

DIFFERENT LEAF PHYSIOLOGICAL ADAPTIVE TOLERANCE OF *PTEROCELTIS TATARINOWII* AGAINST EXTREME DROUGHT AND WATERLOGGING

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Abstract

Pteroceltis tatarinowii Maxim, an important limestone canopy tree endemic to China, is the vital material for the famous “Xuan Paper”. However, knowledge of the water adaptation of this species is essential for its natural regeneration and its related economic utilization, but it is still limited. Here, two extreme water stresses, i.e., drought and waterlogging, were applied to the one-, two-, and three-year individuals of *P. tatarinowii*, and the leaf physiological indices, including related antioxidants osmoregulatory substances, and photosynthetic parameters, were investigated and compared on different days. The results indicated that at the 5th day of water stress, leaves of three ages of *P. tatarinowii* generally showed increased antioxidant enzyme contents, and osmoregulatory substances, yet decreased net photosynthesis rates in drought and waterlogging treatments, compared with the control group. These changes in physiological parameters intensified with the extension of stress duration. Overall, waterlogging induced more severe oxidative stress and photosynthetic inhibition than drought in *P. tatarinowii* individuals. Besides, the older individuals of *P. tatarinowii* showed a stronger tolerance but a slower response than the younger ones. These results are instructive and helpful for seedling regeneration in wild populations, forestation in limestone mountains, and water management in cultivated forests of *P. tatarinowii*.

Key words: Drought; Waterlogging; Different age levels; Physiological response; Adaptive tolerance; Antioxidant enzymes; Osmoregulatory substances.

Introduction

With frequently oscillating fluctuations in rainfall and temperature, which exacerbate drought and flooding at the regional scale, plants are constantly facing severe challenges in the abiotic environment that pose far-reaching threats to ecosystem composition, functioning, and carbon balance (Gessler *et al.*, 2017; Rukh *et al.*, 2023). Plants, particularly long-lived trees, use diverse regulatory mechanisms to withstand unfavorable conditions, and these responses can differ even among species growing in the same environment (Kreuzwieser *et al.*, 2014; Hasanuzzaman *et al.*, 2022; Chen *et al.*, 2023). Adequate soil moisture is required for normal plant metabolism and physiological activity, whereas water deficits beyond a plant's tolerance threshold progressively impair these functions (Gambetta *et al.*, 2020). However, continuous precipitation inevitably leads to excessive soil moisture content, which also hinders the respiration and nutrient uptake of plant roots. The metabolites accumulated by aerobic respiration of the root system aggravate the decay or even death of the root system (Bakshi *et al.*, 2023). Therefore, extreme drought and waterlogging are one of the crucial disasters that jeopardize the normal life activities of plants.

The deciduous plant *Pteroceltis tatarinowii* Maxim, which falls under the family Cannabaceae, occupies a broad range of habitats across mainland China. The species

is valued both ornamentally and economically, particularly because its bark is a principal raw material for traditional “Xuan Paper”, which has international cultural-heritage recognition. *P. tatarinowii* commonly occurs on foothills, along forest margins and streams, and in rocky crevices at elevations of about 200-1500 m, thus water stress is an essential and noteworthy limiting factor for the growth and cultivation of their seedlings. During our field investigation, we observed a few natural seedlings from wild populations. Meanwhile, in the cultivated field, the bark yield and quality were severely affected during drought or flooding years, especially in the cultivation base (Tata *et al.*, 2022; Yusriadi *et al.*, 2024). Nevertheless, the role of water stress as an ongoing impediment to the normal growth and development of *P. tatarinowii* seedlings and saplings remains unknown.

To date, due to the effective utilization and urgent protection of wild *P. tatarinowii* germplasm resources, research on this species has mainly focused on seed dormancy and germination, propagation and nursery techniques, cultivation and ground conditions, as well as genetic diversity (Zhang *et al.*, 2007; Li *et al.*, 2019). Nevertheless, research regarding the impact of extreme water stress on this economically important species remains limited (Zhang *et al.*, 2024). Hou *et al.*, (2003) revealed that annual *P. tatarinowii* seedlings were more resistant to drought than *Fraxinus tomentosa* and *Taxodium distichum*. Wei *et al.*, (2007) dealt with annual sown seedlings of *P. tatarinowii*. They found an

overall trend from response to non-adaptation in three alternating wet and dry treatment cycles. For young trees of *P. tatarinowii*, whether they are able to endure waterlogging damage and to what extent they can endure it is unknown, which is a necessary prerequisite for the cultivation of seedlings and natural regeneration. In short, the physiology of *P. tatarinowii* under water stress, especially waterlogging, is underexplored and need further investigation.

Here, we imposed two extreme water-stress treatments, i.e., extreme drought and waterlogging, for three age levels of *P. tatarinowii* individuals under a simulated cultivation condition. Leaf physiological responses, including reactive oxygen species metabolism and photosynthesis were measured in individuals of different ages on the 5th, 10th, and 15th days of stress. The investigation therefore focused on two main questions: (1) Do both extreme water stresses obstruct the leaf physiology of *P. tatarinowii* individuals equally? If not, which stress has a more substantial effect, drought or waterlogging? (2) Do young *P. tatarinowii* individuals respond consistently in time and degree toward two designed water stresses like the old ones? If not, which age individuals of *P. tatarinowii* show the most significant resistance to water stress? Based on the above results, we propose to establish effective and specific guidelines for the management of *P. tatarinowii* young trees and the natural regeneration of this species.

Materials and Methods

Study site and materials: Situated in East China's lower Yangtze River reaches, where a subtropical humid monsoon climate prevails, the experimental site was based at Anhui Normal University (118°22'E, 31°20'N) in Wuhu, Anhui Province. Temperature extremes at the study area include a maximum of 38°C in July–August and a mean of 3°C in January, while the annual average is 16°C. The annual precipitation is uneven with an average of 1200 mm. The plant growing season (April–September) often encounters the plum rain season, which is continuous rainy weather (mid-June to early July), and the frost-free season lasts for 219–240 days per year.

