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Male *Populus cathayana* than female shows higher photosynthesis and less cellular injury through ABA-induced manganese transporting inhibition under high manganese condition

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Abstract

Key message **High Mn poisoned male and female *Populus cathayana*. The toxicity could be alleviated by exogenous ABA application. Intriguingly, ABA granted higher resistance to males than to females under high Mn stress because ABA could induce more blocking of Mn translocation to leaf in males than in females.**

Abstract Abscisic acid (ABA) is involved in plants' adaptive responses to various environmental stresses. However, little is known about the sex-related detoxification of ABA in plants under excess manganese (Mn) conditions. To reveal potentially different ABA detoxification mechanisms between *Populus cathayana* males and females against excess Mn exposure, photosynthesis performance, Mn^{2+} concentrations and morphologic changes were investigated. High Mn stress led to a more severe chloroplast destruction and, thus, greater reduction in the photosynthesis of *P. cathayana* females when compared to males. Under high Mn conditions, Mn reallocated mainly to leaves in females, while in males, it was distributed equally to roots and leaves. With the application of ABA, photosynthesis was restored more in males and more integrated grana in males than in females. It should be noted that Mn concentrations in males were lower in leaves and higher in roots and stems than those in females when treated with the combination of Mn and ABA. Conclusively, due to the reduction of root–shoot Mn transportation induced by ABA in *P. cathayana* males, males experienced less physiological injuries than do females, which suggest that males possess greater ABA-inducible resistance to Mn stress than do females.

Keywords Abscisic acid · Mn toxicity · Dioecy · Poplar · Sexual differences

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Introduction

Manganese (Mn) is an essential element for plant growth and development. It is needed during photosynthesis and it functions in the decomposition of superoxide (Chen et al. 2013c; Millaleo et al. 2010; Pittman 2005). However, in acidic soils and mine spoils, the presence of excessive Mn leads to serious heavy metal toxicity for plants (Foy et al. 1978; Reis and Junior 2011). Previous studies believed that once excessive Mn is transported to the shoot, especially to leaves, the photosynthetic apparatus will be harmed and the gas-exchange process will be disturbed (Doncheva et al. 2005; Lidon et al. 2004; Reis and Junior 2011). Afterwards, necrotic and brown spots will emerge in leaves (Rezai and Farboodnia 2008). Yet, to counteract Mn stress, plants have evolved sophisticated mechanisms to cope with high Mn stress, such as blocking off Mn transportation from roots to leaves (Dorling et al. 2011; Fernando et al. 2008; Xue et al. 2004). It has been demonstrated that blocking off Mn transportation is related to the presence of the endogenous stress-related phytohormone, ABA (De Vleesschauwer et al. 2010; Raghavendra et al. 2010).

ABA is indispensible in plants' abiotic and biotic stress resistances (Peleg and Blumwald 2011; Pieterse et al. 2012; Ryu et al. 2010; Wang et al. 2011; Xu et al. 2011). During heavy metal detoxification, ABA can protect plants through the inhibition of the transportation of toxic heavy metal from root to aerial parts. Previous study has found that cadmium uptake was inhibited by exogenous ABA application in Arabidopsis (Fan et al. 2014). Among woody plants, exogenous ABA alleviates zinc uptake and accumulation in *Populus × canescens* under excess zinc conditions (Shi et al. 2015). In different cadmium resistant cultivars of rice, exogenous ABA decreases the cadmium content, and enhances the cadmium tolerance of cadmium-sensitive cultivars (Hsu and Kao 2003). However, whether ABA functions differently in Mn resistance between different sexes is still unclear.

