average linkage hierarchical clustering was created by topological overlap-based dissimilarity measures. In this study, we identified 12 modules (Fig.

S2) by a dynamic tree-cutting algorithm and merging threshold function at 0.2. The Blast2go (v3.1) and Python goatools package (https://github.com/tanghaibao/goatools) were used for Gene Ontology (GO) annotations and GO enrichment analysis, respectively. Heatmaps were plotted with up-regulated or down-regulated genes between "females versus males" from the results of GO functional enrichments. The *P*-value was set to <0.01 (heatmap.2 in the 'gplots' package in R).

2.8 Quantitative PCR analyses

The total RNA of the bark was extracted using TRIzol reagent (Invitrogen). The first cDNA strand was synthesized from RNA samples using the PrimeScript reverse transcription (RT) reagent kits (Takara). The mRNA levels were detected with One Step TB Green™ PrimeScript™ RT-PCR kits in a 25 μl reaction system following pairs of gene-specific primers (Table S2). The relative expression levels of genes were calculated according to $\Delta\Delta C_t$ (Liu et al., 2017). *TUB4.1* was chosen as the housekeeping gene (He et al., 2013).

2.9 Statistical analyses

Statistical analyses were conducted 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 $P < 0.05$.

3. Results

3.1 Sexually different stress tolerance and plant growth characters

Salt stress induced leaf chlorosis in females but not in males, irrespective of N forms (Fig. 1A). Leaf photosynthesis, leaf potential and stomatal conductance in females were higher than those in males under both NO_3^- and NH_4^+ supply under salt stress (Fig. 1). The chlorosis of leaves in females caused by salt stress was more pronounced under an NH_4^+ supply than under a NO_3^- supply, while no significant chlorosis was found in males under either N form (Fig. 1A). Both the photosynthesis rate and leaf water potential decreased significantly in females under both N forms when exposed to salt stress, while there was no significant difference in males under either N form (except for leaf photosynthesis under NH_4^+ supply)

(Fig. 1B-C). Salt stress reduced the stomatal conductance by 59% and 36% in females and males, respectively, under NO_3^-, and by 84% in females under NH_4^+ when compared to controls (Fig. 1D).

Additionally, salt stress increased the malondialdehyde (MDA) and superoxide radical ($O_2^{\cdot-}$) levels in leaves and barks, but did not affect these two parameters in leaves and barks of males under NO_3^- and NH_4^+ supply (Table S3). Moreover, salt stress-induced increases in MDA and $O_2^{\cdot-}$ levels of leaves and barks were more significant in females than in males under both N forms. In roots, salt stress in females increased MDA and $O_2^{\cdot-}$ levels under NO_3^- supply, and $O_2^{\cdot-}$ levels under NH_4^+ supply, but it did not affect MDA under NO_3^- supply (Table S3). In males, salt stress increased MDA and $O_2^{\cdot-}$ levels in roots under both N forms (except for root $O_2^{\cdot-}$ levels under a NO_3^- supply). Moreover, females had higher root $O_2^{\cdot-}$ levels than males under NO_3^- supply, whereas there was no difference in root $O_2^{\cdot-}$ levels between the sexes when exposed to salt stress. Salt stress induced a higher root MDA in males than in females under an NH_4^+ supply, but there was no difference in root MDA between the sexes under salt stress (Table S3).

3.2 Tissue Na^+, and K^+/Na^+ ratio

Salt stress increased the Na^+ concentrations in the leaves, bark, wood and roots of both sexes (Fig. 2). Females had higher leaf, bark, wood and root Na^+ concentrations under both NO_3^- and NH_4^+ supply when exposed to salt stress (Fig. 2). The NH_4^+ supply in females increased the Na^+ accumulation in leaves, wood and roots compared to a NO_3^- supply under salt stress, whereas it decreased Na^+ accumulation in bark when exposed to salt stress (Fig. 2). In contrast, males had greater Na^+ accumulation in leaves, bark, wood and roots under NO_3^- supply than under NH_4^+ supply when exposed to salt stress. In addition, salt stress significantly decreased the ratio of K^+ to Na^+ in leaves, bark and roots of males and females under both N forms (Fig. 2). Salt stress decreased the K^+/Na^+ ratios in the wood of females under both N forms and in the wood of males under NO_3^- supply, but it increased the wood K^+/Na^+ in males under NH_4^+ supply when compared to controls (without a 50 mM NaCl treatment) (Fig. 2). Moreover, females had lower K^+/Na^+ ratios in leaves, roots, bark and wood than males under salt stress. In males, the K^+/Na^+ ratios of leaves, bark, wood and roots

were higher under an NH_4^+ supply than under a NO_3^- supply, while there was no significant difference in K^+/Na^+ ratio of female leaves, bark, wood and roots between NH_4^+ and NO_3^- supplies (Fig. 2).

Females had higher shoots to root, leaves to bark and leaves to shoot Na^+ ratios than males when exposed to salt stress under both N forms (Fig. 3A,B,D). However, the bark to shoot Na^+ ratio of females was lower than that of males under both N forms (Fig. 3C). Females receiving NH_4^+ rather than NO_3^-, had higher Na^+ levels in shoots relative to roots, in leaves relative to bark and in leaves relative to shoot, but lower Na^+ levels in bark relative to shoot. In contrast, NH_4^+-fed males had higher Na^+ levels in leaves relative to shoot, but a lower Na^+ level in bark relative to shoot, and similar Na^+ levels in shoot, roots, leaves and bark when compared to those under NO_3^- supply.

