

Effects of exogenous NO on antioxidant system of *Taxus* plants under simulated acid rain stress

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Abstract

Taxus is a famous medicinal and landscape tree species. The aim of this study was to explore the effect of exogenous nitric oxide (NO) on the resistance of *Taxus* plants to acid rain stress and to identify *Taxus* species with strong acid rain resistance by principal component analysis and comprehensive evaluation. In this study, sodium nitroprusside (SNP) was used as the exogenous NO donor. The effects of different SNP solution concentrations on the antioxidant systems of three *Taxus* species subjected to simulated acid rain stress (pH = 3.0) were compared. In order to achieve this goal, we determined the rate of O²⁻ production, the ASA and GSH contents in leaves of three *Taxus* plants (*Taxus mairei*, *Taxus chinensis*, and *Taxus yunnanensis*). At the same time, the active leaves of some antioxidant enzymes (SOD, POD, CAT, APX and GR) were determined. For *Taxus chinensis* plants subjected to acid rain stress, treatment with an SNP concentration of 0.25 mmol·L⁻¹ led to the most significant improvements in the antioxidant system. For *Taxus mairei* and *Taxus yunnanensis*, the treatment with the SNP concentration of 0.50 mmol·L⁻¹ was best for improving their antioxidant systems under stress. Meanwhile, *Taxus chinensis* had the strongest resistance to simulated acid rain, followed by *Taxus mairei* and *Taxus yunnanensis*.

Keywords: acid rain; antioxidant system; nitric oxide (NO); sodium nitroprusside (SNP); *Taxus* plants

Introduction

Taxus is a genus in the Taxaceae family, which mostly consists of evergreen shrub species. Most *Taxus* species have a beautiful tree shape and strong germination ability. *Taxus* plants is well known for its high value in the treatment of various cancers (Fei *et al.*, 2019). They grow quickly, are well adapted to their environment and are easy to trim and shape (Rathore *et al.*, 2019). *Taxus* is an economically important and an endangered gymnosperm genus and *Taxus* is an important ornamental plant for horticulture and gardening (Fei and Tang, 2018). Therefore, *Taxus* species have been increasingly used in urban landscaping. Rainwater acidification is considered to be one of the most serious environmental problems affecting transboundary natural environments. Due to the interaction of these acids with other components in the atmosphere, protons are released. This proton release increases the acidity of the soil and the utilization of toxic heavy metals, reduces

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soil fertility, and ultimately negatively affects the growth and productivity of gardens and crops (Singh and Agrawal, 2008). Nitric oxide (NO), as a signaling molecule, usually interacts with plant hormones and other endogenous molecules during early plant growth and development (Freschi and Luciano, 2013). Many reports have indicated that the application of exogenous NO donors confers tolerance to various abiotic stresses (Mirza *et al.*, 2010; Hasanuzzaman *et al.*, 2011; Hasanuzzaman *et al.*, 2013). Sodium nitroprusside (SNP) is an exogenous NO donor. In this study, the effects of SNP on the antioxidant substance content and antioxidant enzyme activity of *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* were analyzed under acid rain stress (pH = 3.0). Choosing *Taxus* species with strong acid rain resistance is of great significance for the future promotion and application of *Taxus* in landscape beautification. It is also of practical significance to explore the effect of exogenous NO on mitigating the effects of acid rain stress on *Taxus* plants.

Materials and Methods

Experimental setup and treatment conditions

The experiment was conducted in the greenhouse of the Botanical Garden of the College of Horticulture and Gardening, Yangtze University, China. In November 2016, 200 2a seedlings of each of three species, *Taxus mairei*, *Taxus chinensis*, and *Taxus yunnanensis*, were introduced from outside of the university. A mixed matrix, with a loam-to-fine sand-to-wood chips ratio of 3:1:1, was used to plant the seedlings in. The seedlings were planted in a flower pot with an inner diameter of 10 cm and placed in a greenhouse with normal water and fertilizer management. On August 9, 2018, approximately 180 robust *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* seedlings were selected. The seedlings were randomly divided into six groups, as shown in Table 1. Four times (on August 9, 11, 13, and 15), in the evening, each group of seedlings was sprayed with SNP concentrations of 0 mmol·L⁻¹ (CK1), 0 mmol·L⁻¹ (CK2), 0.10 mmol·L⁻¹ (N1), 0.25mmol·L⁻¹ (N2), 0.50mmol·L⁻¹ (N3) and 0.75mmol·L⁻¹ (N4). The control groups (CK1 and CK2) were sprayed with clear water. A repeat was set for every 10 plants, each treatment was repeated three times, and the leaves were evenly sprayed. The purpose of CK1 was to provide a control for CK2, to show to what extent the simulated acid rain solution with a pH of 3.0 could damage the *Taxus* plants.

Acid rain stress treatment began on August 20, and 500mL of simulated acid rain solution (Ju *et al.*, 2017) (pH = 3.0) was sprayed evenly on the seedling leaves of each stress group (CK2, N1, N2, N3 and N4). An equal volume of simulated acid rain with a pH of 5.6 was used as a control (CK1). The leaves were sprayed once a week, and the SNP solutions of various concentrations were used to prevent decomposition and deterioration. Normal water and fertilizer management was maintained throughout the test period. All the treatments ended on September 10, 2018, and samples were taken after 10 days of growth. An appropriate amount of functional leaves was collected and stored at -80 °C in an ultra-low temperature refrigerator.