Females and males of dioecious plants show different tolerances to environmental stresses, which is largely due to their different investments to reproduction (Barrett and Hough 2012; Case and Barrett 2004; Freeman et al. 1976; Wallace and Rundel 1979). Since females allocate more nutrients and energy into reproduction than to stress resistance, they are generally more susceptible to adverse environmental stresses than males (Chen et al. 2015; Obeso 2002; Stark et al. 2000). Nevertheless, few studies have reported differences between females and males in the perception of a stress signal (Barrett and Hough 2012; Chen et al. 2010; Crossgrove and Zheng 2004; De Vleesschauwer et al. 2010; Hultine et al. 2013; Lee et al. 2004; Wallace and Rundel 1979). In our study, the following hypotheses were tested: (1) the toxicity could be alleviated by an exogenous ABA application; (2) ABA could grant greater resistance to males than to females under high Mn stress by blocking off Mn translocation to leaves more effectively in males than in females. We aim to uncover whether ABA, the stress-related signal, functions differently in *P. cathayana* males and females under high Mn conditions.

Materials and methods

Plant materials and experimental design

Cuttings of *P. cathayana* males and females were selected among F_1 individuals, which were derived from a controlled intraspecific cross between two *P. cathayana* genotypes selected from their natural habitat (Ledu, 36°31′N, 102°28′E, 3160 m alt.) in the Qinghai Province, China. Of each sex, 45 healthy and uniform cuttings germinated from 2-year old rootstocks were selected and grown in a naturally lit, well-ventilated greenhouse (average temperature and relative humidity being 27 °C and 70%, respectively) for 4 weeks. Each cutting grew in a 10-L plastic pot filled with 8 kg nursery soil and 8 g slow-release fertilizer (13% N, 10% P and 14% K). When cuttings reached about 50 cm in height, they were treated with ABA and high Mn stress. In Mn stress experiments, cuttings were irrigated with 1 L deionized water containing 2 mM $MnCl_2$ every day during the treatment according to our previous studies with changes (Lei et al. 2007). In the ABA experiment, leaves of cuttings were daily sprayed evenly with 10 mL 50 μM ABA in 0.5% (v/v) Tween 20 (Yin et al. 2004). In the combined Mn and ABA experiment, plants were sprayed simultaneously with 10 mL 50 μMABA in 0.5% (v/v) Tween 20 and irrigated with 1 L 2 mM $MnCl_2$. In control experiments, cuttings were sprayed and irrigated with equal volumes of deionized water and 0.5% (v/v) Tween 20. The treatments lasted 4 weeks.

Gas-exchange assay

Gas-exchange was measured at the end of ABA and Mn treatments, from 08:00 to 11:30 h with the Li-6400 portable photosynthesis measuring system (Li-Cor Inc., Lincoln, NE, USA) on the fourth or fifth fully expanded leaf of each sample. The measuring conditions were as follows: leaf temperature 27 °C, leaf-air vapor pressure deficit 1.5 ± 0.5 kPa, photosynthetic photon flux 1400 μmol m^{-2} s^{-1}, relative air humidity 70%, and ambient CO_2 concentration 390 ± 8 μmol mol^{-1}. Chlorophyll fluorescence of the same-year leaves was measured with the PAM chlorophyll fluorometer (PAM 2100, Walz, Effeltrich, Germany). ETR and Φ were calculated (Baker 2008).

Ultrastructural observations of mesophyll cells

For ultrastructural observations, about 1 × 2 cm fresh leaf segments without major veins were cut from the fourth or fifth fully expanded leaf. Leaf segments were fixed in 3% glutaraldehyde (in 0.2 M sodium phosphate buffer, pH 7.2) for 6–8 h, post fixed in 2% osmium tetraoxide for 2 h and dehydrated in ethanol with concentrations increasing by 10% from 50 to 100%, followed by acetone. Leaf segments were then embedded in epon-araldite. Ultra-thin sections (80 nm) were sliced, mounted and counterstained with 3% (w/v) uranyl acetate in ethanol and lead citrate. Images were collected by a transmission electron microscope (H-600IV, Hitachi Co., Japan) at an accelerating voltage of 60.0 kV (Chen et al. 2010).