3.3 Na^+ and K^+ fluxes in roots

Salt stress caused a net Na^+ efflux in the root apical zones of females exposed to a NO_3^--N treatment, and in those of males treated with either N form (Fig. 4). Furthermore, higher net Na^+ effluxes in root apical zones were observed in males than in females under an NH_4^+-N treatment, and there was no significant difference between sexes under a NO_3^--N treatment. Salt stress induced a high Na^+ influx in females and Na^+ efflux in males in mature zones of roots under both N forms (Fig. 4A). In addition, salt stress promoted K^+ effluxes in root apical zones of females under both N forms, while K^+ influx in root apical zones of males decreased by salt stress under an NH_4^+-N treatment but was not affected by salt stress under a NO_3^--N supply (Fig. 4B). However, females exhibited an enhanced K^+ efflux under NH_4^+ but not under NO_3^- in mature root zones under salt stress (Fig. 4B). In comparison, salt stress-induced large net K^+ influxes in the mature root zones of males under both N forms.

After exposure to salt stress, the root apical and mature zones showed an immediate increase in the Na^+ efflux, followed by a continuous drift toward stability in both sexes under both N forms (Fig. 4C). Salt shock treatments induced a greater Na^+ efflux in the apical root zones of males under an NH_4^+-N supply than under NO_3^--N (Fig. 4C). In contrast, females exhibited a greater Na^+ efflux in apical root zones under NO_3^--N than under NH_4^+-N (Fig. 4C). Males had a greater Na^+ efflux in the apical root zones than females in response to salt shock under both

N forms. In addition, salt-shocked males exhibited a great net Na^+ efflux in mature root zones under a NO_3^--N supply. In contrast, salt shock slightly increased the Na^+ efflux in mature root zones of females for a short time, followed by a stable level under both N forms.

A NaCl shock induced net K^+ effluxes in apical root zones of both sexes under both N forms, and such effects were more significant in females supplied with NH_4^+-N instead of NO_3^--N (Fig. 4D). Salt shock-induced net K^+ effluxes were greater in the apical root zones of females than in those of males, especially under an NH_4^+-N supply. Additionally, salt shock caused greater K^+ effluxes in the mature root zones of males under a NO_3^--N supply than under NH_4^+-N; the contrary was true for females under both N forms.

3.4 Transcriptional regulation mechanisms in female and male bark affect acclimation to salt stress under N forms

The numbers of differentially expressed genes (DEGs) identified in female and male barks were 4784 between FA (Female+NH_4^+) and MA (Male+NH_4^+), 2335 between FAS (Female+NH_4^++NaCl) and MAS (Male+NH_4^++NaCl), 1317 between FN (Female+NO_3^-) and MN (Male+NO_3^-), and 6198 between FNS (Female+NO_3^-+NaCl) and MNS (Male+NO_3^-+NaCl), while 3709 common genes between FA versus MA and FAS versus MAS", and 2368 common genes between FN versus MN and FNS versus MNS were detected (Fig. 5A-B). In addition, 4966 DEGs between FA and FAS, 1440 DEGs between MA and MAS, 2151 DEGs between FN and FNS, and 750 DEGs between MN and MNS were discovered, and 382 common genes between FA versus FAS and MA versus MAS, and 234 common genes between FA versus FAS and MA versus MAS were identified in bark (Fig. 5A-B).

We then analyzed the functional enrichment of DEGs based on the corresponding GO annotations. This study found that the significantly up-regulated GO terms between controls and salt stress concerned mainly males, especially under an NH_4^+ supply (Fig. 5C). Conversely, the significantly down-regulated GO terms in response to salt stress were enriched in females, especially under an NH_4^+ supply (Fig. 5D). Furthermore, the up-regulated genes in NH_4^+-fed males under salt stress were mainly involved in the synthesis and metabolism of primary and secondary cell walls (xylan, lignin, polysaccharides and

cellular carbohydrates), as well as in cell construction and protein modification (Fig. 5C). The up-regulated genes in males under salt stress under a NO_3^- supply were involved in cell wall synthesis and metabolism, as well as in aldehyde catabolic processes (Fig. 5C; Table S4). In contrast, the down-regulated genes under salt stress in females under NH_4^+ were mainly involved in cell wall synthesis and metabolism, light-harvesting and photosynthesis, organic acid transport, thiosulfate and phosphate transmembrane transport, responses to abiotic stimulus and protein modifications (Fig. 5D; Table S5). The down-regulated genes in NO_3^--fed females under salt stress were enriched in cell wall synthesis and metabolism, organic acid transport, thiosulfate and phosphate transmembrane transport, responses to abiotic stress and regulation of immune responses (Fig. 5D; Table S5). In addition, we validated the data of RNA-seq by qRT-PCR and obtained a good correlation between RNA-seq and qRT-PCR results, which confirmed the reliability of the RNA-seq profiling (Figs S3-S4).