Individuals representing three age classes, i.e., one-year-old seedlings, two- and three-year-old saplings, were obtained from the Xuancheng planting base. Individuals of three different ages were transplanted in a ventilated shelter on March 3, 2024, with one and two-year-old plants placed in 28×25 cm perforated plastic pots and three-year-old saplings in 35×35 cm pots of the same material. All pots were filled with 2 kg, 3 kg, and 6 kg of soil, respectively, so that all the roots of *P. tatarinowii* individuals were well covered. The soil was taken from Zheshan Mountain behind Anhui Normal University, mixed with an appropriate amount of planting nutrient soil and sand in equal proportions (mountain soil: nutrient soil: sand=10:3:4). The cultivated soil exhibited the following properties: pH 7.4, total organic matter 65.8 g·kg⁻¹, total nitrogen 3.4 g·kg⁻¹, total phosphorus 0.6 g·kg⁻¹, total potassium 16.7 g·kg⁻¹, calcium 10.8 g·kg⁻¹, available potassium 292 mg·kg⁻¹, and available phosphorus 19.1 g·kg⁻¹. Following this, all individuals were appropriately watered, regularly maintained, and weeded after transplantation for more than two months till they cured completely and grew normally.

Experimental design: The experiment was conducted from May 29, 2024, in a ventilated shelter, and healthy *P. tatarinowii* individuals of the same age with uniform growth were chosen and treated with a controlled soil water content using a weighing method. Three treatments were set up: control (CK) (normal water supply with a 50% saturated water content), drought stress (D) (reduced water supply with a 25% saturated water content), and waterlogging stress (W) (excessive water supply with a 4 cm water level above the soil surface). For each treatment, no less than 15 individuals were treated to meet the requirements for the subsequent experiments. Measurements of leaf physiological indicators and photosynthetic parameters for individuals at three ages under three treatments were carried out three times successively on the 5th, 10th, and 15th day after the treatment.

Measurements and Methods

Measurement of physiological indicators: Leaves from three individuals (n = 3) of each age class under three treatments were sealed in sampling bags, kept on ice, and transported immediately to the laboratory. After surface debris was removed by rinsing and gentle wiping, the leaves were first marked with aluminum foil, then flash-frozen in liquid nitrogen, and finally preserved at -80°C pending analysis.

When measured, the leaf sample (0.5 g) was weighed and quickly pulverized. Subsequently, the powdered material was combined with 10 mL of extraction buffer in a centrifuge tube and spun at 4°C and 10,000 rpm for 20 min. The enzyme extraction solution was made by dissolving 500 mL of 50 mmol/L pH 7.0 PBS solution, dissolved in 0.0146 g EDTA (Ethylenediaminetetraacetic acid) and 2 g PVPP (Polyvinylpyrrolidone). The supernatant was then assayed for superoxide dismutase (SOD) using the nitrogen blue tetrazole (NBT) method, for peroxidase (POD) using guaiacol, for malondialdehyde (MDA) using thiobarbituric acid (TBA), for proline using acid ninhydrin, for soluble sugars using anthrone, and for soluble proteins using Coomassie Brilliant Blue G-250, while catalase (CAT) was measured spectrophotometrically.

Measurement of photosynthetic parameters:

Photosynthetic parameters were measured via a LI-6400 portable photosynthesis system (LI-COR, Lincoln, USA). Three healthy leaves from one individual of three age classes in each treatment were labeled and measured first between 9:00 and 11:30 a.m. The photosynthetic result of this individual was then taken as the average of measurements from these three leaves, and three individuals (n = 3) for each age class in each treatment were included. During measurements, a photosynthetically active radiation (PAR, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) of 1200 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ was applied, along with an air flow of 500 $\mu\text{mol s}^{-1}$; meanwhile, the CO₂ concentration was kept near 420 $\mu\text{mol mol}^{-1}$ with the aid of an external vial. The measurements comprised Pn (net photosynthetic rate), Gs (stomatal conductance), Ci (intercellular CO₂ concentration), and Tr (transpiration rate), all expressed in the following units: $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for Pn, and $\text{mol m}^{-2} \text{ s}^{-1}$ for Gs, $\mu\text{mol mol}^{-1}$ for Ci, and $\text{mmol m}^{-2} \text{ s}^{-1}$ for Tr.

Besides, acetone extraction was employed to determine the leaf pigment levels, namely chlorophyll a, chlorophyll b, and carotenoids. μmol

Data processing: For statistical analysis, the normal distribution test and one-way ANOVA were applied using IBM SPSS Statistics 26. Duncan's test ($p < 0.05$) was employed to separate the differences between treatments and age classes, with results presented as mean $\pm$ SD. Origin 2024 software was used for graphing. Besides, to further explore the correlation among all physiologically measured traits of *P. tatarinowii* under water stress, PCA analysis of 13 leaf indexes at three age levels under drought and waterlogging was performed.

Results

Changes in antioxidant enzyme activities of *P. tatarinowii* leaves of three aged individuals under water stress: The SOD activities of *P. tatarinowii* leaves in the drought group (D) and waterlogging group (W) were significantly elevated relative to the control (CK) within each age class on days 5 and 10 ($p < 0.05$, Fig. 1a, b). Nevertheless, on the 15th day,

the SOD activity decreased slightly in both the drought group (D) and waterlogging group (W), turning insignificant with the control group (CK) (Fig. 1c). In addition, SOD activity did not differ significantly among age classes within a given treatment (Fig. 1).

Similarly, the POD content of *P. tatarinowii* leaves in drought group (D) and waterlogging group (W) of the same age showed no significant difference during water stress, except that one-year-old seedlings in the waterlogging group (W), for which POD activity exceeded both CK and drought (D) on days 10 and 15 (Fig. 1e, f). Besides, one-year-old seedlings also maintained greater POD activity than the older age classes under each treatment (Fig. 1d, e, f).

Interestingly, CAT activity in *P. tatarinowii* leaves from different age groups under waterlogging stress exceeded the drought treatment (D) before day 10 and subsequently declined (Fig. 1g, h, i). Under drought stress, one-year-old seedlings exhibited higher CAT activity relative to older saplings, partly consistent with the POD activity.

