Adaptive changes of ROS/RNS redox and melatonin synthesis under salt and waterlogging stresses in Pittosporum tobira

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Abstract

Plants have evolved a variety of complex mechanisms to resist the environmental factors including salt and waterlogging stresses. In this study, we described adaptive changes of *Pittosporum tobira* toward salt and/or waterlogging stresses by mediating ROS (reactive oxygen species)/RNS (reactive nitrogen species) redox and melatonin synthesis. When the *P. tobira* seedlings were subjected to salt stress, waterlogging stress, and salt-waterlogging stress, both the photosynthetic capacity and antioxidant capacity were significantly inhibited, accompanying with the alterations of MDA, H_2O_2, O_2^- and NO levels and melatonin metabolism. These observations were correlated with the changes in the activities of antioxidant enzymes (SOD, CAT, POD and APX) and melatonin biosynthetic enzymes (MEL, TDC, SNAT, SER, and 5-MT) as wells as in the expression of their encoding genes. Lower melatonin content was found in the seedlings treated by salt-waterlogging stress than in those treated by salt or waterlogging stress. Furthermore, the tolerances of the seedlings grown at Zhejiang province to salt and waterlogging stress were stronger than those grown at Fujian province. Our findings suggested that the MEL/ROS/RNS redox network induced by salt stress, waterlogging stress, salt-waterlogging stress may be a crucial mechanism for coping with adverse conditions in *P. tobira*.

Introduction

Global warming is a significant factor contributing to the rise in sea levels in coastal regions, and it has also brought about changes in rainfall patterns, leading to the occurrence of waterlogging hazards and the expansion of land salinization(Martin et al. 2011; Bennett et al. 2009; Hirabayashi et al. 2013). Extensive studies in various species show that waterlogging and soil salinization significantly impede plant growth, development and distribution on a global scale(Su et al. 2021; Gill et al. 2019; Antonelli et al. 2021). Waterlogging stress induces hypoxia in submerged tissues, hindering the transport of water and nutrients from roots to shoots and leaves, disrupting endogenous metabolism, and retarding the growth rate(Colmer 2003). During this period, there is an excessive accumulation of reactive oxygen species (ROS, e.g. hydrogen peroxide) and reactive nitrogen species (RNS, e.g. superoxide anion) in plants. These molecules act as signaling molecules to trigger defense responses at the onset of stress but have detrimental effects on the body when present in excessive amounts(Apel and Hirt 2004; Arnao and Hernández-Ruiz 2019). For instance, high levels of ROS and RNS lead to lipid peroxidation, protein oxidation, and DNA strand breakage(Lee et al. 2004). Salt stress can disrupt the osmotic balance and induce an overproduction of ROS and RNS in plants, leading to protein structure degradation and hastening plant mortality(Maathuis and Amtmann 1999; Xie et al. 2011).

Plants have evolved various stress response mechanisms to mitigate the harmful effects of oxidative damage. This includes the production of antioxidant enzymes and non-enzymatic antioxidant substances(Miller et al. 2010; Sadeghnezhad et al. 2016). Melatonin, N-acetyl-5-methoxy-tryptamine, was identified as a free radical scavenger and broad-spectrum antioxidant in response to abiotic stresses in plants(Zhan et al. 2019; Xu et al. 2016). Under salt and waterlogging stresses, melatonin has been shown to effectively scavenge the excessive accumulation of ROS and RNS in plants by an interdependent

feedback pathway(Liu et al. 2015; Corpas et al. 2019; Liang et al. 2015). Furthermore, the melatonin biosynthetic genes play an important role in enhancing plant resistance. For instance, Arabidopsis lines overexpressing *VvSNAT1* from grape exhibited significant improvements in leaf coloration, growth, and germination rates when subjected to salt stress, compared to wild-type lines(Wu et al. 2021).

Pittosporum tobira, a landscape plant, is widely planted in the zone of southern China, possessing numerous ecological functions and medicinal properties. It is known that salinity and submerged conditions affect growth and development of *P. tobira*. However, there is limited knowledge regarding the investigation of *P. tobira* in relation to these abiotic stresses(El Dib et al. 2015).In this study, we investigated how *P. tobira* responds to waterlogging and/or salt stresses by mediating the melatonin/ROS/RNS regulatory network. Our findings may be able to provide a theoretical reference for the selection of suitable forest sites for *P. tobira*.

Materials and methods

Plant materials and growth conditions

The *P. tobira* seedlings grown at two different locations Zhejiang (120.27ºE, 30.17ºN) and Fujian (117.35ºE, 24.52ºN) provinces were used as the materials. The healthy seedlings were subjected to stress treatments as follows: (1) control (CT), in which soil relative water content was maintained at 70%-80% of the pot's holding capacity; (2) salt stress (ST), in which NaCl concentration was set at 1.5%; (3) waterlogging stress (WL), in which 1–2 cm water layer was maintained above soil surface; and (4) a combination of ST and WL (ST + WL), in which waterlogging stress was applied after 3 days of salt stress. For each treatment, three replicates were set in the experiment.

Determination of photosynthesis and chlorophyll fluorescence parameters

The photosynthesis and chlorophyll fluorescence parameters of *P. tobira* seedlings were measured at fifth day of each treatment. The net photosynthetic rate (Pn) was detected between 9:00–10:00 AM using an LI-6400 photosynthesizer (LI-6400, LICOR, USA), following the method described by Hao et al(Hao et al. 2022). The maximum photochemical efficiency of PSII (Fv/Fm), the actual photosynthetic efficiency of PSII [$Y_{(PSII)}$], photochemical quenching (qP), and non-photochemical quenching (qN) were assessed following a 20-minute period of dark acclimation. Measurements were conducted using a portable pulse amplitude modulation fluorometer (FMS2, Hansatech, King’s Lynn, Norfolk, UK) at the same time.

Determination of lipid peroxidation (MDA), superoxide anions radical (O_2^-), hydrogen peroxide (H_2O_2), and enzymatic antioxidants

The *P. tobira* seedlings were sampled for measurement at fifth day of each treatment. Superoxide anion radical (O_2^-) was quantified using the hydroxylamine oxidation method(Elstner and Heupel 1976). The malondialdehyde (MDA) content was determined using the thiobarbituric acid method(Dhindsa et al. 1981). The content of hydrogen peroxide (H_2O_2) was measured using the dimethoate orange method([CSL STYLE ERROR: reference with no printed form.]).