Mn^{2+} content analysis

To detect the Mn concentration, roots, stems and leaves of *P. cathayana* males and females were segmented and cleaned with water. After that, the samples were soaked in 0.2% EDTA for 2 h and then rinsed thoroughly with deionized water to eliminate possible chemical contamination. Afterwards, they were dried at 80 °C (48 h) and weighed. The dried samples were digested in a mixture of $HNO_3/HClO_4$ (4:1), and the Mn concentration was determined using an inductively coupled argon plasma emission spectrometry (Model 1CAP 61E; Thermo-Jarrell Ash, Waltham, MA, USA) (Chen et al. 2013a). The Mn^{2+} ratio were calculated by dividing the average Mn^{2+} concentration of different tissues to average whole Mn^{2+} concentration of each sex and treatment separately.

Statistical analysis

All data were analyzed with the software Statistical Package for the Social Sciences (SPSS) version 19.0. Three-way analyses of variance (ANOVAs) were employed to test the overall effects of sex, Mn and ABA on physiological and biochemical parameters. Before ANOVAs, data were checked for normality and the homogeneity of variances. For Mn concentrations in roots, stems and leaves, which showed heterogeneous variances, the data were ln-transformed to obtain a normal distribution and homogeneity of variances. Post hoc comparisons were conducted using the Duncan test at a significance level of $P \leq 0.05$.

To determine whether high Mn and exogenous ABA affect Mn concentrations of *P. cathayana* males and females, we organized the data into three traits × 2 sexes under three treatments matrix. To find out whether female responses to Mn and ABA were similar to those of males, we compared Mn concentrations using a principal component analysis (PCA). For PCA, three biological replicate data of Mn^{2+} concentrations in root, stem and leaf were standardized and subsequently computed by SIMCA version 13.0 (http://umetrics.com/products/simca).

Results

Photosynthetic changes

To determine the effects of high Mn and ABA spraying on gas-exchange in *P. cathayana* males and females, we measured the net photosynthesis rate (*A*), stomatal conductance (g_s) and transpiration rate (*E*). Table 1 shows that the exposure to high Mn led to significant decreases of *A*, g_s and *E* in both male and female seedlings. However, the effects of ABA spraying alone did not dramatically change the net photosynthesis rate in either sex. It should be noted that *A*, g_s and *E* decreased more in females than in males when grown under high Mn compared to control condition. When

Table 1 Net photosynthesis rate (*A*), stomatal conductance (g_s) and transpiration rate (*E*) in *P. cathayana* males and females, as affected by manganese, ABA and their combination

$MnCl_2$ (mM)	ABA (μM)	Sex	*A* (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})
0	0	Male	20.462 ± 0.365 ab	0.542 ± 0.092 a	9.822 ± 0.813 a
0	50	Male	23.035 ± 0.239 a	0.521 ± 0.030 a	9.488 ± 0.239 a
2.0	0	Male	6.901 ± 0.265 c	0.027 ± 0.004 c	0.913 ± 0.109 c
2.0	50	Male	9.016 ± 1.522 c	0.042 ± 0.014 c	1.311 ± 0.401 c
0	0	Female	17.235 ± 0.778 b	0.588 ± 0.016 a	11.121 ± 0.247 a
0	50	Female	17.989 ± 1.031 b	0.255 ± 0.063 b	6.000 ± 1.017 b
2.0	0	Female	3.112 ± 0.557 d	0.011 ± 0.001 c	0.388 ± 0.095 c
2.0	50	Female	7.949 ± 2.540 c	0.040 ± 0.022 c	1.305 ± 0.652 c
		F_s	***	NS	NS
		F_{Mn}	***	***	***
		F_{ABA}	**	*	*
		$F_{s\times Mn}$	NS	NS	NS
		$F_{s\times ABA}$	NS	*	*
		$F_{Mn\times ABA}$	NS	**	***
		$F_{s\times Mn\times ABA}$	NS	*	**