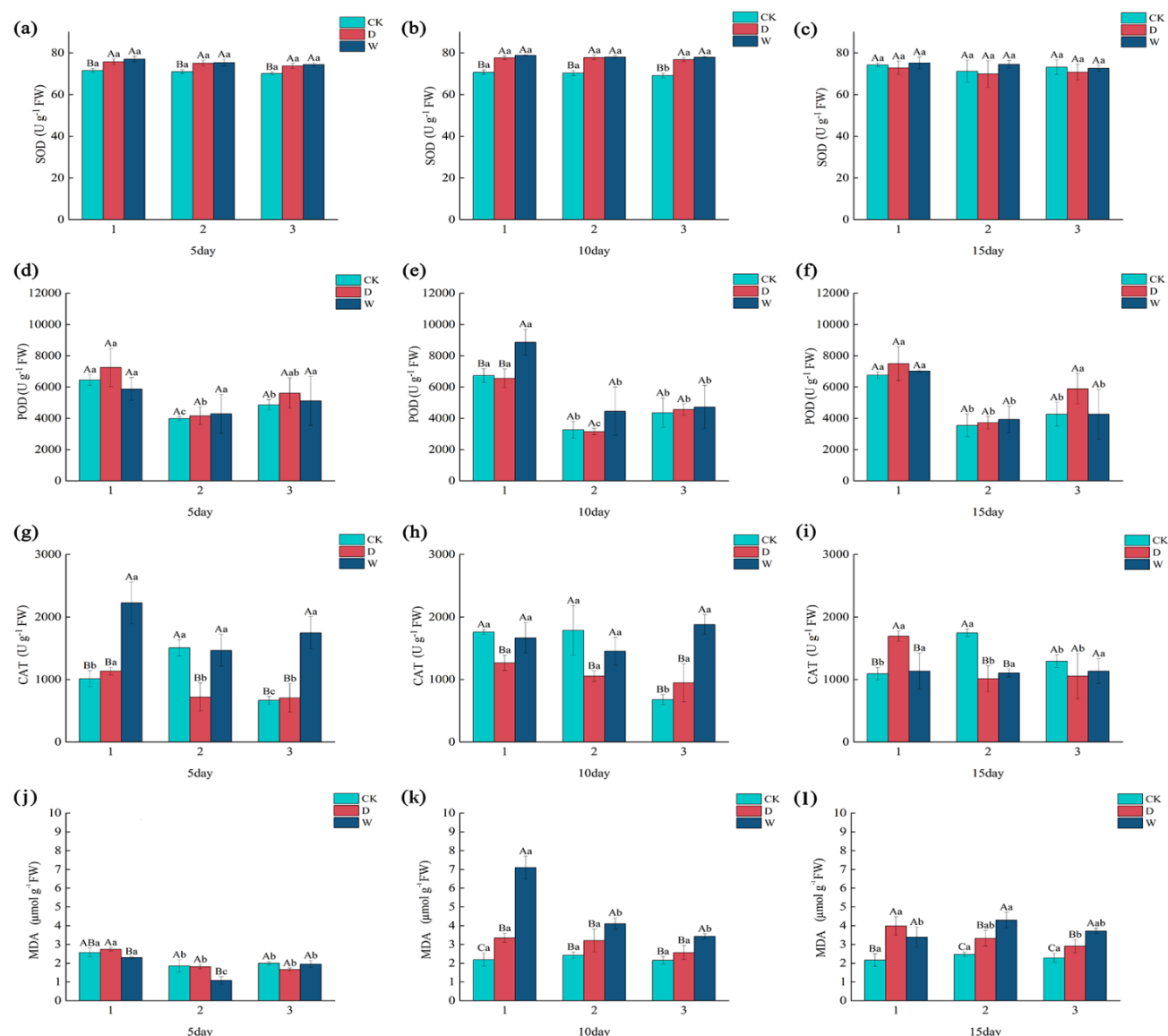


Fig. 1. Differences in protective enzyme activities and MDA content of *P. tatarinowii* leaves from three age groups (1, 2, and 3 years old) individuals under drought (D, red), waterlogging (W, blue), and control (CK, green) treatments on the 5th, 10th, and 15th day. (a-c) SOD activities. (d-f) POD activities. (g-i) CAT activities. (j-l) MDA content. Uppercase letters denote treatment differences within an age class, whereas lowercase letters denote differences among age classes within the same treatment. Different letters indicate significant differences at $p < 0.05$.