Fresh leaves of treated seedlings were collected for detecting the contents of four enzymatic antioxidants. Superoxide dismutase (SOD) activity was assessed using the nitro tetrazolium blue chloride (NBT) method, with measurements taken at a wavelength of 560 nm(Pereira et al. 2002). The activity of CAT was determined following the method of Aebi(Aebi 1984). Peroxidase (POD) activity was determined using the guaiacol method by recording the change in absorbance at 470 nm wavelength for 1 min([CSL STYLE ERROR: reference with no printed form.]). Ascorbate peroxidase (APX) activity was determined by the H_2O_2 scavenging reaction method. The change in absorbance was measured at 290 nm wavelength(Peng et al. 2015).

Determination of nitric oxide (NO), nitrate reductase (NR), and nitric oxide synthase (NOS)

Leaf tissues were dissected under ice bath conditions, and then homogenized into a 10% tissue homogenate using the SCIENTZ-48 high-throughput tissue grinder. After centrifuging for 15 minutes at 4°C and 4000 $r \cdot min^{-1}$, the resulting supernatants were collected for subsequent analysis. The concentration of NO was measured using a nitric oxide (NO) assay kit (Nanjing Jiancheng Bioengineering Institute, China). NR and NOS activities were assessed using the nitrate reductase (NR) assay kit and nitric oxide (NOS) assay kit, respectively (Nanjing Jiancheng Bioengineering Institute, China).

Determination of the activities of key enzymes and the levels of intermediate metabolites involved in melatonin biosynthesis

Leaf samples were pulverized into a fine powder using liquid nitrogen and dissolved in cooled methanol that contained 0.1% formic acid. After vortexing for 30 min and ultrasounding for 30 min, the extracted samples were subjected to centrifugation at 10,000×g for 10 minutes at 4 °C, and the supernatant was filtered using a membrane filter with a pore size of 0.22 μm. The contents of tryptophan (TRP), tryptamine (TRY), 5-hydroxytryptamine (Ser), N-Acetyl-5-hydroxytryptamine (NAS), N-Acetyltryptamine (NAT), 5-hydroxytryptophan (5-HTP), 5-methoxytryptamine (5-MT), and melatonin (MEL) were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS, LC: LCAD AB SCIEX; MS: 5500QTRAP AB SCIEX).

Leaf tissues were ground into a 10% tissue homogenate using a SCIENTZ-48 tissue grinder. After centrifuging for 15 minutes at 4°C and 4000 $r \cdot min^{-1}$, the supernatants were extracted for subsequent analysis using an enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Youxuan Biotech, China) with an Epoch enzyme marker (Bio Tek Instruments, USA). The activities of tryptophan decarboxylase (TDC), tryptamine-5-hydroxylase (T5H), serotonin-N-acetyl transferase (SNAT), tryptophan hydroxylase

(TPH), and N-acetylserotonin methyltransferase (ASMT) were measured at 450 nm using an Epoch ELISA (Bio Tek Instruments, USA).

qRT-PCR assays

Total RNAs were isolated from leaf samples using a plant total RNA extraction kit (BioTeke Corporation, China), and first-strand cDNA synthesis was performed using a SMART™ reverse transcription kit (Clontech, USA). The expression levels of *TDC*, *SNAT*, *SOD*, *APX*, and *CAT* were detected on a 7500fast real-time fluorescence quantitative PCR (Applied biosystems, USA) with 18s rRNA as an internal reference gene. The specific gene primers are shown in Supplementary Table 1. The relative expression levels of target genes were determined using the $2^{-\Delta\Delta CT}$ method(Livak and Schmittgen 2001), and three biological replicates were established.

Result

Symptoms changes of P. tobira seedlings under salt and/or waterlogging stresses

The leaves of *P. tobira* seedlings showed different degrees of curling under salt stress, and showed symptoms of curling, wilting and green loss under waterlogging stress (Fig. 1). Under the combined treatment of salt stress and waterlogging stress, the symptoms of green loss, wilting, and leaf curling in the seedlings were aggravated.

Effects of salt or/and waterlogging stresses on the photosynthesis in P. tobira leaves

To examine the effect of salt or/and waterlogging stresses on the photosynthesis in *P. tobira*, the net photosynthetic rate (Pn) and five chlorophyll fluorescence parameters were detected in stress-treated leaves. As shown in Fig. 2, the levels of Pn, PSII original light energy conversion efficiency (Fv/Fm), and PSII actual light energy conversion efficiency [$Y_{(PSII)}$] were significantly lower in the seedlings with different combinations of treatments than those in the controls (CT). Compared to CT, the levels of photochemical quenching coefficient (qP) in *P. tobira* seedlings at Zhejiang province were reduced by 20.68% under ST, by 22.13% under WL, and by 51.86% under ST + WL. However, the levels of non-photochemical quenching coefficient (qN) were individually increased by 305.67%, 402.52%, and 192.45% under ST, WL, and ST + WL. In contrast, the levels of qP in *P. tobira* seedlings at Fujian province were increased by 19.76% and the levels of qN, an indicator of plant's capacity for photoprotection(Hao et al. 2022), were decreased by 31.48% under ST, relative to CT. The levels qP showed the decrease of 9.43% under WL and of 41.77% under ST + WL in *P. tobira* seedlings at Fujian province, compared to the CT. qN levels exhibited the increase of 145.61% under WL and 170.18% under ST + WL.

Effects of salt or/and waterlogging stresses on the oxidation in P. tobira leaves

To determine whether salt or/and waterlogging stresses affect the oxidation in *P. tobira*, three oxidation-associated parameters were detected in stress-treated leaves. Under stress conditions, the levels of malondialdehyde (MDA, a quantitative indicator for assessing the degree of lipid peroxidation in

plants(Dehghan-Harati et al. 2022)), hydrogen peroxide (H_2O_2), and superoxide anion (O_2^-) in the seedlings grown at Fujian were significantly higher than those at Zhejiang (Fig. 3). Compared with the controls, the levels of MDA in the seedlings at Zhejiang were increased by 18.95% under ST, by 102.96% under WL, and by 112.41% under ST + WL, and those at Fujian were increased by 27.17% under ST, by 89.23% under WL, and by 134.01% under ST + WL. The levels of H_2O_2 in the seedlings at Zhejiang were increased by 10.66% under ST, by 23.62% under WL, and by 31.29% under ST + WL, and those at Fujian were increased by 12.3% under ST, by 24.18% under WL, and by 33.52% under ST + WL. The levels of O_2^- in the seedlings at Zhejiang were increased by 2.48% under ST, by 11.31% under WL, and 18.85% under ST + WL, and those at Fujian were increased by 2.16% under ST, by 9.29% under WL, and by 15.20% under ST + WL. These results suggested that the seedlings grown at Fujian under stress treatments might have more oxidation than those at Zhejiang.

Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) serves as the primary defense mechanism for scavenging ROS in plants. Here, we investigated the effects of ST, WL, and ST + WL on SOD, POD, CAT, and APX in *P. tobira* leaves. Under ST, WL, and ST + WL, the activities of SOD, CAT, and APX in the seedlings showed a declining trend at both Zhejiang and Fujian, but their activities were significantly higher at Zhejiang than those at Fujian (Fig. 4). The activities of the four antioxidants were higher under ST than those under WL. Interestingly, POD activities in the seedlings were declined at Fujian under ST, WL or ST + WL, while its activities exhibited a significant increase at Zhejiang under ST + WL.

The expression of *SOD*, *CAT* and *APX* genes was detected by qRT-PCR in *P. tobira* seedlings under ST, WL, and ST + WL. The results revealed that ST inhibited the expression of *SOD* by 11.30% of *CAT* by 24.48%, and of *APX* by 29.37% in the seedlings at Zhejiang (Fig. 5). Similarly, the expression levels of *SOD*, *CAT* and *APX* decreased by 71,20%, 37.60% and 32.83% under WL, and decreased by 94.51%, 72.62%, and 80.89% under ST + WL at Zhejiang. In contrast, in the seedlings at Fujian the expression levels of *SOD*, *CAT*, and *APX* exhibited decreases of 14.17%, 35.16%, and 15.7% under ST, decreases of 70.58%, 79.85%, and 46.83% under WL, and decreases of 84.89%, 80.93%, and 75.51% under ST + WL.

Effects of salt or/and waterlogging stresses on NO, NR, and NOS levels in P. tobira leaves

In plants, the nitric oxide (NO) biosynthesis pathways include the catalysis of L-arginine by nitric oxide synthase (NOS), the reduction of nitrite (NO_2^-) by cytoplasmic nitrate reductase (NR), and non-enzymatic pathways(Xia Haiwei et al. 2015). We here examined how ST, WL, and ST + WL affects the accumulation of NO, NOS and NR in *P. tobira* seedlings. As shown in Fig. 6, the seedlings grown at Zhejiang exhibited corresponding increases of NO levels by 9.89% under ST, by 8.91% under WL, and by 3.57% under ST + WL, decreases of NR levels by 1.49%, 3.16%, and 6.06%, and decreases of NOS levels by 6.44%, 11.76%, and 15.90%. In contrast, the seedlings grown at Fujian under ST, WL, and ST + WL had reduced NO, NR, and NOS levels.

Effect of salt or/and waterlogging stresses on melatonin biosynthesis in P. tobira

The melatonin biosynthetic enzymes melatonin (MEL), tryptophan decarboxylase (TDC), serotonin-N-acetyl transferase (SNAT), 5-hydroxytryptamine (SER), and 5-methoxytryptamine (5-MT) were measured in *P. tobira* seedlings subjected to ST, WL and ST + WL. As shown in Fig. 7, the activities of MEL, TDC, SNAT, SER, and 5-MT were significantly reduced under ST, WL, and ST + WL, and their activities showed larger changes in the seedlings at Zhejiang than those in Fujian. The activities of MEL, TDC, SNAT, SER, and 5-MT in the seedlings at Zhejiang were visibly higher under WL than under ST, while the activities of SNAT, SER and 5-MT were higher under ST or WL than under ST + WL.

The tryptophan (TRP) levels in *P. tobira* seedlings grown at Zhejiang exhibited increases by 29.54% under ST, by 33.85% under WL and by 7.69% under ST + WL, and the levels at Fujian were correspondingly increased by 19.72%, 26.34%, and 0.94%. The tryptophan hydroxylase (TPH) levels in the seedlings at Zhejiang were increased by 5.5% under ST and by 2.04% under WL, while the TPH levels at Fujian decreased by 7.43% under ST and 10.55% under WL. ST + WL induced increases of TPH levels by 29.67% at Zhejiang and by 6.82% at Fujian. The tryptamine (TRY) levels in *P. tobira* seedlings grown at Zhejiang decreased by 6.90% under ST, but those at Fujian were increased by 7.42%. Under WL, the TRY levels were increased by 9.97% at Zhejiang and by 17.87% at Fujian. The TRY levels were found to be significantly decreased in the seedlings under ST + WL cultivated at Zhejiang and Fujian. Under ST or WL, the levels of 5-hydroxytryptophan (5-HTP) exhibited a declining pattern at Zhejiang or Fujian but were increased by 26.3% at Zhejiang and by 8.31% at Fujian. ST induced increases of tryptamine-5-hydroxylase (T5H) levels in *P. tobira* seedlings at Zhejiang and Fujian with higher levels at Fujian than at Zhejiang. Under WL conditions, the T5H levels at Zhejiang exhibited an increase by 6.79%, but no significant alteration was observed in the seedlings grown at Fujian. Under ST, the content of N-acetylserotonin methyltransferase (ASMT) in *P. tobira* seedlings was reduced by 4.28% at Zhejiang but was elevated by 6.87% at Fujian. Under WL, the ASMT levels exhibited an increase by 9.96% at Fujian, but no significant changes at Zhejiang. Under ST + WL, the ASMT levels were reduced by 23.71% at Zhejiang and by 9.59% at Fujian. The levels of N-Acetyltryptamine (NAT) in *P. tobira* seedlings were decreased under ST by 12.98% at Zhejiang and by 15.40% at Fujian, but the levels of N-Acetyl-5-hydroxytryptamine (NAS) showed an increase of 5.32% at Zhejiang and of 4.18% at Fujian. WL and ST + WL induced an 11.04% and 11.54% increase in NAT levels but led to a reduction of 2.59% and 11.48% in NAS levels at Zhejiang.

The expression levels of *SNAT* and *TDC* genes in *P. tobira* seedlings at Zhejiang exhibited decreases of 8.51% and 36.28%, respectively, under ST, decreases of 25.51% and 24.92% under WL, and decreases of 54.57% and 63.14% under ST + WL. In the seedlings grown at Fujian, the expression levels of *SNAT* and *TDC* were reduced by 33.46% and 21.02%, respectively, under ST, by 61.76% and 60.64% under WL, and by 75.33% and 81.22% under ST + WL.