Each value is the mean ± SE ($n = 3$)

F_s sex effect, F_{Mn} manganese treatment effect, F_{ABA} ABA treatment effect, $F_{s\times Mn}$ the interactive effect of sex and manganese, $F_{s\times ABA}$ the interactive effect of sex and ABA, $F_{Mn\times ABA}$ the interactive effect of manganese and ABA, $F_{s\times Mn\times ABA}$ the interactive effect of sex, manganese and ABA, *NS* not significant, $P > 0.05$; *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; and ***, $P \leq 0.001$. Different letters represent statistical significance between treatments at $P < 0.05$ according to Duncan multiple range tests sprayed with ABA at the same time, A in males was greater than that in females. To examine the possible role of ABA in photosynthetic responses to high Mn stress, chlorophyll fluorescence parameters, which are indicators of photosynthetic capacity, were measured. When treated with Mn alone, F_m (maximum fluorescence), F_v/F_m (ratio of variable to maximum fluorescence – the quantum efficiency of open photosystem II centers), ETR (photosynthetic electron transport rate), and Φ (effective quantum yield of photosystem II) decreased in male and female leaves (Table 2). By contrast, F_m, F_v/F_m, ETR and Φ increased in both females and males when treated with the combination of Mn and ABA compared to the Mn treatment alone. The results indicated that stress caused by high Mn reduced photosynthetic performance in both *P. cathayana* males and females, while ABA spraying could relieve the stress effect.

Differences in Mn location

Mn contents in male and female leaves, stems, and roots were assayed. Mn alone and the combined treatment of Mn and ABA led to a significant increase in the Mn contents of leaves in both sexes. However, there were no differences between control and ABA-treated seedlings (Fig. 1a). In stems, the Mn treatment alone and Mn × ABA treatment reduced Mn contents (Fig. 1b), and in roots, Mn × ABA led to a greater reduction of Mn contents in females than in males (Fig. 1c). It should be noted that the Mn concentration in female leaves when sprayed with ABA under high Mn conditions was significantly higher than that in males but lower in stems and roots (Fig. 1a–c). Concerning the Mn distribution in leaves, stems and roots, females and males showed dramatic differences (Fig. 1d). In control females, 58.3 and 41.7% of Mn was distributed in roots and above-ground parts, respectively, but only 1.9% in leaves. While in control males, most Mn (85.8%) was in stems and only 8.7% in roots (Fig. 1d), indicating that under optimal conditions, most Mn accumulated in above-ground parts in males, while in females it was distributed evenly in above- and below-ground parts. When facing with high Mn, the proportion of Mn increased to 92.7% in female leaves, while only 6.9% remained in roots. On the contrary, in males, the proportion of Mn in leaves and roots increased from 5.5 to 47.7% and from 8.7 to 51.6%, respectively. These data indicate that Mn stress could induce the root-oriented relocation in males and aerials-oriented relocation in females of Mn^{2+}. Furthermore, ABA spraying induced most Mn, about 95.5% of all Mn, to concentrate in male roots when exposed to high Mn. On the contrary, Mn concentrations were the same in Mn-treated female leaves whether treated with or without ABA. Our result indicated that ABA might block off Mn transportation from roots to leaves in males but not in females.

Table 2 The maximum fluorescence (F_m), the ratio of variable to maximum fluorescence (F_v/F_m), the apparent photosynthetic electron transport rate (ETR) and the effective quantum yield of photosystem II (Φ) in *P. cathayana* males and females, as affected by manganese, ABA and their combination