In addition, we employed a weighted gene co-expression network analysis (WGCNA) to analyze Pearson correlations between module eigengenes (MEs), sexes, N forms and salt stress (Fig. 6). We defined 12 modules containing highly correlated variables, which are potentially involved in the same biological process (Fig. 6A,C). These 12 modules were defined and color-labeled based on similar expression patterns. Among them, seven modules were significantly correlated with sex, salt stress and N form (P<0.05) (Fig. 6B). By analyzing gene functions and pathway enrichment, we found that sex-dependent and salt-independent modules were blue, brown and green. Genes in blue, brown and green modules were enriched in protein metabolism and modification, isoprenoid biosynthesis, as well as in responses to proteins and stimulus (Fig. 6C). The sex- and salt-dependent genes were enriched in red and yellow modules, which participated in pyrimidine nucleotide metabolism, tissue development, mitotic cell cycle, cell wall synthesis and metabolism. The sex-independent and salt stress-dependent modules were enriched in lignin metabolism, photosynthesis and microtubule nucleation (purple, black and grey modules) (Fig. 6C). Some genes relate to Na^+ efflux, such as *SOS1*, *SOS3* and *NHX2*, K^+ transporter *KT1*, H^+-ATP ase *VHA1.1* were up-regulated under salt stress in males, while genes related to K^+ transporter *KAT1*, *KUP1* and *KUP2/6* were up-regulated under salt stress in females, under both N forms

(Fig. 7A-B).

4. Discussion

4.1 N forms affect sex-specific K^+/Na^+ homeostasis

We discovered that *Populus cathayana* displays sex-specific responses to abiotic stresses and females usually exhibit stronger tolerance to abiotic stresses than males (Liu et al., 2021a). In this study, females have a higher sensitivity to salt stress than males under both N forms, especially when receiving NO_3^- rather than NH_4^+, while males have similar tolerance to salt stress under NO_3^- relative to NH_4^+ supply (Fig. 1). The higher salt sensitivity of females largely depended on the lower tissue K^+/Na^+ ratio when compared to males under both N forms (Fig. 2). Higher K^+/Na^+ sensitivity is regarded as a critical protective mechanism against salt stress, and it is a good criterion for evaluating tolerance to salt stress in plants (de Souza-Miranda et al., 2016).

The roots, as the first checkpoint for the Na^+ and K^+ uptake from the soil, have a key role in reducing the overall plant Na^+ over-accumulation and achieving K retention (Chakraborty et al., 2016; Khan et al., 2016). Females had more thin roots and larger root biomass, which intensively explored the soil around their roots (Liu et al., 2021b), thus having a higher ability for nutrient absorption. However, this acquisitive strategy in females may not distinguish ions, which are toxic or necessary for plants (Liu et al., 2021a). Additionally, sex-specific root K^+/Na^+ ratios could be explained by K^+ and Na^+ fluxes in the roots. The lower K^+ retention and lower Na^+ efflux in females relative to males are the main reason for the lower K^+/Na^+ ratio in roots (Fig. 4). A lower Na^+ accumulation in the root apex is expected because of the predominant expression of Na^+/H^+ exchangers SOS1 in the root apexes (Shi et al., 2000). Therefore, the root apex should have a better ability of Na^+ exclusion relative to other root regions. Indeed, the root apical zone is the main region determining Na^+ exclusion in both sexes under both N forms (Fig. 4). The higher Na^+ efflux in the root apex of males would lead to a lower tissue Na^+ level relative to females (Figs 2, 4). Correspondingly, the salt shock-induced instantaneous Na^+ efflux was greater in the apical zones of male roots, especially under an NH_4^+ supply relative to females, further demonstrating the role of root apex in Na^+ efflux (Fig. 4). Interestingly, we found that females and males exhibited opposite

directions of root Na^+ flux in mature zones under both N forms. Roots in mature zones of males still exclude Na^+ at the lower flux rate relative to the root apex, while females exhibit a higher net Na^+ influx, suggesting that the mature zones of female roots play critical roles in absorbing Na^+. Such roles of the mature zone of roots in the Na^+ uptake are probably associated with the dense root hairs in these regions, but this requires further research.

Effective K^+ retention in roots is another important strategy for achieving salt tolerance in plants (Shabala et al., 2016). Consistently, the strong efflux of K^+ in apical and mature zones of female roots induced by salt stress would lead to a loss of K^+ in roots and a decreased K^+ ratio in tissues under both N forms. Males with a lower K^+ efflux in roots show stronger K^+ retention in roots when compared to females (Fig. 4). Under a salt shock treatment, males recovered more easily their capacity of K^+ retention and had a stronger buffer capacity in response to salt stress (Fig. 4). These results suggested that K^+ influx was enough to offset K^+ efflux in both root regions of males; the contrary was true for females under both N forms. It appeared that the sexual difference under salt stress differs greatly depending on K^+ flux (efflux in females and influx in males); N forms mainly affected the ability of K^+ efflux in the roots of females.

An adequate N application under salt stress is an effective strategy to sustain or improve the production of salt-polluted soils and plant tolerance (Ashraf et al., 2018; Soliman et al., 2020). The NH_4^+ and NO_3^- forms have protective roles against salt stress (Ashraf et al., 2018). In this study, females receiving NH_4^+ rather than NO_3^- have higher K^+/Na^+, mainly derived from a higher Na^+ efflux in root apex and a lower Na^+ uptake, as well as a lower net K^+ flux, while males receiving NH_4^+ rather than NO_3^- have similar net K^+ influx levels in both root regions and a higher apical Na^+ exclusion. The N form-specific differences in root net Na^+ and K^+ flux, thus shoot Na^+ accumulation, might be explained by the absorption and metabolism of NH_4^+ and NO_3^-. Females had higher energy requirements for growth and reproduction (Juvany and Munne-Bosch, 2015). Such higher energy requirements in females relative to males probably inherently existed before reproductive maturity (Randriamanana et al., 2014). Moreover, NH_4^+ assimilation takes place primarily in the roots, while NO_3^- assimilation takes place in the shoots (Ashraf et al., 2018). The higher energy requirement for NH_4^+ assimilation in the roots of females may lead to the reduced energy for Na^+ exclusion, thus higher Na