Discussion

Physiological responses of P. tobira under different stress treatments

Photosynthesis of plants is susceptible to environmental factors such as salt (ST) and waterlogging (WL) stress. This study revealed that ST, WL and ST + WL inhibited Pn, $Y_{(PSII)}$ and Fv/Fm of *P. tobira* seedlings to different extents, and that Pn, $Y_{(PSII)}$, and Fv/Fm were lower under ST + WL than those under ST or WL, which was consistent with previous studies(Zheng et al. 2009). It is possible that ST + WL leads to an increase of Na^+ content in *P. tobira* seedlings, which affects the metabolism and efficiency of photosynthesis and produces more necrosis and green leaves(Zeng et al. 2013). Another reason for the changes in photosynthesis is that ST + WL may cause increases of Fe and Mn levels in *P. tobira* seedlings, which results in excessive accumulation of ROS in leaves, damaging the cell membrane and hindering the efficiency of light energy conversion(Keunen et al. 2011; Katerji et al. 2004). Under ST, the qP levels of *P. tobira* seedlings grown at Fujian were increased, suggesting that plants may enhance salt resistance through elevating PSII electron transfer activity. Under ST and WL, the qN levels of *P. tobira* seedlings at Zhejiang were increased, implying that plants may safeguard the photosynthetic organs against damage caused by excessive light energy absorption.

It has been demonstrated that melatonin enhances chlorophyll content and light energy conversion efficiency of PSII through the reprogramming of polyamine and ethylene metabolism under stressful conditions in alfalfa and cucumber(Zhang et al. 2019; Jahan et al. 2021; Liu et al. 2022). Our results indicated that melatonin levels of *P. tobira* seedlings were reduced under ST, WL and ST + WL, compared to the controls, which was consistent with a previous study(Li et al. 2022). These results suggested that the interaction between melatonin and photosynthetic pigments may confer *P. tobira* salt and waterlogging stresses.

The changes of redox homeostasis and melatonin biosynthesis may contribute to the resistance of P. tobira to stress treatments

P. tobira is present in the intertidal zone of the southern coastal region of China, and subject to submergence during high tide and emerges during low tide. Under stressful conditions, the contents of ROS, mainly including OH^-, O_2^-, and H_2O_2, are elevated in mangrove(Zhong et al. 2023). Here, we found that O_2^- and H_2O_2 levels were significantly increased under ST and WL, compared to the controls. This observation suggested that salt and waterlogging stress have a detrimental impact on *P. tobira*, leading to a toxic effect. Higher levels of O_2^- and H_2O_2 were observed in *P. tobira* seedlings under ST + WL than those under ST or WL, indicating more pronounced oxidative damage under synergistic salt-waterlogging stresses relative to single stress, similar to the findings reported by Zheng et al(Zheng et al. 2009). Elevated MDA contents in *P. tobira* seedlings under ST, WL, and ST + WL indicated an imbalance in redox homeostasis. Under stressful conditions, SOD specifically scavenges O_2^- and serves as an indicator of the plant's resistance capacity(Ahmad et al. 2017). Our current results revealed that salt stress, waterlogging stress, and waterlogging-salt stresses induced a significant increase of SOD activity in *P. tobira* seedlings, accompanying with the reduction of three antioxidants CAT, POD, and APX. These results implied that *P. tobira* responds to salt and waterlogging stress by mediating ROS activity.

Under stress conditions, ROS enhances the production of endogenous melatonin, which efficiently scavenge ROS by enhancing the activities of antioxidant enzymes such as SOD and CAT, thus reducing their detrimental impacts[9,16,43–46]. In this study, we found that ST, WL, and ST + WL resulted in lower ROS levels in *P. tobira* seedlings grown at Zhejiang than those grown at Fujian. By contrast, melatonin contents of the seedlings were higher at Zhejiang than at Fujian under control group, salt stress, and salt-waterlogging stress. This finding suggested that *P. tobira* seedlings grown at Zhejiang could accumulate more melatonin that cleans over-accumulation of ROS under stress conditions. It is known that NO acts as a signaling molecule to regulate stress responses in plants[47]. Our result revealed that the activities of NR and NOR were significantly reduced under ST, WL, and ST + WL, suggesting that *P. tobira* may respond to these stresses by NO signaling.

Conclusion

In summary, we provided evidence showing that *P. tobira* could withstand salt stress and waterlogging stress by mediating the MEL/ROS/RNS redox network (Fig. 8). These results are helpful for genetic modification of salt and waterlogging stresses in *P. tobira*.

Declarations

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Author Contribution: Xiaojiao Pan: Writing - original draft preparation, review, and editing; Pengcheng Wang: Formal analysis and investigation; Mingjun Teng: Experimental operations; Manzhu Bao: Supervision