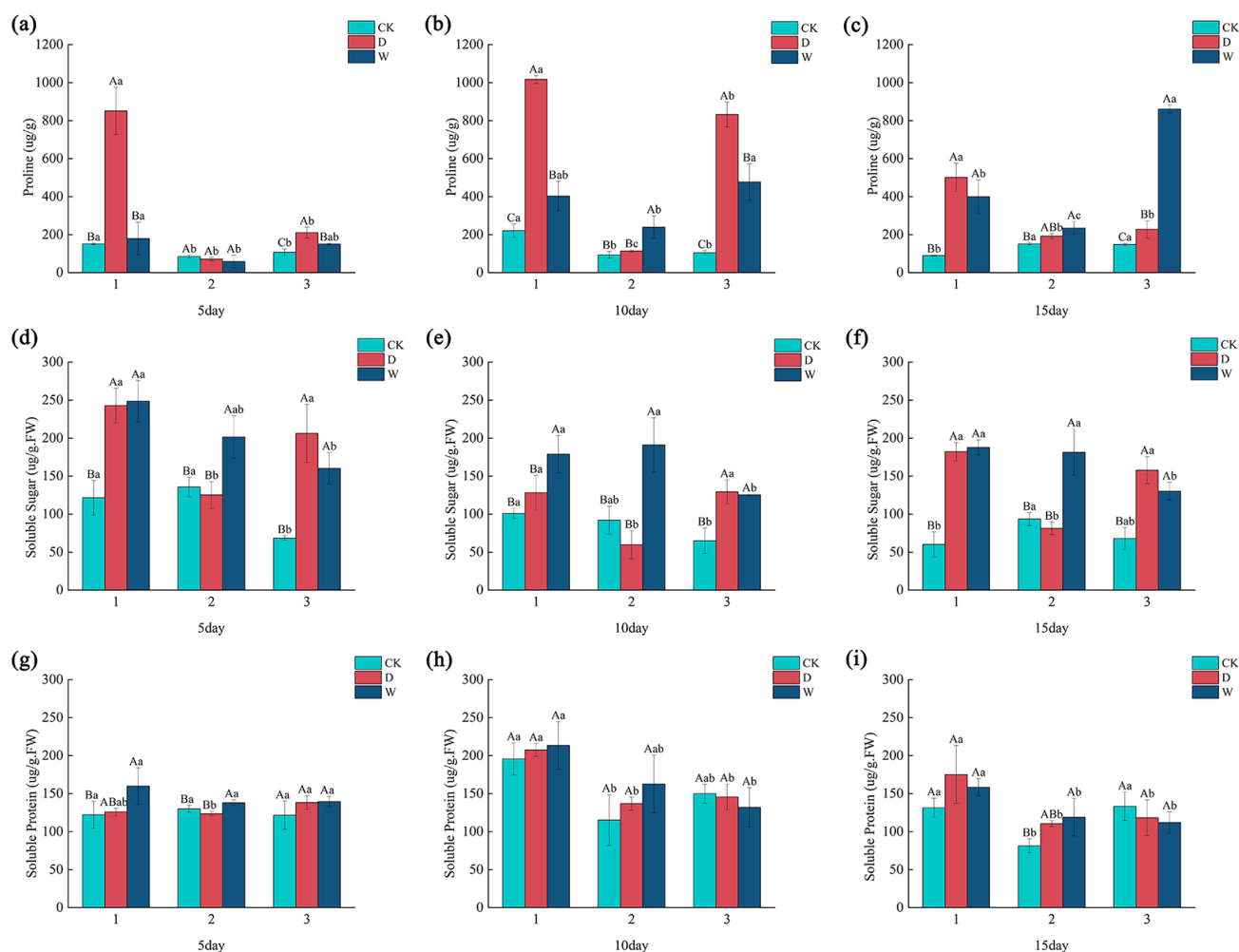


Fig. 2. Osmoregulatory substances response to water stress in *P. tatarinowii* leaves from three age groups (1, 2, and 3 years old) individuals under drought (D, red), waterlogging (W, blue), and control (CK, green) treatments on the 5th, 10th, and 15th day. (a-c) Proline level responses to water stress in different ages of *P. tatarinowii* leaves. (d-f) Soluble sugar level responses to water stress in different ages of *P. tatarinowii* leaves. (g-i) Soluble protein level responses to water stress in different ages of *P. tatarinowii* leaves. Uppercase letters indicate treatment differences within the same age class, while lowercase letters indicate differences among different age groups. Different letters indicate significant differences at $p < 0.05$.

MDA level responses to water stress in *P. tatarinowii* individuals of different ages: The MDA content of the drought and waterlogging group was significantly above the control on days 10 and 15 in all three aged individuals (Fig. 1k, l). Meanwhile, the drought group generally showed higher MDA than the waterlogging group on days 10 and 15, except for the one-year-old seedlings on the 15th day. Besides, MDA content of individuals in two stressed groups at three ages all showed an increasing trend with the continuous stress time, except that a maximum value was found in one-year-old seedlings under waterlogging stress on the 10th day (Fig. 1k).

Osmoregulatory substances response to water stress in *P. tatarinowii* individuals of different ages: With the delay of different water stress times, leaf proline content responded differently among various age levels (Fig. 2a-c). Under drought and waterlogging stress, the proline content of the leaves of one-year-old *P. tatarinowii* increased initially, peaked on day 10, and then declined, especially in the drought treatment. Increases of 156.18% (on day 10) and 55.12% (on day 15) in leaf proline were observed in the

two-year-old waterlogged saplings (W) relative to the control (CK). Similarly, the proline level in the leaves of three-year-old *P. tatarinowii* under the waterlogging group (W) also showed a significant increasing trend as the delay of the stress day increased, but the drought group (D) initially increased and then decreased by the 15th day. Proline accumulation in leaves under drought stress was significantly higher in one-year-old seedlings, exceeding that in two- and three-year-old saplings by a notable margin.

For almost all individual ages, control soluble sugar concentrations were significantly above those under the two stress regimes, except in two-year-old saplings subjected to drought (Fig. 2d-f). Meanwhile, in one- and three-year-old *P. tatarinowii* leaves subjected to either drought (D) or waterlogging (W), soluble sugar contents decreased initially and then rose moderately.

The drought stress did not significantly alter the soluble protein content of *P. tatarinowii* leaves for three aged individuals (Fig. 2g-i). Similarly, the soluble protein under the waterlogging stress generally did not differ significantly from the control, except for one-year-old seedlings at day 5 and two-year-old saplings at days 5 and 15.