$MnCl_2$ (mM)	ABA (μM)	Sex	F_m	F_v/F_m	ETR	Φ
0	0	Male	1.423 ± 0.041 a	0.808 ± 0.003 a	93.167 ± 1.692 a	0.740 ± 0.013 a
0	50	Male	1.417 ± 0.051 a	0.802 ± 0.008 a	92.900 ± 0.656 a	0.737 ± 0.005 a
2.0	0	Male	1.181 ± 0.103 b	0.752 ± 0.009 b	88.500 ± 0.436 bc	0.703 ± 0.004 bc
2.0	50	Male	1.287 ± 0.069 ab	0.789 ± 0.011 a	90.633 ± 1.450 ab	0.719 ± 0.011 ab
0	0	Female	1.423 ± 0.051 a	0.806 ± 0.010 a	91.833 ± 1.677 ab	0.729 ± 0.013 ab
0	50	Female	1.295 ± 0.131 ab	0.797 ± 0.010 a	91.067 ± 1.474 ab	0.723 ± 0.012 ab
2.0	0	Female	1.268 ± 0.032 b	0.766 ± 0.022 b	87.167 ± 3.855 c	0.692 ± 0.030 c
2.0	50	Female	1.295 ± 0.050 ab	0.792 ± 0.007 a	90.767 ± 0.289 ab	0.720 ± 0.002 ab
		F_s	NS	NS	NS	NS
		F_{Mn}	***	***	***	***
		F_{ABA}	NS	*	NS	NS
		$F_{s\times Mn}$	NS	NS	NS	NS
		$F_{s\times ABA}$	NS	NS	NS	NS
		$F_{Mn\times ABA}$	*	***	*	*
		$F_{s\times Mn\times ABA}$	NS	NS	NS	NS

Each value is the mean ± SE ($n = 3$)

F_s sex effect, F_{Mn} manganese treatment effect, F_{ABA} ABA treatment effect, $F_{s\times Mn}$ the interactive effect of sex and manganese, $F_{s\times ABA}$ the interactive effect of sex and ABA, $F_{Mn\times ABA}$ the interactive effect of manganese and ABA, $F_{s\times Mn\times ABA}$ the interactive effect of sex, manganese and ABA, *NS* not significant, $P > 0.05$; *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; and ***, $P \leq 0.001$. Different letters represent statistical significance between treatments at $P < 0.05$ according to Duncan multiple range tests