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Figures

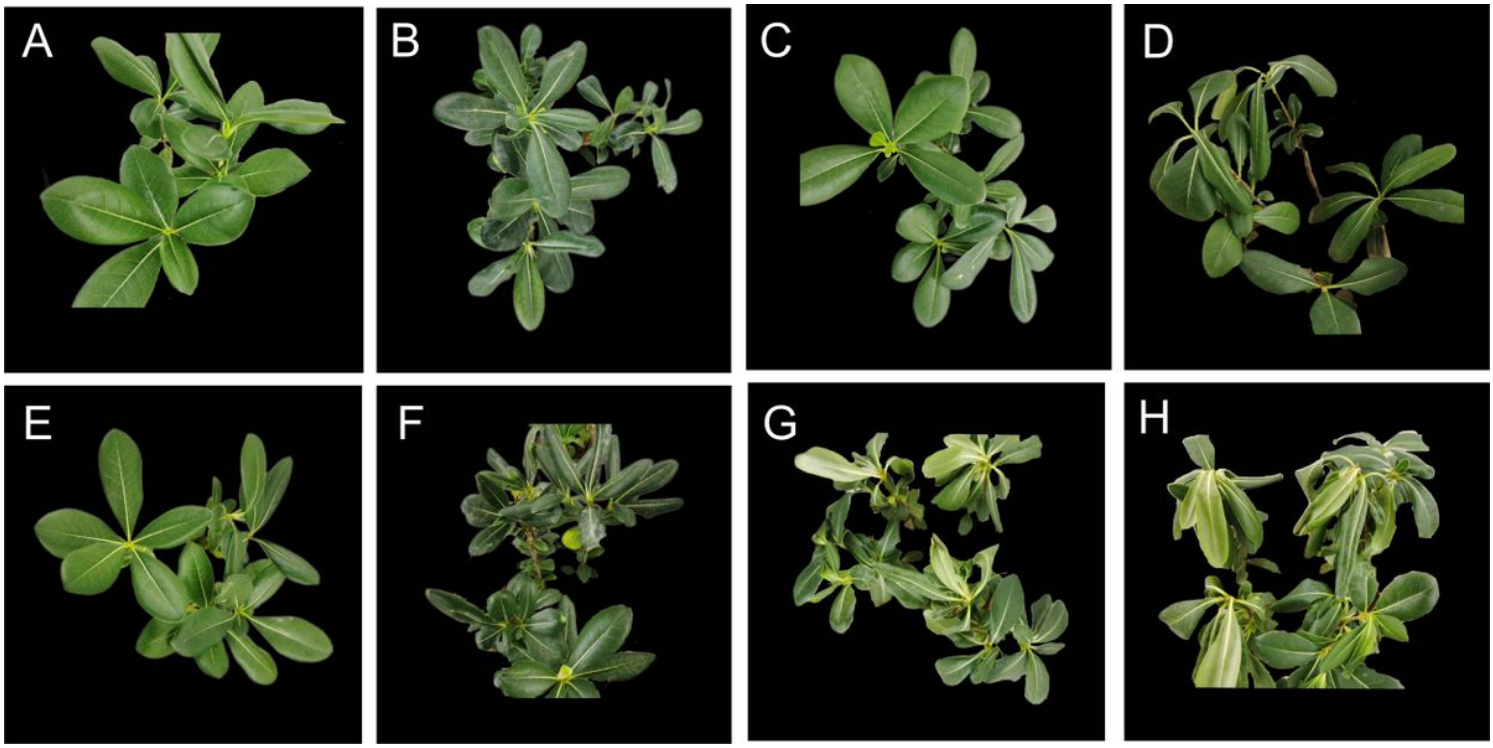

Figure 1

Physiological changes of *P. tobira* seedlings under salt or/and waterlogging stresses at Zhejiang and Fujian provinces. Figures A-D illustrate the phenotypic changes in the leaves of *P. tobira* seedlings grown in Zhejiang under control, salt stress, waterlogging stress, and combined salt-waterlogging stress, respectively and figures E-H illustrate the phenotypic changes in the leaves of *P. tobira*seedlings grown in Fujian under control, salt stress, waterlogging stress, and combined salt-waterlogging stress, respectively.

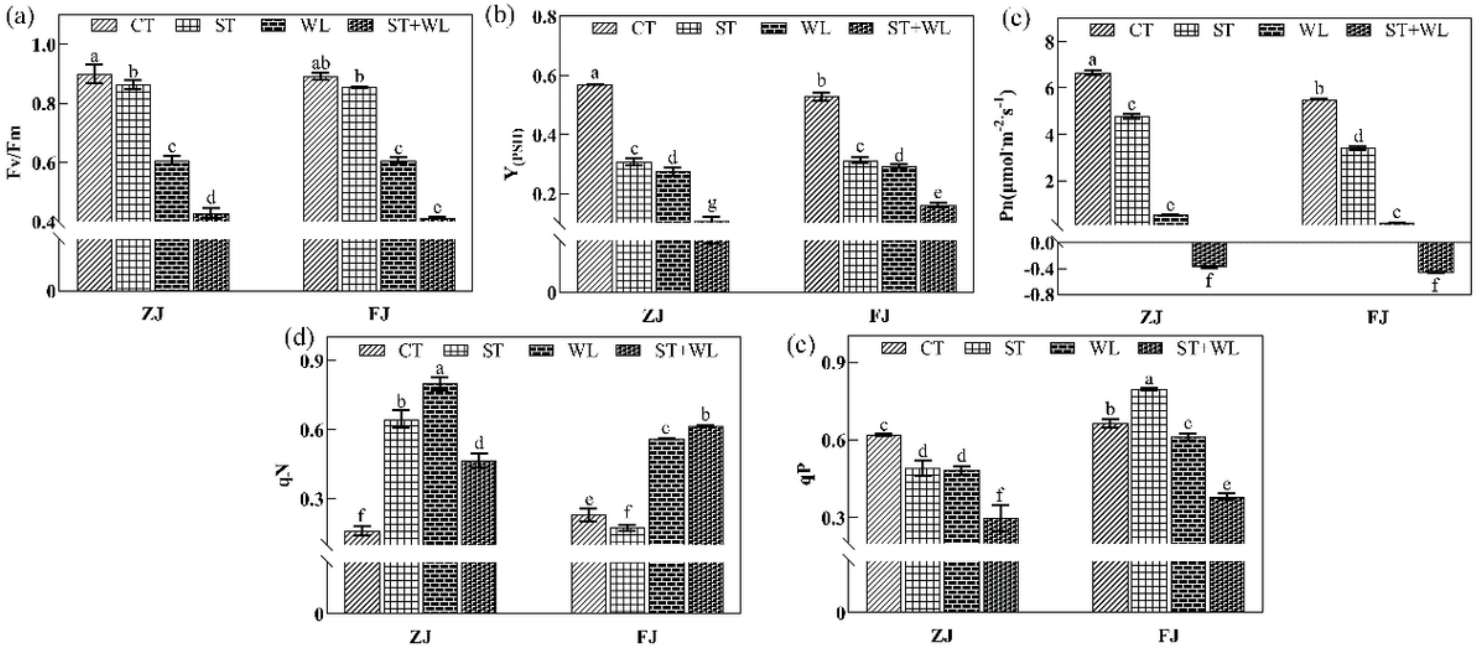

Figure 2

The effects of salt stress (ST), waterlogging (WL) and salt-waterlogging stress (ST+WL) on chlorophyll fluorescence parameters in *P. tobira* seedlings at Zhejiang (ZJ) and Fujian (FJ) provinces. Fv/Fm (a), $Y_{(PSII)}$ (b), Pn (c), qN (d), and qP (e). Data are means of three biological replicates ± standard error (S.E). Different letters denote statistically significant differences in the two-way analysis of variance (ANOVA) followed by Duncan's test ($p < 0.05$) for each stress treatment.