accumulation in females under NH_4^+ supply than NO_3^- supply. Besides, the higher preference for NO_3^- relative to NH_4^+ in poplar females (Chen et al., 2014) promoted plant growth and elevated plant resistance to salt stress (Fig. 1). For males, the lower energy requirement for growth and reproduction relative to females gives them more energy for Na^+ efflux in roots under NH_4^+ supply than NO_3^- supply, as shown by higher Na^+ efflux in the apical root (Fig. 4). NH_4^+ competed with Na^+ for the absorption sites in plasma membranes of roots, thus reducing Na^+ uptake and causing an elevated K^+/Na^+ ratio (Alvarez-Pizarro et al., 2011; Ashraf et al., 2018). However, whether there is an antagonistic effect of NH_4^+ on Na^+ uptake in males still needs to be clarified in future studies. Overall, these results suggested that the effects of N forms on K^+ and Na^+ influx and thus on the maintenance of the K^+/Na^+ ratio largely depend on sex-specific responses to salt stress.

4.2 Responses in bark under different N forms reflect the molecular mechanism to salt stress tolerance

It is notable that males receiving NH_4^+ rather than NO_3^- have similar Na^+ levels in the shoots and roots, and higher leaf Na^+ despite their higher root Na^+ efflux and similar Na^+ uptake. In females, the ratio of Na^+ levels in shoots to roots under NH_4^+ was 13% higher and the ratio of Na^+ in leaves to roots was 34% higher under $NH4^+$ than under NO_3^- supply under salt stress (Figs 2, 3). These results imply that other pathways and/or mechanisms may participate in the shoot Na^+ reallocation. The bark is composed of phloem and other tissues, functioning in nutrient delivery to sink tissues, thereby resulting in an internal Na^+ reallocation from the shoots to roots (Wolf et al., 1991; Wu et al., 2015). Na^+ accumulation in the bark does not only reduce leaf Na^+ accumulation, but also more Na^+ becomes reallocated into new developing leaves and roots (Shabala and Munns, 2012). Na^+ recirculation from shoots to roots via the phloem has been shown to play an important role in the salt tolerance of plants (Berthomieu et al., 2003; Tester and Davenport, 2003).

To gather support for sex-specific patterns in the role of tree barks in salt tolerance and more detailed insight into the corresponding molecular mechanism, we conducted detailed transcriptional profiling in females and males under both N forms. Among differentially expressed genes, we found that males' transcriptional responses were greater and more

positive than in females when exposed to salt stress, especially under an NH_4^+ supply; the contrary was true for females, especially under an NH_4^+ supply (Fig. 5). These data further supported the conclusion that males show more positive responses to salt stress relative to females. Overall, females and males exhibited sexual dimorphism in transcriptional profiling of tree barks, which was salt-and N-independent (Fig. 6). Females usually had higher expressions of genes (brown and green modules) related to the isoprenoid biosynthetic process, transmembrane transport, lipid metabolism, responses to light and protein folding relative to males (salt-independent), which might be associated with a higher demand for nutrients and secondary metabolites. In contrast, males had a higher expression of genes (blue module) related to signal transduction, protein phosphorylation and metabolism, transcription regulation (salt-independent response), which are associated with faster responses of males to stresses.

Salt stress affects sex-specific transcriptional changes in the bark, which might be associated with a sexual difference in salt sensitivity. Genes (red module) related to pyrimiding nucleoside metabolism, cytidine catabolism, and RNA modification are up-regulated by salt stress in females, but these genes show higher expressions in males irrespective of salt stress. These results suggest that males could trigger molecular and phenotypic responses much earlier than females, irrespective of salt stress. By contrast, most genes up-regulated by salt stress in males (yellow module) were related to cell wall biogenesis and metabolism, as well as to cellular polysaccharide biosynthesis. Most of these expressions were higher in males than in females, indicating that males perceived salt stress more effectively and, consequently, modified the expression of genes in the downstream signaling network (Figs 5, 6). Moreover, cell wall integrity is a critical factor determining plant growth, salt tolerance, and salt stress sensing and responses (Feng et al., 2018; Zhao et al., 2018). Cell wall remodeling and integrity facilitate Na^+ sequestration into the apoplast space from the cell cytoplasm and enhance salt tolerance (Oliveira et al., 2020; Rui and Dinneny, 2020).