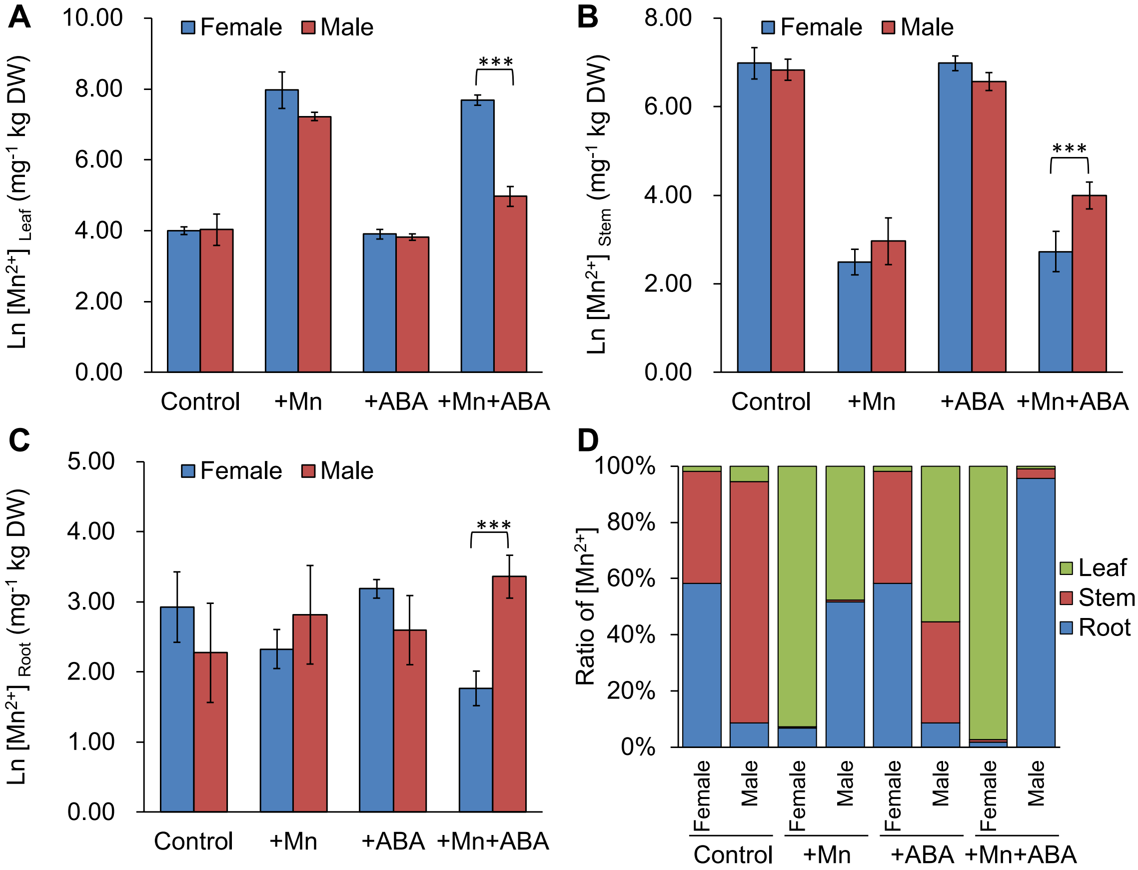

Fig. 1 Mn^{2+} concentration in **a** leaves, **b** stems and **c** roots, and proportions of Mn^{2+} in those three parts in *P. cathayana* females and males (**d**). Data represent the mean ± SE ($n=3$). Asterisks indicate a significant difference ($P<0.01$) between females and males

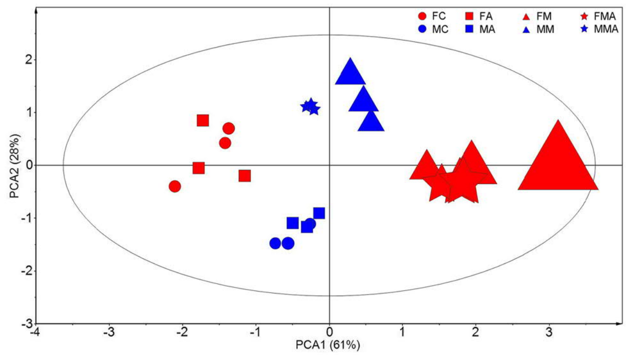

Fig. 2 PCA plots of Mn concentration in *P. cathayana* females and males in control, high Mn stress, ABA spraying and ABA × Mn combination conditions. *MC* control male, *MA* ABA-treated male, *MM* manganese-treated male, *MMA* manganese- and ABA-treated male, *FC* control female, *FA* ABA-treated female, *FM* manganese-treated female, *FMA* manganese- and ABA-treated female. Symbol sizes indicate the Mn concentration in leaves

PCA analysis of Mn concentrations in females and males

To determine whether females and males respond similarly to high Mn and ABA treatments, we compared the responses to Mn using the principal component analysis (PCA). The first PCA axis (PCA1) was identified as an axis of Mn concentrations, accounting for 61% of the total variance. PCA1 clearly separated control and Mn-treated females (Fig. 2), while in males, the separation by PCA1 was not clear, especially between the ABA-sprayed individuals with or without Mn treatment (Fig. 2). The results indicated that under ABA spraying, the Mn location pattern of Mn-treated males returned to that of control males. However, ABA spraying did not influence much the Mn location pattern of Mn-treated females. The Mn concentration in female leaves stayed high even when sprayed with ABA, but in males, the Mn concentration of leaves was reduced by ABA spraying (Fig. 2).