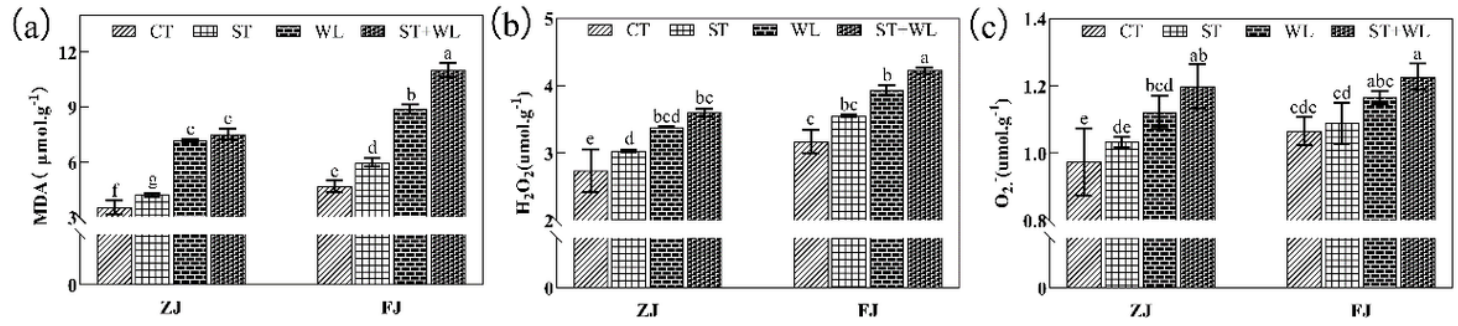

Figure 3

The effect of salt stress (ST), waterlogging stress (WL) and salt-waterlogging stress (ST + WL) on the levels of MDA, H_2O_2, and O_2^- in *P. tobira* at Zhejiang (ZJ) and Fujian (FJ) provinces. Data are means of three biological replicates ± standard error (S.E). Different letters denote statistically significant differences in the two-way analysis of variance (ANOVA) followed by Duncan's test ($p < 0.05$) for each stress treatment.

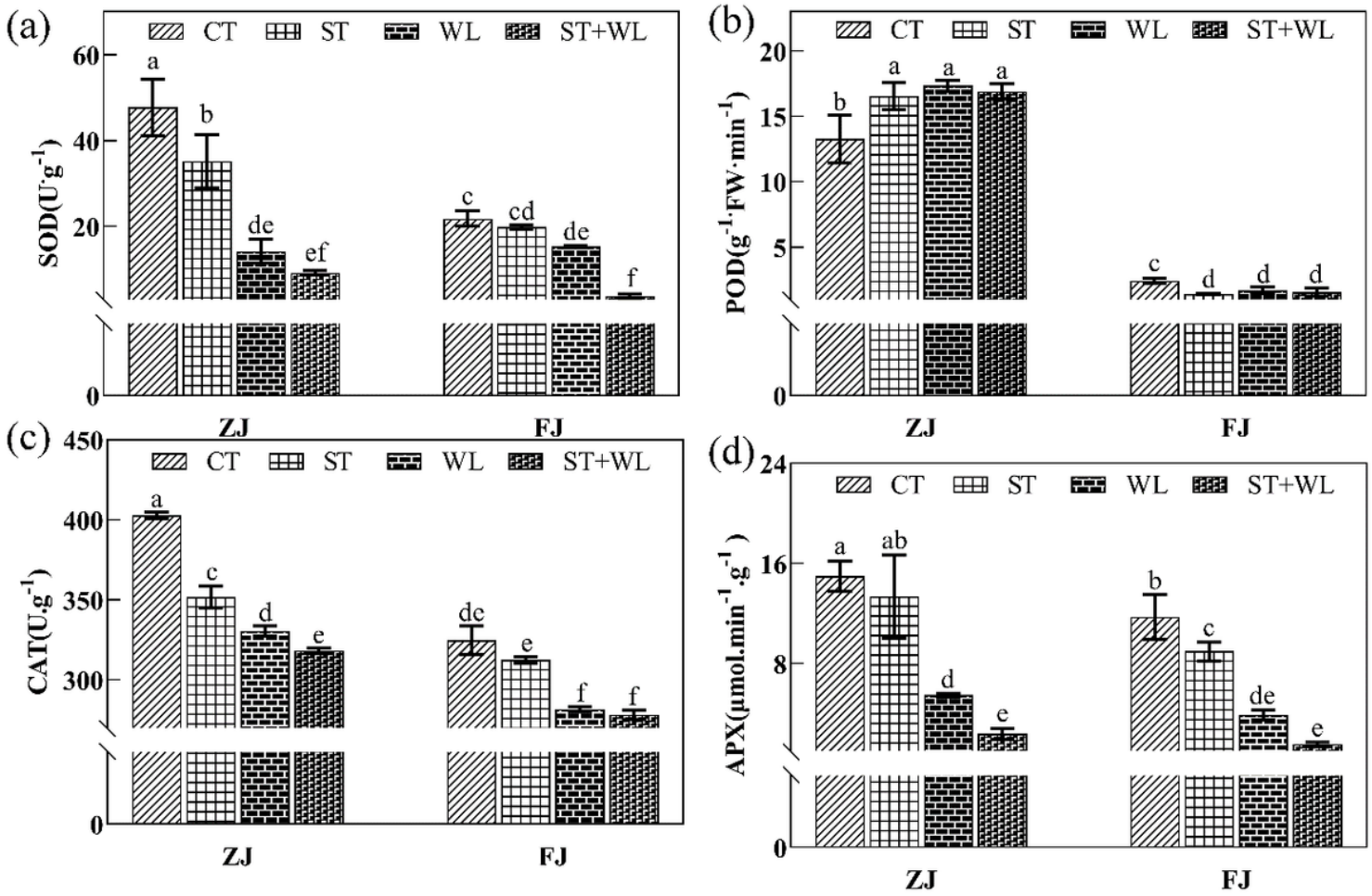

Figure 4

The effect of salt stress (ST), waterlogging stress (WL) and salt-waterlogging stress (ST + WL) on antioxidant enzymes (SOD, POD, CAT, and APX) in *P. tobira* at Zhejiang (ZJ) and Fujian (FJ). Data are means of three biological replicates ± standard error (S.E). Different letters denote statistically significant differences in the two-way analysis of variance (ANOVA) followed by Duncan's test ($p < 0.05$) for each stress treatment.