Since the cell wall is highly important for salt stress tolerance, one would expect that genes related to cell wall biosynthesis and metabolism would be down-regulated in females and up-regulated in males. Indeed, the most up-regulated genes induced by salt stress exist in the male barks and are mainly enriched in cell wall synthesis and metabolism, as well as in

carbohydrate metabolism, especially under NH_4^+ supply, which is probably involved in salt stress tolerance in males (Fig. 5; Tables S4). Cell wall integrity is a highly important factor for plant growth and salt stress tolerance. The mutants with defectivecell wall integrity are sensitive to salt stress in plants (Byrt et al., 2018). For example, *CESA6*, a key component of the cellulose synthase complex, is involved in the maintenance of cell wall intensity (Kim and Barbara, 2008; Zhang et al., 2016). Indeed, we found that the expression of *CESA6* was significantly higher in males than in females, suggesting that males maintained better the cell wall intensity and had higher salt tolerance (Fig. S5). Moreover, the maintenance of cell turgor plays a critical role in cell growth and acclimation to salt stress. Adaptation to osmotic changes needs to alter the extensibility of cell wall, which involves the expansins, endo-1,4-β-glucanases (EGases) and xyloglucan endo-β-transglucosylase/hydrolases (XETs/XTHs) and pectin methylesterases. We found that the expression of genes related to some members of the XTH family, such as *XTH8*, *9*, *10*, *28*, *32*, *33* and endo-1,4-β-glucanases genes *EGL1* was significantly higher in males than females irrespective of salt stress under both N forms, underpinning the roles of cell wall component in acclimation to salt stress for males (Fig. 3; Fig. S5). The higher expression of genes related to lignin and suberin, such as *C4H*, *CLI1*, *IRX1* and *IRX3*, in males with salt stress under both N forms than in females can benefit the poplar male by preventing water loss and Na^+ transport (Fig. S5; Byrt et al., 2018). Moreover, the binding of Na^+ ions to negatively charges cell wall components, such as the cross-linking of some pectin molecules, may limit its movement to the stele and/or the shoots. The higher expression of genes related to pectins, such as *RHD3* and *GAUT12*, might sequestrate Na^+ in the cell aplastic space and reduce its toxicity in males under both N forms in response to salt stress. Thus, the common up-regulation of the cell wall synthesis and metabolism in the bark under both N forms might reflect a higher tolerance to salt stress relative to females.

Furthermore, the higher sensitivity to salt stress in females may be partly associated with the disturbance of proteostasis and photosynthesis processes in the bark (Fig. 5). Salt stress causes protein denaturation and damage in plants, and the removal of degraded proteins is considered as one of the critical strategies for cell survival (Smalle and Vierstra, 2004; Cui et al., 2012; Tan et al., 2016). Indeed, we found that salt stress down-regulated genes related to

protein degradation in the barks of females, which probably increased the denaturized proteins accumulation in cells, and thus leading to cell damage and sensitivity to salt stress in females. The genes related to response to unfolded protein, protein phosphorylation, de novo protein folding, such as genes related to IRE1-mediated unfolded protein response, *IRE1A* and *IRE1B*, were down-regulated by salt stress in the barks of females (Table S5). The down-regulation of genes related to unfolded protein response, such as *BZIP28*, inhibited the degradation of unfolded proteins. Additionally, the damage to the photosynthetic process in the female bark, especially under an NH_4^+ supply, is probably correlated with the greater female sensitivity to salt stress. In addition, bark photosynthesis may have a role in carbon fixation and maintenance of physiological activities in woody tissues (Cernusak and Cheesman, 2015; Vandegehuchte et al., 2015).

In addition, barks play an important role in the maintenance of K^+/Na^+ sequestration and reallocation to roots. Na^+/H^+ exchangers located in the plasma membrane (*SOS1*) and tonoplast *(NHX*) participate in the removal of excess Na^+ from the cytosol, which is activated by the SOS2-SOS3 complex (Bassil et al., 2012; Miranda et al., 2017; Liu et al., 2019). Salt stress induced the up-regulation of *SOS1*, *NHX* and *SOS3* genes in males under both N forms, which reduced the Na^+ accumulation in the cytoplasm and enhanced salt stress tolerance, especially under NO_3^- (Fig. 7A-B). Moreover, the phloem in the barks facilitates the Na^+ reallocation into roots. Therefore, it is reasonable to suggest that the accumulation of Na^+ in the barks not only sequestrated Na^+ in the intracellular space, but also promoted Na^+ translocation to roots and thus excreted outside the roots. In contrast, females had higher expression of genes related to K^+ uptake, such as *KAT1*, *KUP2*, *KUP6* and *KUP7* under both N forms in response to salt stress. However, salt stress induced K^+ leakage from the cytoplasm by up-regulating *SKOR* genes in the barks. The uptake of K^+ did not offset its leakage from the cytoplasm, as shown by the lower K^+/Na^+ ratio in the barks (Fig. 2). Meanwhile, the reduced Na^+ efflux certainly increased Na^+ sensitivity in females under NH_4^+ supply. Aquaporin *PIP1;1* and *PIP2;1* were down-regulated, but *PIP1;4* and *TIP2;1* were up-regulated under salt stress in females under both N forms (Fig. 7A-B). Aquaporin facilitates the regulation of water balance and increases salt tolerance in plants (Sreedharan et al., 2015; Saddhe et al., 2021). In contrast, the up-regulation of genes encoding aquaporin

PIP2;7 and *TIP1:1* was greater in males than in females, suggesting that males have a sophisticated regulation of water transport, which also enhances salt stress tolerance.