Sexual differences in ultrastructural damage

To examine the effect of ABA on Mn-induced cell damage, the cellular ultrastructure was investigated. Mn toxicity caused more grievous vacuolation in female mesophyll chloroplasts than in male poplars while ABA alone treatment did not influence both females and males much (Fig. 3b, c, f, g). However, ABA spraying offset the Mn-induced vacuolation (Fig. 3d, h). In addition, when facing with the combination of high Mn and ABA, grana were fewer in females than in males (Fig. 3d, h). The results indicated that high Mn stress causes damage to the mesophyll cell structure in both sexes, while ABA protects male mesophyll cells more than those in females.

Discussion

Although many studies have investigated sex-related tolerances to abiotic stresses (Barrett and Hough 2012; Case and Barrett 2004; Groen et al. 2010; Rozas et al. 2009; Wang and Curtis 2001), little is known about the function of ABA on stress mitigation in dioecious plants. In the present study, we analyzed photosynthesis, Mn reallocation and ultrastructure changes in *P. cathayana* females and males under a single treatment with high Mn or ABA spraying, and under the combined treatment of high Mn or ABA spraying. Our results showed that an exogenous application of ABA induced sexually different responses in Mn reallocation to roots, stems and leaves that could induce different degrees of damage on the subcellular structure and photosynthesis capacity in males and females. The data presented here offered insight into the effects of ABA on dioecy plants.

ABA contributes to higher photosynthesis capacity in males when facing high Mn

ABA is known to improve photosynthetic capacity when plants face abiotic stresses (Ashraf and Harris 2013; Zhang et al. 2008). ABA was believed to cause stomatal closure in some plants, and induce a decrease in *E* afterwards (Cutler et al. 2010). It is also proposed that ABA plays a redox-retrograde role in the *APX2* expression signaling network, which is harmful to the photosynthetic apparatus (Galvez-Valdivieso et al. 2009), while ABA might improve the PSII thermo-tolerance (Hao et al. 2012). Our results show that high Mn stress led to a decrease in the photosynthetic capacity of both male and female *P. cathayana*, while ABA prevented such decreases. Intriguingly, the photosynthetic capacity of Mn-treated males was a little higher than that of Mn-treated females when sprayed with ABA (Tables 1, 2), which might result from the fact that males protect their photosynthetic apparatus better via ABA-induced blocking of Mn transportation from roots to aerial parts (Dučić et al. 2006; Shao et al. 2017) (Figs. 1, 3).