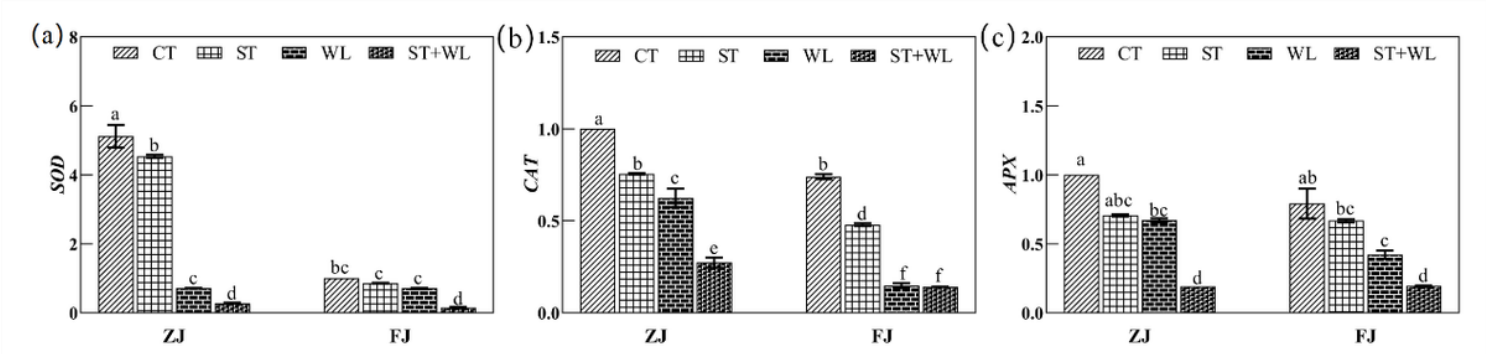

Figure 5

The effect of salt stress (ST), waterlogging stress (WL) and salt-waterlogging stresses (ST + WL) on *SOD*, *CAT*, and *APX* expression in *P. tobira* at Zhejiang (ZJ) and Fujian (FJ). Data are means of three biological replicates ± standard error (S.E). Different letters denote statistically significant differences in the two-way analysis of variance (ANOVA) followed by Duncan's test ($p < 0.05$) for each stress treatment.

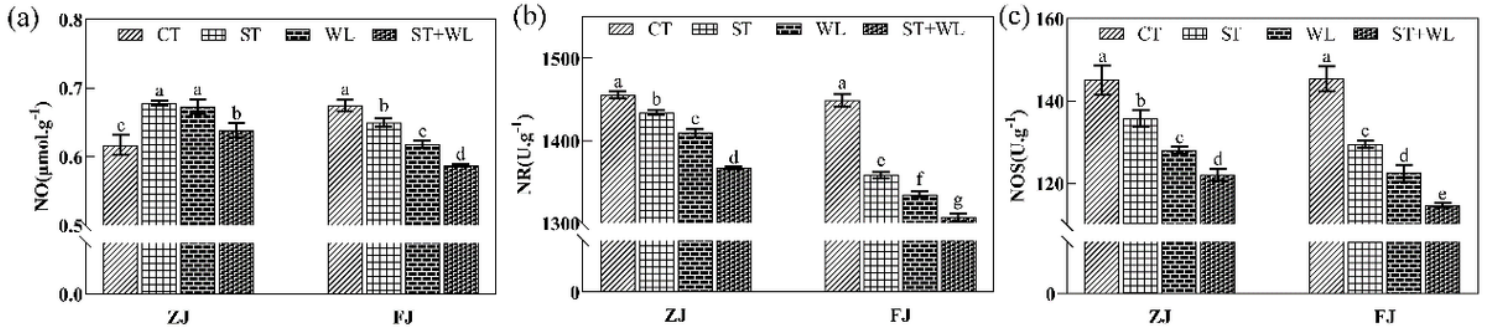

Figure 6

The effect of salt stress (ST), waterlogging stress (WL) and salt-waterlogging stresses (ST + WL) on NO, NR, and NOS contents in *P. tobira* at Zhejiang (ZJ) and Fujian (FJ). Data are means of three biological replicates ± standard error (S.E). Different letters denote statistically significant differences in the two-way analysis of variance (ANOVA) followed by Duncan's test ($p < 0.05$) for each stress treatment.