5. Conclusions

This study shows that females are more sensitive to salt stress under an NH_4^+ supply than under a NO_3^- supply, and males are more tolerant to salt under both N forms (Fig. 8). The higher salt stress tolerance in males than females was mainly derived from the maintenance of a higher K^+/Na^+ ratio achieved by the promotion of the root Na^+ efflux and K^+ retention (Fig. 8). We also demonstrated that root apex is the main region to perform Na^+ efflux. Females receiving NO_3^- rather than NH_4^+ have a higher Na^+ efflux in the root apexes, lower net Na^+ influx and higher K^+ influx in both root regions, as well as higher Na^+ translocation from roots to shoots, and thus a lower salt sensitivity. In contrast, males had a lower Na^+ efflux in root apexes and higher Na^+ translocation to shoots, but a similar translocation rate of Na^+ in leaves to bark as well as salt tolerance (Fig. 8). The transcription analysis conducted for the bark suggests that the lower salt stress tolerance in females probably correlates with the reduced Na^+ sequestration into extracellular spaces and disturbed scavenging of denaturized proteins, especially under an NH_4^+ supply (Fig. 8). In contrast, the promotion of cell wall modification and Na^+ accumulation in the apoplast in male bark, especially under an NH_4^+ supply, would elevate salt sequestration and tolerance. Thus, our results suggest that an orchestrated anatomical, physiological and transcriptional regulation is probably required for salt tolerance and male poplar are superior under both inorganic N forms.

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Figure legends

Figure 1 Effects of salt stress on (A) leaf phenotype, (B) leaf photosynthetic rate (*A*), (C) leaf water potential (ψ), (D) and stomatal conductance (g_s) in *P. cathayana* females and males as affected by salt stress (0, 50 mM NaCl), N forms (NH_4^+, NO_3^-) and their combinations. The experiment consisted of eight treatments, four replications for each treatment. The treatments included two sexes (females and males) × two salt regimes (0, 50 mM NaCl) × two N regimes (3.75 mM NO_3^- and 3.75 mM NH_4^+) (Fig. S1; Table S1). Differences between means were analyzed by Duncan's tests. Values are means ± SD (n = 4 replicates). Different letters indicate significant differences among treatments ($P < 0.05$). Bar, 2 cm.

Figure 2 Na^+ concentration in roots (g kg^{-1} DW), wood (mg kg^{-1} DW), bark (mg kg^{-1} DW) and leaves (mg kg^{-1} DW), and the corresponding K^+/Na^+ ratio of *P. cathayana* females and males as affected by salt stress (0, 50 mM NaCl), N forms (NH_4^+, NO_3^-) and their combinations. Values are means ± SD (n = 4 replicates). Different letters indicate significant differences among treatments ($P < 0.05$).

Figure 3 Shoot Na^+ concentration relative to that of roots (A), leaf Na^+ concentration relative to that in bark (B), bark Na^+ concentration relative to that in shoot (C) and leaf Na^+ concentration relative to that in shoot (D) in *P. cathayana* females and males affected by salt stress (0, 50 mM NaCl), N forms (NH_4^+, NO_3^-) and their combinations. Differences between means were analyzed by Duncan's tests. Values are means ± SD (n = 4 replicates). Different letters indicate significant differences among treatments ($P < 0.05$).

Figure 4 The mean flux of net Na^+ (A) and K^+ (B), and transient Na^+ (C) and K^+ (D) kinetics

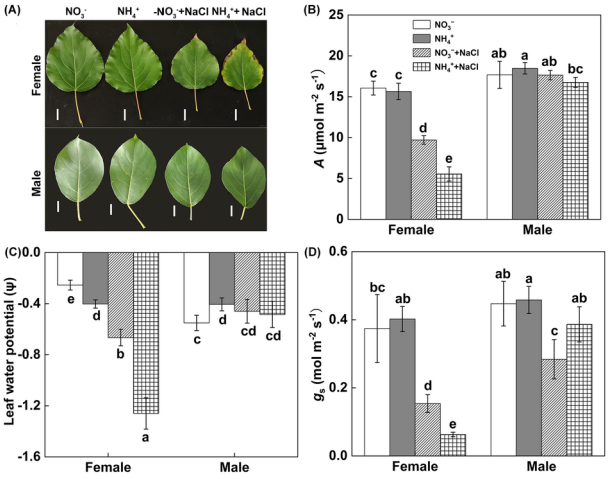
of salt shock (100 mM NaCl) in apical and mature zones of roots in *P. cathayana* females and males as affected by salt stress (0, 50 mM NaCl), N forms (NH_4^+, NO_3^-) and their combinations. Before salt shock, steady Na^+ and K^+ fluxes of roots were examined for approximately 5 min. Salt shock (100 mM NaCl) was performed by adding NaCl stock (10 M, pH, 6.0). Differences between means were analyzed by Duncan's tests. Values are means ± SD (n = 4 replicates). For (A) and (B), different letters on the bars indicate significant differences between the treatments ($P < 0.05$).

Figure 5 Comparative profiling of transcriptional salt stress responses under NH_4^+ and NO_3^- supply in barks of *P. cathayana* females and males. Venn diagrams showing the distribution of differentially expressed genes identified between females and males (A), as well as between control and salt stress (B) under both N forms. Circle representation shows the GO terms enriched in up-regulated (C) (numeric data in Table S4) and down-regulated genes (D) (numeric data in Table S5) between control and salt stress in females and males under NO_3^- and NH_4^+ supply. FA, female+NH_4^+; FAS, female+NaCl+NH_4^+; MA, male+NH_4^+; MAS, male+NaCl+NH_4^+; FN, female+NO_3^-; FNS, female+NaCl+NO_3^-; MN, male+NO_3^-; MNS, male+NaCl+NO_3^-.