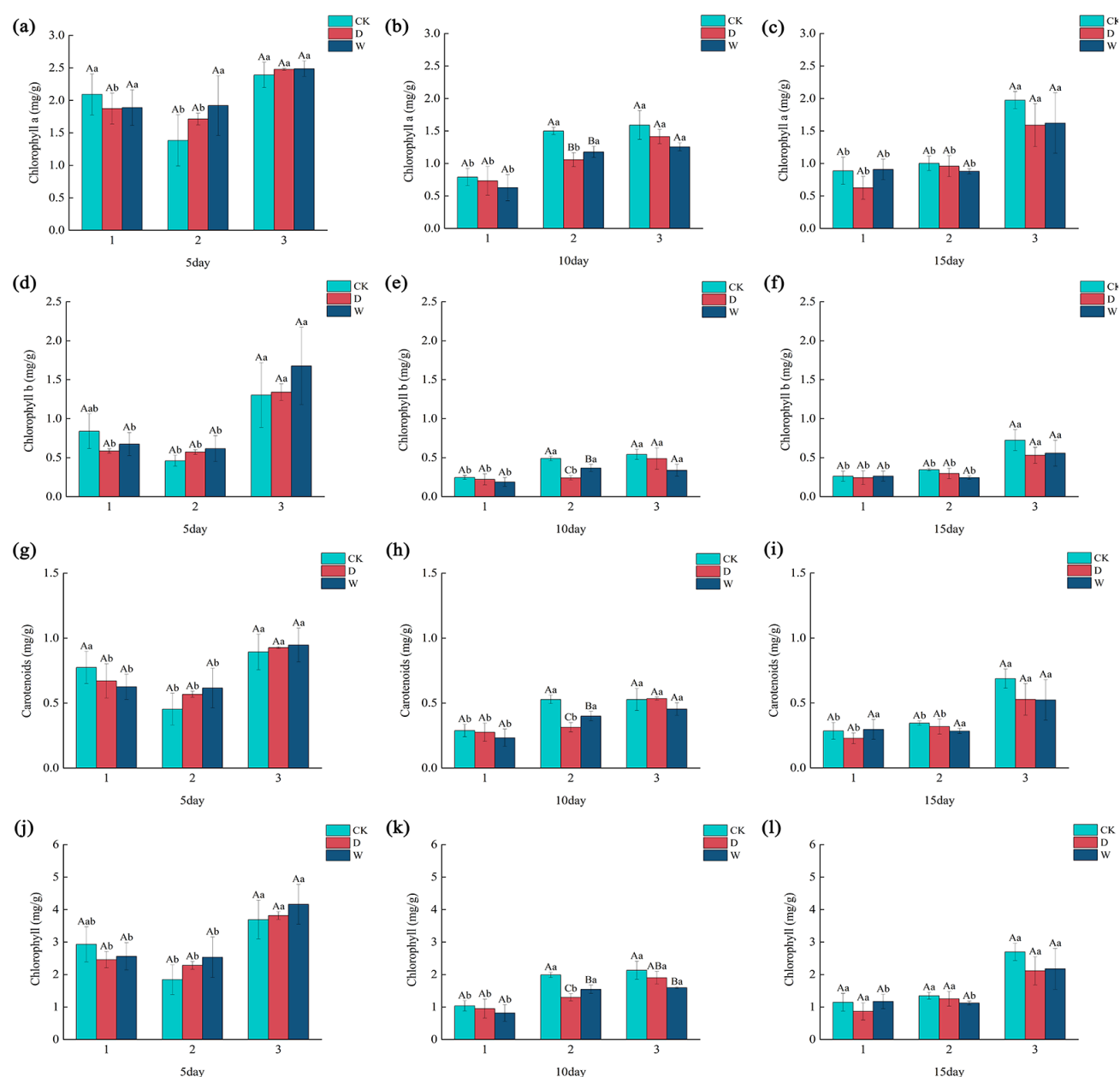


Fig. 3. Chlorophyll level responses to water stress in *P. tatarinowii* leaves from three age groups (1, 2, and 3 years old) individuals under drought (D, red), waterlogging (W, blue), and control (CK, green) treatments on the 5th, 10th, and 15th day. (a-c) Leaf chlorophyll a level responses to water stress in different ages of *P. tatarinowii*. (d-f) Leaf chlorophyll b level responses to water stress in different ages of *P. tatarinowii*. (g-i) Leaf carotenoid level responses to water stress in different ages of *P. tatarinowii*. (j-l) Leaf chlorophyll level responses to water stress in different ages of *P. tatarinowii*. Uppercase letters indicate differences among different processes at the same age, and lowercase letters indicate differences among different age groups. Different letters indicate significant differences at $p < 0.05$.

Chlorophyll level responses to water stress in *P. tatarinowii* individuals of different ages: Across all individual ages, chlorophyll a and chlorophyll b were lower on days 10 and 15 than on day 5 under both stress treatments (Fig. 3). Under waterlogging conditions at day 15, these two pigments in three-year-old *P. tatarinowii* leaves significantly exceeded those in one- and two-year-old leaves. Under drought and waterlogging, the carotenoid levels in leaves of all age classes did not differ significantly from those in the control, with the exception of the two-year-old saplings at day 10 (Fig. 3h). However, following the delay in stress, the carotenoid content decreased for each age group under both drought and waterlogging stress. The

leaf total chlorophyll content showed a similar change to the carotenoid content.

Leaf photosynthesis response to water stress in *P. tatarinowii* individuals of different ages: Compared with the control, all age groups under both drought and waterlogging exhibited reduced P_n ; in the control, three-year-old saplings had higher P_n than the younger ones, whereas such age-related differences were absent under stress conditions (Fig. 4a and c).

The leaf Gs of three age groups under the two stressed treatments were below control values on days 10 and 15. In the control group, the Gs of two- and three-year-old saplings exceeded that of one-year-old seedlings, but this trend was not consistent in the stressed groups.