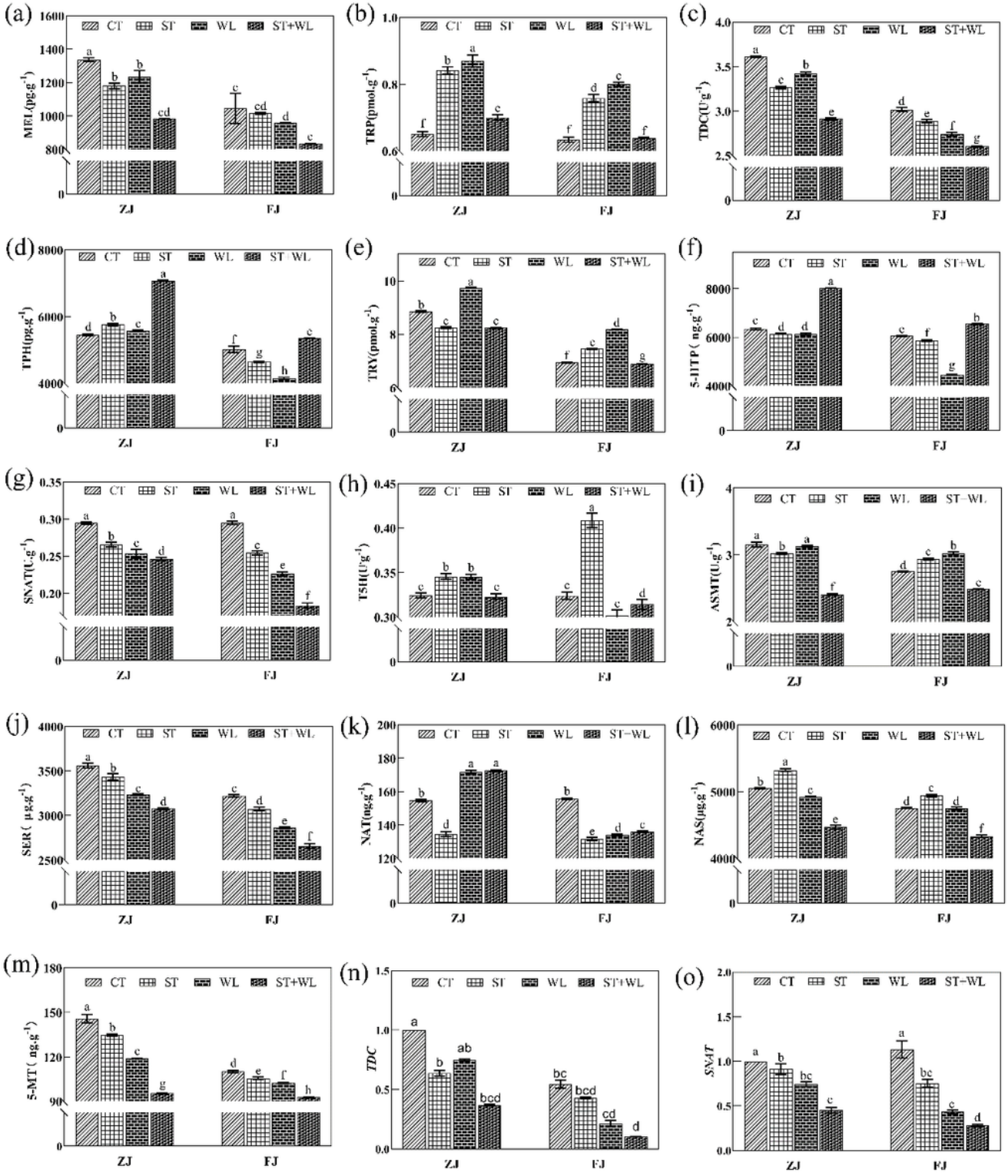

Figure 7

The effects of salt stress (ST), waterlogging (WL) and salt-logging stress (ST+WL) on melatonin (MEL), intermediate metabolites of melatonin biosynthesis (TRP, TRY, SER, NAS, NAT, 5-HTP, 5-MT), melatonin biosynthesis catalytic enzymes (TDC, T5H, SNAT, TPH, ASMT,) and melatonin synthesis-regulated genes (*TDC*, *SNAT*) in *P. tobira* at Zhejiang (ZJ) and Fujian (FJ). Data are means of three biological replicates ±

standard error (S.E). Different letters indicate significant differences in two-way ANOVA followed by Duncan's test ($p < 0.05$) under each stress treatment.

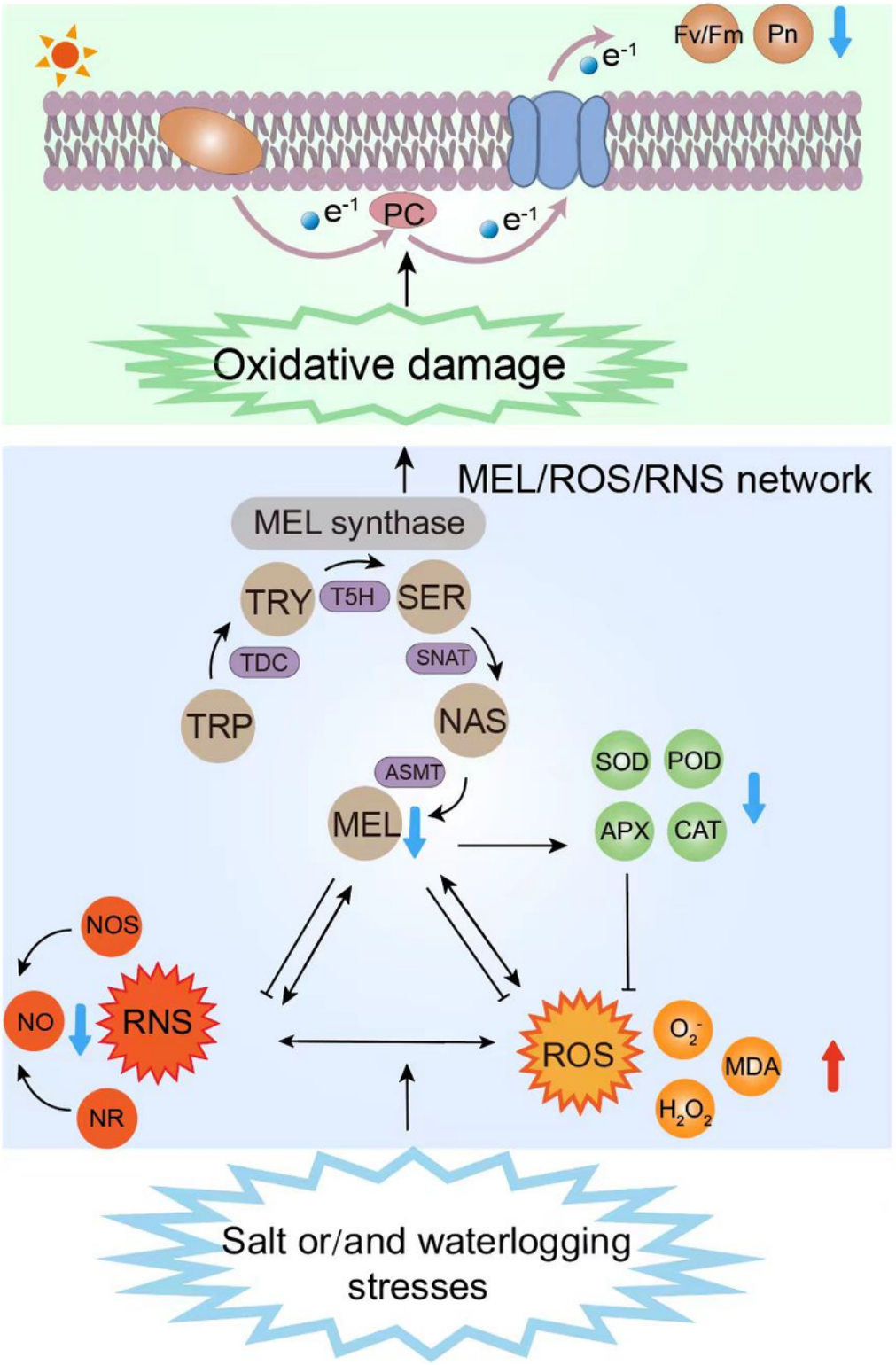

Figure 8

The MEL/ROS/RNS regulatory network. At the beginning of salt stress or waterlogging stress, ROS act as signaling molecules to activate the melatonin/ROS/RNS redox network. With the aggravation of stress,

the synthesis of RNS and melatonin was inhibited in *P. tobira* seedlings, indirectly causing a decrease in the activity of antioxidant enzymes. The imbalance in the accumulation of ROS caused oxidative damage in the plant and a decrease in its photosynthetic capacity.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.xlsx