Figure 6 Clustering analyses of expressed mutual orthologs (EMOs) in the bark of *P. cathayana* females and males (Table S6). (A) Heatmap analysis showing the Pearson correlation coefficient determined between the module eigengenes in the bark of females and males, salt stress and different N forms by a weighted gene co-expression network analysis. The number of genes in each module is shown on the left. Each color module represents a trait. Each module was identified based on its eigengene calculated as the first principal component of the gene expression pattern. Cells represent the correlation coefficient and the corresponding *P*-value (bracket). (B) Correlations of significant modules with sexes, salt and N form treatments shown as an intersection chart. These modules were selected based on the condition of $P < 0.05$. (C) Heatmaps of gene expression profiles under modules shown in (B). FA, female+NH_4^+; FAS, female+NaCl+NH_4^+; MA, male+NH_4^+; MAS, male+NaCl+NH_4^+; FN, female+NO_3^-; FNS, female+NaCl+NO_3^-; MN, male+NO_3^-; MNS, male+NaCl+NO_3^-.

Figure 7 Gene expression dynamics of K^+ and Na^+ transport genes (A) and the corresponding schematic model of the mediation of root K^+ and Na^+ dynamics (B) in the barks of *P. cathayana* females and males. The green arrow indicates the down-regulation of genes related to K^+ and Na^+ transport between females and males; the red arrow indicates the up-regulation of genes related to K^+ and Na^+ transport between females and males. The width of the arrow indicates the levels of gene expression. *AKT2*, *KAT1*, *KUP2/6/7*, *KT1* and *SKOR* genes are involved in K^+ transmembrane transport. *KCO1* and *KCO6* genes encode vacuole membrane-localized proteins involved in K^+ uptake. *AHA1.1* and *AHA10* genes encode a plasma membrane proton ATPase. *VHA1.1* gene encodes a vacuole membrane proton ATPase. *SOS1* gene encodes a plasma membrane-localized Na^+/H^+ antiporter. *SOS2* and *SOS3* genes encode proteins involved the activation of SOS1. *CPK11* gene encodes a protein involved Ca^{2+} transport. FA, female+NH_4^+; FAS, female+NaCl+NH_4^+; MA, male+NH_4^+; MAS, male+NaCl+NH_4^+; FN, female+NO_3^-; FNS, female+NaCl+NO_3^-; MN, male+NO_3^-; MNS, male+NaCl+NO_3^-.

Figure 8 A schematic model for root net Na^+ and K^+ flux in root apical zones (AZ) and mature zones (MZ), root to shoot Na^+ translocation and bark transcriptional responses to salt stress under NH_4^+ or NO_3^- supply in *Populus cathayana* females and males. The width of the cyan arrow represents the strength of net root Na^+ (red dots) flux. The width of the orange arrow indicates the strength of net root K^+ (blue dots) flux. For shoot, the width of red arrow indicates the ratio of shoot Na^+ to that of root (A, female+NaCl+NO_3^-; B, female+NaCl+NH_4^+; E, MNS, male+NaCl+NO_3^-; F, male+NaCl+NH_4^+). The width of sky blue arrow indicates the ratio of leaf Na^+ to that of bark (C, female+NaCl+NO_3^-; D, female+NaCl+NH_4^+; G, MNS, male+NaCl+NO_3^-; H, male+NaCl+NH_4^+). ES, extracellular space; IS, intracellular space.



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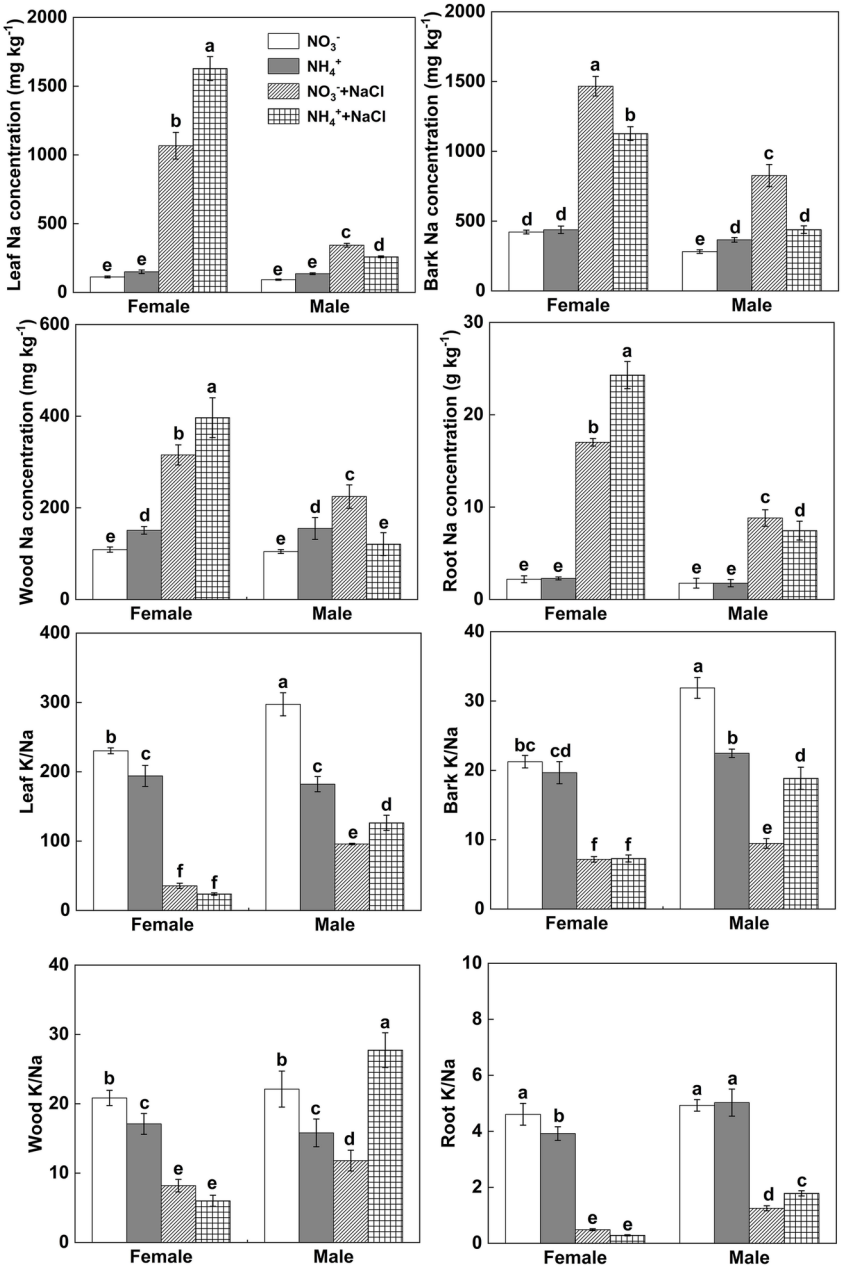