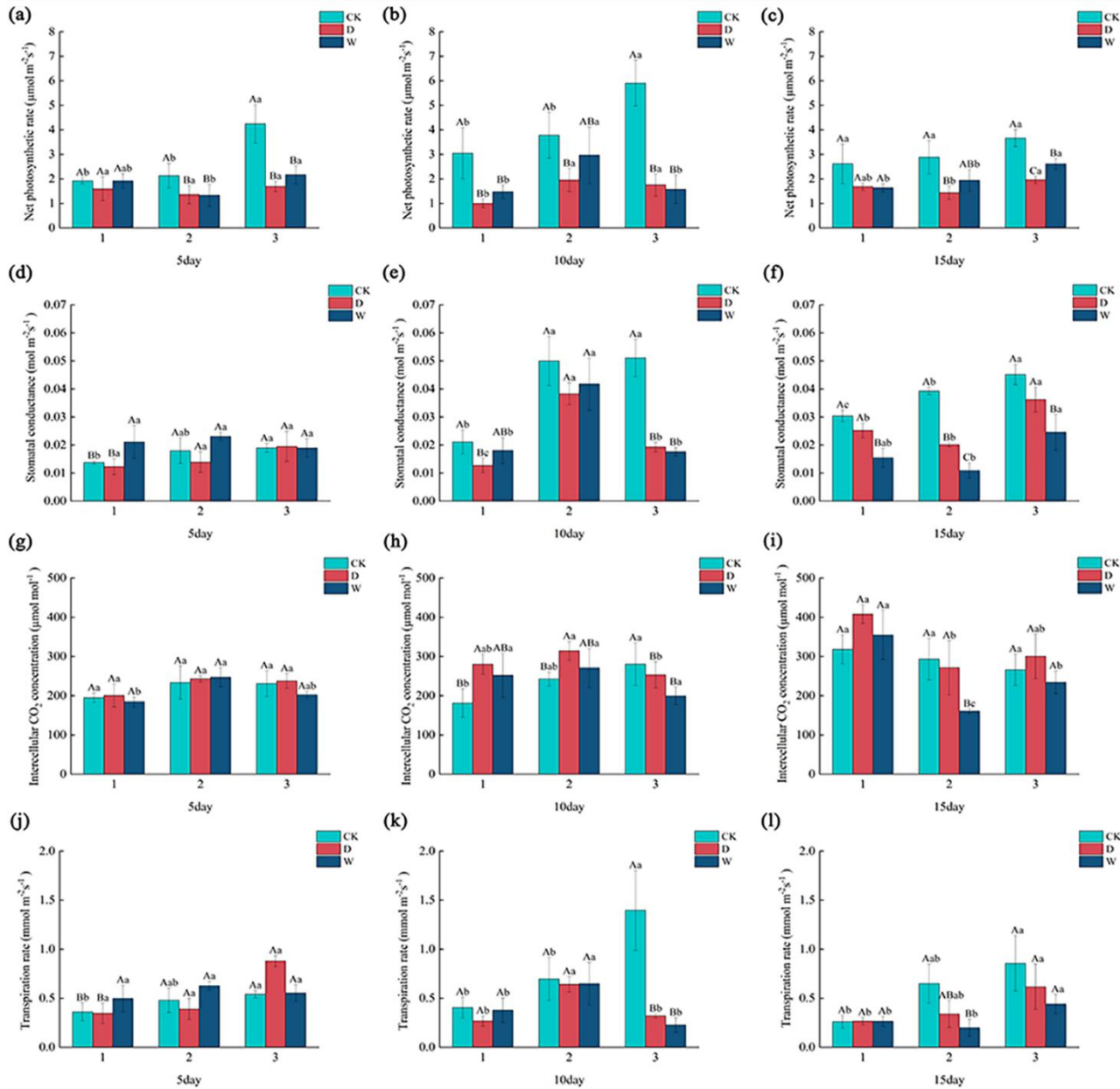


Fig. 4. Photosynthesis response to water stress in *P. tatarinowii* leaves from three age groups (1, 2, and 3 years old) individuals under drought (D, red), waterlogging (W, blue), and control (CK, green) treatments on the 5th, 10th, and 15th day. (a-c) Pn response to water stress in *P. tatarinowii* leaves of different ages. (d-f) Gs response to water stress in *P. tatarinowii* leaves of different ages. (g-i) Ci response to water stress in *P. tatarinowii* leaves of different ages. (j-l) Tr response to water stress in *P. tatarinowii* leaves of different ages. Uppercase letters denote treatment differences within the same age, and lowercase letters denote age-class differences. Different letters indicate significant differences at $p < 0.05$.

Similar to Gs, the Ci in leaves of different ages showed a certain dynamic change under water stress. Leaves from the two stressed treatments showed similar Ci with the control for all age groups on the 5th day, but this trend was not consistent with the 10th and 15th day. Additionally, under the same stress, the leaf Ci of two- and three-year-old saplings were significantly lower than in one-year-old seedlings on day 15.

From the 5th to the 15th day, the Tr of all age groups under the two stressed treatments declined relative to the control on days 10 and 15, except for one-year-old seedlings on the 15th day (Fig. 4l). In the control treatment, the Tr of two- and three-year-old saplings consistently and significantly exceeded that of one-year-old seedlings, whereas this pattern was not observed under the two stress treatments.

PCA of 13 indications of *P. tatarinowii* under water stress:

According to the PCA analysis, there were differences in 13 leaf indexes of *P. tatarinowii* among different age grades under two water stresses (Fig. 5). Under the drought stress, five indexes of MDA, CAT, soluble protein (SP), Ci, and SOD had a large positive load value with PC1, whereas chlorophyll (Ch), transpiration rate (Tr), and carotenoid (CAR) had a large negative load value with PC1. There were four indexes that were highly correlated with PC2: proline (Pro), soluble sugar (SS), and POD, which showed large positive load values, while Gs showed large negative load values. Under the waterlogging stress, the positive load values of MDA, POD, soluble protein (SP), and SOD were larger, while the negative load values of chlorophyll (Chl) and carotenoid (CAR) were larger for PC1. There was a total of 5 indicators

that were highly correlated with PC2. Among them, soluble sugar (SS), Tr, CAT, and Gs had large positive load values, and proline (Pro) had a large negative load value. The enzyme activity and osmotic substances of one-year-old *P. tatarinowii* were more obviously deviated from the older individuals; the latter responding factors contributed more to the photosynthetic parameters, especially in the drought group.

Discussion

Changes in antioxidant enzyme activities of *P. tatarinowii* leaves under water stress: Plants rely on several antioxidant enzymes to maintain cellular redox balance. Among these enzymes, SOD, POD, and CAT function to detoxify reactive oxygen species (ROS) arising from normal metabolism, thereby diminishing oxidative injury (Akbari *et al.*, 2025; Pandey *et al.*, 2025). In the absence of stress, ROS generation and scavenging maintain a dynamic equilibrium. Still, once they are subjected to unfavorable conditions or severe stresses, the antioxidant enzyme protection system responsible for scavenging reactive oxygen radicals is ruptured. In this case, ROS and free radicals can accumulate in cells, causing oxidative injury to biomolecules and ultimately restricting plant growth (Kang *et al.*, 2012; Zhang *et al.*, 2015). Thus, antioxidant enzyme activity, in terms of both magnitude and variation, can serve as a reliable indicator of stress severity and plant resistance capacity.

In this study, SOD activity in leaves of different ages of *P. tatarinowii* under two extreme water stresses increased significantly relative to the control on days 5 and 10, which is in accordance with Zheng *et al.*, (2019). It is plausible that SOD is a key player in the early stage of water stress. In contrast, POD activity exhibiting species-specific trends that are modulated by senescence and organ developmental progression (Liang *et al.*, 2018). Chang *et al.*, (2023) found that the younger *Platycladus orientalis* trees contained more POD than older individuals. However, Yao *et al.*, (2024) revealed that POD activity in poplar trees at different growth stages decreased with the increase of leaf maturity. In *P. tatarinowii*, one-year-old seedlings consistently exhibited significantly higher leaf POD activity than the two older age classes, regardless of the presence or absence of water stress. And this may indicate that the POD response rate of one-year-old *P. tatarinowii* was more sensitive than that of older individuals.