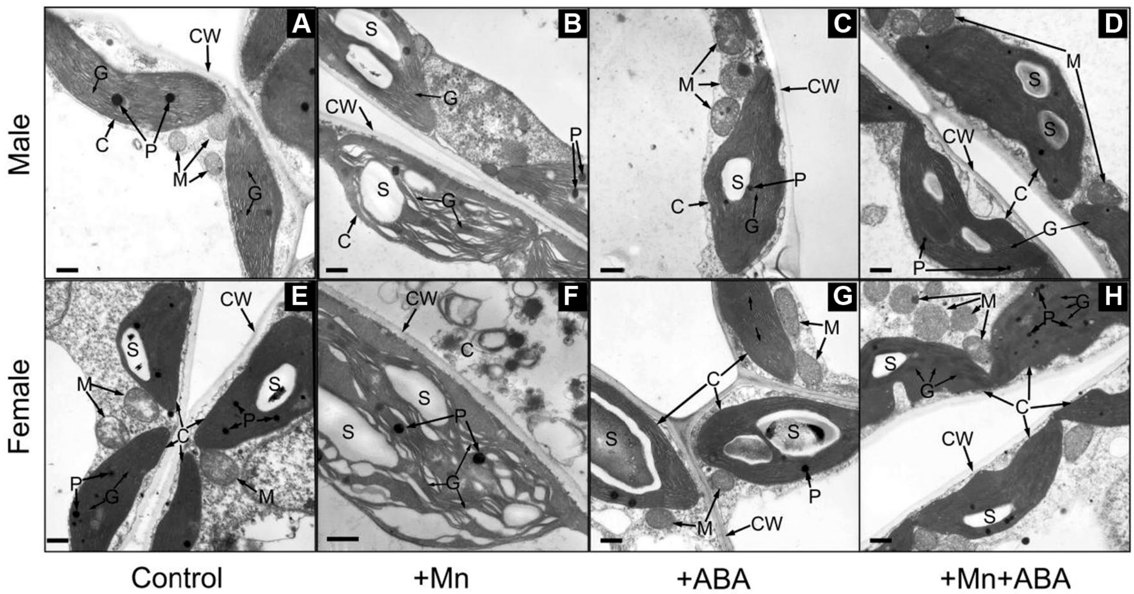

Fig. 3 Transmission electron microscopy observations of mesophyll cells in *P. cathayana* males (**a–d**) and females (**e–h**) under control conditions (**a, e**), and as affected by Mn stress alone (**b, f**), ABA alone (**c, g**) and the combined treatment (**d, h**). The bars shown are 1 μm. *C* chloroplast, *CW* cell wall, *G* granum, *M* mitochondrion, *P* plastoglobuli, *S* starch granule

ABA inhibits Mn transportation from roots to aerial parts more in males

Plants have evolved various transportation networks to reduce the damage caused by excessive levels of ions (Horie et al. 2009; Krämer et al. 2007; Millaleo et al. 2010; Ryan et al. 1997). ABA-inducible gene expression can be immediately triggered by abiotic stress and subsequently followed by stomatal closure that could further lead to lower transpiration (Peleg and Blumwald 2011). As a result of lower transpiration, the Mn transportation from roots to aerial parts decreases (Millaleo et al. 2010; Scoffoni et al. 2015). Our results showed that ABA spraying induced Mn reallocation to take place mainly in female leaves and in male roots (Figs. 1, 2). This result indicates that with the help of ABA, males could inhibit more Mn transport from roots to aerial parts, consequently causing less Mn accumulation in aerial parts, especially in leaves, when compared to females. As a result, the leaf photosynthetic apparatus of males could be protected better than that of females when grown under excess Mn.

Male chloroplasts are less damaged by excess Mn when sprayed with ABA

Excess metal ions are harmful to plant cells, especially to the photosynthetic apparatus (Potters et al. 2007). Mn treatments have been identified to disrupt the thylakoid system arrangement, to induce chloroplast swelling, to increase the amounts of plastoglobuli, and to reduce starch accumulation in chloroplasts (Najeeb et al. 2009). Similarly, *P. cathayana* showed severe loose thylakoid membranes and swollen chloroplasts under high Mn stress (Fig. 3b, f). However, female mesophyll cells were injured more severely compared to male cells. The same situation has been observed in previous studies on poplars exposed to excess salt, drought and heavy metals (Chen et al. 2011, 2013b; Zhang et al. 2010). Intriguingly, toxic symptoms caused by excess Mn were alleviated by ABA (Fig. 3d, h). Combined with the lower Mn abundance in male leaves, we concluded that males could protect chloroplast from excess Mn toxicity better than females through ABA-mediated inhibition of Mn transportation from roots to aerial parts.

Conclusions

Our results demonstrated that the Mn transportation from roots to aerial parts was blocked off by exogenous ABA more in *P. cathayana* males than females. As a result, especially the photosynthesis apparatus of males was less damaged by Mn than that of females. Consequently, the photosynthesis capacity of males suffered little when faced with high Mn. In summary, Mn stress resistance could be enhanced by exogenous ABA more in males than in females. Further studies should focus deeper on the question why ABA provides better resistance to Mn in males than in females.

Author contribution statement FC developed the initial research idea and designed the experiment. He also conducted the field work and was responsible for the statistical analysis and manuscript writing. JS, DM, LK, XT and HK contributed largely to the writing of the manuscript and provided useful suggestions and comments. CL acquired the funding for the project that was performed in his laboratory. He also contributed to the writing of the paper.

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Compliance with ethical standards

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

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