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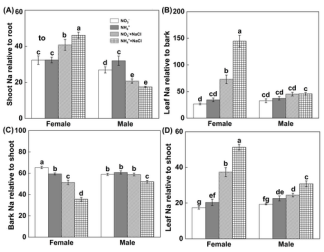

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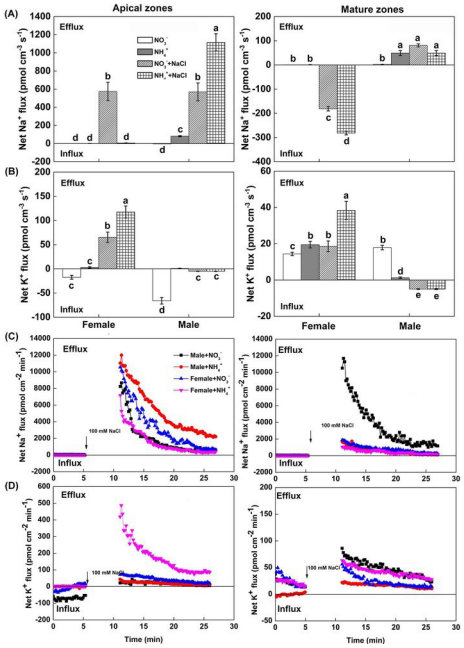

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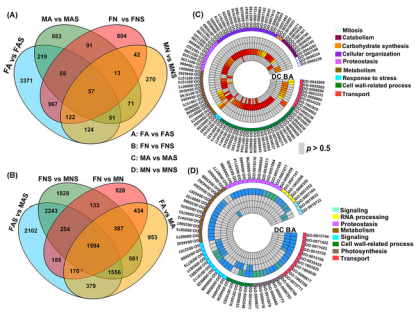

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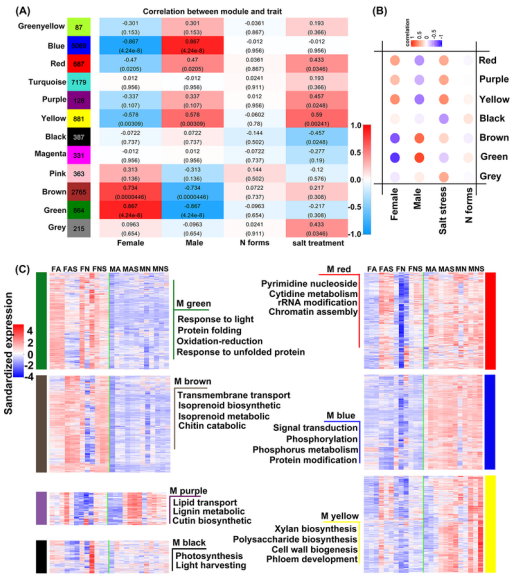

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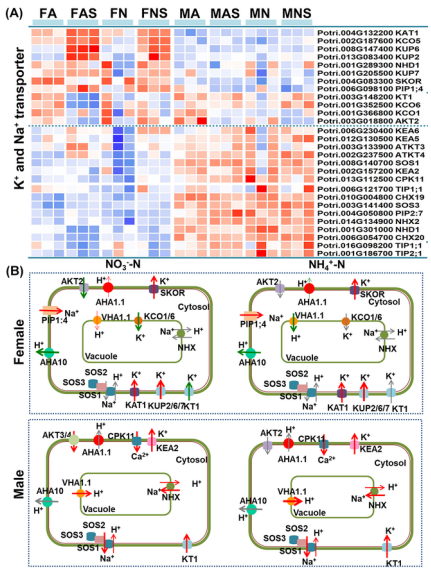

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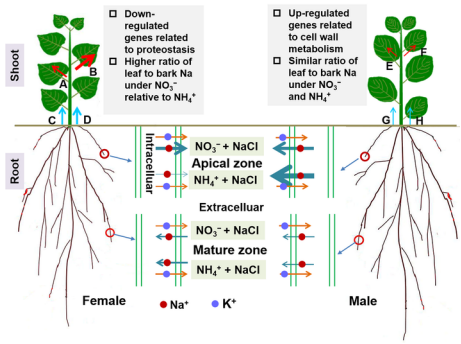

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