Incompatible with SOD and POD, the CAT activity in *P. tatarinowii* leaves from different age groups under waterlogging stress exceeded the drought treatment before day 10 and then declined (Fig. 1g, h, i). When plants face water stress, CAT might buffer or increase the plants' resistance in the early stage. Once the damage exceeds plants' tolerance, CAT could no longer release its potential abilities (Akbari *et al.*, 2025). In the same way, CAT activity remained higher in one-year-old seedlings than in the older age classes.

Overall, the changes of the three antioxidant enzymes under water stress were not completely synchronized in *P. tatarinowii* leaves, indicating that the response degrees of SOD, POD, and CAT were not completely synchronous. Both drought and waterlogging significantly impaired seedling/sapling physiology of *P. tatarinowii*. Antioxidant enzymes responded rapidly, but tolerance varied with age. As stress duration increases, the antioxidant enzyme activities in leaves may reach their limit, the reactive oxygen radicals may be out of balance, and thus different

age classes of *P. tatarinowii* suffered different degrees of damage during various periods of stressed seedlings.

MDA level responses to water stress in *P. tatarinowii* individuals of different ages: Water stress can disturb cellular redox balance and promote ROS accumulation, thereby enhancing membrane-lipid peroxidation and impairing membrane integrity and function (Anjum *et al.*, 2017; Li *et al.*, 2021). Because MDA is formed during lipid peroxidation, its accumulation is commonly used as an indicator of oxidative injury to cell membranes (Imahori *et al.*, 2008; Krasensky *et al.*, 2012). Bai *et al.*, (2021) found that the MDA levels in *Syringa oblata* surpassed the control from day 4 of flooding stress onward, and kept rising during the subsequent period. Under progressive drought stress, *Lotus japonicus* exhibited a peak in MDA content followed by a decline. (Song *et al.*, 2014). In this study, the MDA content of *P. tatarinowii* leaves under both drought and flooding was significantly above the control after 10 days of stress in every age class. On the 10th and 15th day, the MDA contents of *P. tatarinowii* under the flooding treatment were detected nearly all higher than the drought treatment. This might suggest that both water stresses cause damage to the plasma membranes of *P. tatarinowii*, and the menace of flooding was severe than drought. Unexpectedly, a significantly higher MDA content appeared in one-year-old *P. tatarinowii* seedlings than older saplings under waterlogging stress on the 10th day, which is in line with the walnut leaves subjected to low-temperature stress (Turfan *et al.*, 2020). The special physiological character of the one-year-old *P. tatarinowii* seedlings inferred that they were more acutely sensitive than older individuals. This is worth noting in the forest management, as it may be closely related to seedling survival and regeneration potential.

Osmoregulatory substances response to water stress in *P. tatarinowii* individuals of different ages: During environmental stress, plants are able to accumulate compatible solutes, which decrease cellular osmotic potential and thereby assist in sustaining water absorption and intracellular water homeostasis (Koryo *et al.*, 2013; Yang *et al.*, 2019). Proline, soluble sugars, proteins, and betaine have been identified as major osmotic-adjustment compounds in numerous investigations (Munns *et al.*, 2008; Ruiz *et al.*, 2016; Cai *et al.*, 2020). Zhang *et al.*, (2021) examined the osmoregulatory substances in *Hippophae rhamnoides* under drought stress and revealed that proline, soluble sugars, and proteins all exhibited higher contents than in the control. By flooding non-heading Chinese cabbage, Wang *et al.*, (2013) found an elevation in proline, soluble sugar, and protein. Varied decreasing patterns in osmoregulatory substances were observed as the flooding stress treatment progressed. Wan *et al.*, (2024) demonstrated that progressive intensification of stress stimulated the accumulation of osmoregulatory substances, including proline and soluble proteins in *Cyclocarya paliurus*, thereby improving its resistance to adverse external environments. For *P. tatarinowii*, the proline content in the leaves of one-year-old *P. tatarinowii* under drought increased initially and then declined, which was consistent with the curve trend of proline content in *Ginkgo biloba* trees under NaCl stress (Sun *et al.*, 2017). Besides, the content of proline of one-year-old *P. tatarinowii* leaves under drought treatment was also higher than that of older *P. tatarinowii* individuals.

Both drought and flooding stresses caused an increased soluble sugar and protein content in *P. tatarinowii* leaves, especially for one-year-old seedlings, suggesting that these two solute groups may act together in regulating leaf osmotic potential under both stresses. One-year-old seedlings may be the most sensitive among the three age groups due to their stronger ability to accumulate soluble sugars and proteins compared to older saplings (Cheng *et al.*, 2018).

Leaf photosynthetic indexes responses to water stress in *P. tatarinowii* individuals of different ages: Chloroplast pigments capture light for photosynthesis, and changes in chlorophyll concentration can therefore reflect plant physiological status (Flexas *et al.*, 2020; Qiao *et al.*, 2024). In the present study, extended exposure to both drought and flooding stress led to a decline in chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll across all three age classes. This decline in pigments may reflect water-stress damage to chloroplast ultrastructure, with consequent effects on photosynthetic performance (Li *et al.*, 2015; Wu *et al.*, 2018). Besides, the significantly higher chlorophyll content observed in three-year-old *P. tatarinowii* saplings relative to younger ones is likely a reflection of the more abundant chloroplasts present in the leaves of older individuals (unpublished data).

Water stress exerts a significant influence on leaf Pn, Gs, Ci, and Tr, with the observed Pn declines commonly ascribed to both stomatal and non-stomatal constraints (Farquhar, 1982). Under stomatal limitation, reduced Gs restricts CO₂ diffusion into the leaf, which can lower Ci and constrain photosynthetic carbon assimilation (Escalona *et al.*, 1999). Non-stomatal limitation can also arise when Rubisco (ribulose-1,5-bisphosphate carboxylase) activity declines, reducing CO₂ fixation to RuBP (ribulose-1,5-bisphosphate) and thereby restricting the Calvin cycle (Carmo-Silva *et al.*, 2010). Significantly lower Pn was observed in the present study under two water stresses relative to the control for most individuals,

except for one-year-old seedlings on the 5th day, which is broadly consistent with the reduced Gs and Ci for all individuals. The primary factor of stomatal limitation responsible for the reducing photosynthetic performance under water stress is supported by this observation. However, at the early stage of two water stresses, i.e., on the 5th day, two- and three-year-old saplings showed a significant reduction in Pn, but their Gs and Ci did not show any difference with the control treatment. Therefore, the limitation of Pn at the onset of the two stresses is likely attributable to non-stomatal factors (Su *et al.*, 2022).

In addition, distinct impacts of drought and waterlogging on Pn, Gs, Ci, and Tr were observed. Under the condition of waterlogging, one-year-old *P. tatarinowii* seedlings might undergo more severe anaerobic respiration and alcohol production in the underground root system for its reduced quantity root survival (unpublished data), indirectly leading to the decrease of leaves Gs, Pn, and Tr, together with the accumulation of Ci. On the contrary, the drought group (D) affected its Pn by increasing Gs and CO₂ entering plant cells, indicating that one-year-old *P. tatarinowii* was more affected by waterlogging. The Pn, Gs, and Tr of two-year-old *P. tatarinowii* increased first and then decreased, which may be due to the long-term stress breaking the photosynthetic tolerance threshold. The decrease in CO₂ entry into mesophyll cells can reduce Ci and subsequently constrain Pn and Tr. However, the decrease in Ci still could not be ruled out due to the influence of non-stomatal conductance. Besides, compared with younger individuals of *P. tatarinowii*, especially on the 15th day, the Pn and Gs of three-year-old saplings had higher values for all treatments. This is in line with the higher pigment content found in three-year-old saplings and may indicate their stronger photosynthesis ability and resistance to water stress than the younger ones. Furthermore, young, metabolically active tissues in one-year-old seedlings may prioritize rapid osmotic adjustment (e.g., high proline content), while older saplings may rely more on structural stability and maintained photosynthesis.

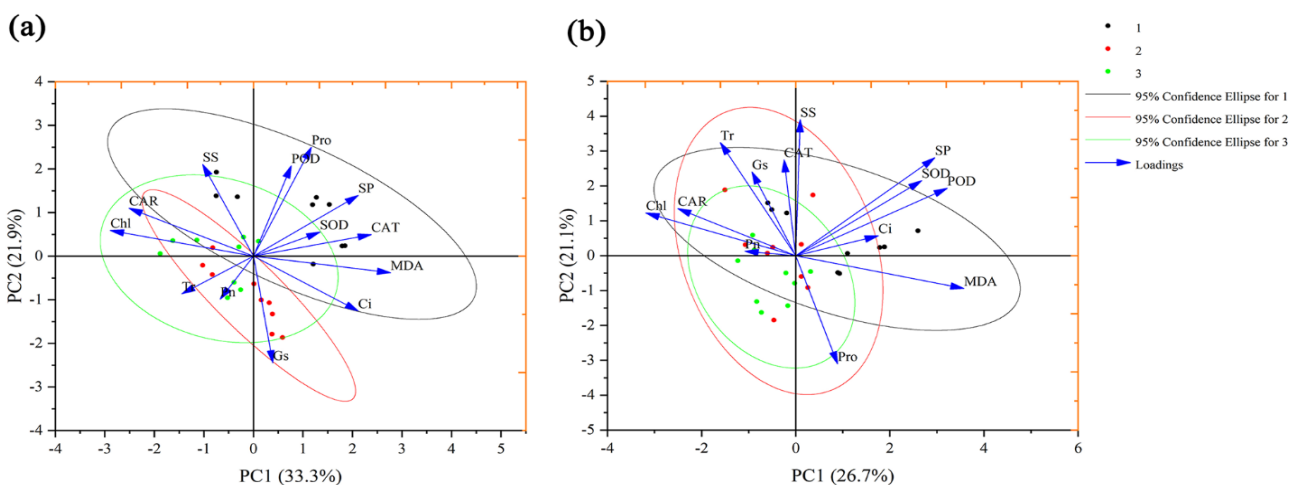


Fig. 5. Principal component score graphs of 13 indicators of different treatments for *P. tatarinowii*. Black, red, and green represent principal component scores during water stress of one-, two-, and three-year-old *P. tatarinowii*, respectively. (a) PCA map of drought treatment. (b) PCA map of waterlogging treatment. Under drought stress, PC1 exhibited large positive loadings for MDA, CAT, soluble protein (SP), Ci, and SOD, and large negative loadings for chlorophyll (Chl), Tr, and carotenoid (CAR); PC2 showed large positive loadings for proline (Pro), soluble sugar (SS), and POD, and a large negative loading for Gs. Under waterlogging stress, PC1 had large positive loadings for MDA, POD, SP, and SOD, and large negative loadings for Chl and CAR; PC2 showed large positive loadings for SS, Tr, CAT, and Gs, and a large negative loading for Pro.

Conclusion

Water availability strongly affected the physiological performance of *P. tatarinowii* seedlings and saplings, with the magnitude of the response varying among age classes. Our present study also showed that *P. tatarinowii* individuals of varying age classes were able to cope with external water stress within a certain time confinement. On the whole, extreme waterlogging was more stressful than drought for these limestone tree seedlings and saplings, although with some incomplete consistency. PCA analysis showed that the antioxidant enzyme activity and osmotic substances played a major role in one-year-old *P. tatarinowii*, while changes in photosynthetic indices were more prominent in the two- and three-year-old saplings, which also reflected the differences in physiological response mechanisms of different ages of *P. tatarinowii* in dealing with water stress.

Our study suggests that the soil water content of the habitat has a certain impact on the renewal and natural population distribution of *P. tatarinowii* seedlings and saplings. As a pioneer tree species in limestone mountains, *P. tatarinowii* appears to tolerate drought better than prolonged waterlogging. Due to the scarcity of waterlogging study on *P. tatarinowii*, our findings provide practical guidelines for seedling regeneration and forest management of this species. It is necessary to rationally control the soil moisture content in terms of water management in artificial cultivation to avoid irreversible damage to the annual *P. tatarinowii* seedlings caused, in particular, by waterlogging.

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