Table1. *Taxus* seedling treatment groups

Treatment characteristics	Treatment group					
	CK1	CK2	N1	N2	N3	N4
Sodium nitroprusside (mmol·L ⁻¹)	0	0	0.10	0.25	0.50	0.75
Simulated acid rain (pH)	5.6	3.0	3.0	3.0	3.0	3.0

Antioxidant system index measurements

The superoxide dismutase (SOD) activity was determined through the aorta-anisidine method (Durak *et al.*, 1993). The activity of peroxidase (POD) was determined via the guaiacol method (Rufino *et al.*, 2008). The formation rate of superoxide radical (O₂⁻) was measured using the hydroxylamine oxidation method (Karpets and Kolupaev, 2018). The procedure given by Huang *et al.* (2005) was used for the determination of

ascorbic acid (ASA) contents. The guaiacol method was used to measure the catalase (CAT) activity (Durak *et al.*, 1999). Ascorbate peroxidase (APX) activity was measured using a 2 mL system measuring instrument (Gadelha *et al.*, 2017). The reduced glutathione (GSH) contents were determined using spectrophotometry (Cao *et al.*, 2018). The glutathione reductase (GR) activity was calculated by measuring the decrease in absorbance at 340 nm caused by the dehydrogenation of NADPH (Gadelha *et al.*, 2017).

Data analysis

The SAS 10.0 statistical software was used to process data for the analysis of variance (ANOVA) and Duncan multiple comparison tests. The Origin 2020 software was used for plotting, and the principal component analysis (PCA) was used to comprehensively evaluate the three *Taxus* plants.

Results

Effect of SNP on the rate of O₂⁻ production in leaves of Taxus spp. under acid rain stress

As shown in Figure 1A, after simulated acid rain treatment, the O₂⁻ generation rate in the leaves of *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* was significantly higher than in the CK1 group ($p < 0.05$). The O₂⁻ generation rate in *Taxus mairei* and *Taxus chinensis* leaves first decreased and then increased as the SNP concentration increased. The O₂⁻ generation rate in *Taxus yunnanensis* leaves decreased with increasing SNP solution concentration. Compared with CK2, the production rate of O₂⁻ in the leaves of all three species was significantly lower in the groups sprayed with SNP solution ($p < 0.05$). The *Taxus mairei* plants in the N3 group displayed the most significant reduction ($p < 0.05$) in O₂⁻ generation compared to those in the CK2 group, with a 36.85% reduction. The *Taxus chinensis* plants in the N2 group displayed the most significant reduction ($p < 0.05$) in O₂⁻ generation compared to those in the CK2 group, with a reduction of 26.00%. For *Taxus yunnanensis*, the N4 treatment (except N1) led to the most significant decrease in O₂⁻ generation at 25.48% ($p < 0.05$). The results show that the application of simulated acid rain with a pH of 3.0 can significantly ($p < 0.05$) increase the rate of O₂⁻ production in the leaves of the three *Taxus* species. Spraying with SNP concentrations of 0.50 mmol·L⁻¹, 0.25 mmol·L⁻¹ and 0.75 mmol·L⁻¹ can significantly ($p < 0.05$) reduce the O₂⁻ production rate in the leaves of *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* plants subjected to acid rain stress, respectively.

Effects of SNP on SOD, POD and CAT activities in leaves of Taxus spp. under acid rain stress

After simulated acid rain treatment (pH = 3.0), the SOD (Figure 1B), POD (Figure 1C) and CAT (Figure 1D) activity in the leaves of all three *Taxus* species were significantly lower than after CK1 treatment ($p < 0.05$). Upon the addition of SNP, the SOD activity of *Taxus mairei* generally increased with increasing SNP solution concentration, while the POD and CAT activity first increased and then decreased. The activities of SOD and CAT in the leaves of *Taxus chinensis* increased first and then decreased as the concentration of SNP increased. While the activity of POD showed a downward trend. The activity of SOD in *Taxus yunnanensis* leaves decreased with increasing SNP concentration, the POD activity first increased and then decreased. While the CAT activity showed a downward trend. For the *Taxus mairei* plants, the activities of SOD, POD and CAT were significantly higher ($p < 0.05$) than CK2. The N3 treatment had the most significant effect on the activities of SOD, POD and CAT in *Taxus mairei* ($p < 0.05$), with increases of 63.59%, 126.30% and 41.65%, respectively. The SOD and POD activities (N1, N2 and N3) in *Taxus chinensis* leaves have been significantly increased ($p < 0.05$), N4 has been significantly decreased ($p < 0.05$). The SOD activity of *Taxus chinensis* leaves increased significantly ($p < 0.05$) after N3 treatment, with an increase of 37.69% compared to after CK2. The POD activity of *Taxus chinensis* leaves increased significantly ($p < 0.05$) after N2 treatment, with an increase of 34.50% compared to after CK2. The leaves of *Taxus yunnanensis* plants subjected to N1 treatment displayed the highest SOD activity, subjected to N3 treatment displayed the highest

POD activity and those subjected to N4 treatment displayed the highest CAT activity ($p < 0.05$), with increases of 37.69%, 123.95% and 29.19%, respectively. The results showed that the application of simulated acid rain with a pH of 3.0 can significantly ($p < 0.05$) reduce the activities of SOD, POD and CAT in the leaves of the three *Taxus* species. After the SNP solutions were sprayed at concentrations of 0.50 mmol·L⁻¹, 0.50 mmol·L⁻¹ and 0.10 mmol·L⁻¹, the leaf SOD activity in stressed *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* plants, respectively, increased significantly ($p < 0.05$). After the SNP solutions were sprayed at concentrations of 0.50 mmol·L⁻¹, 0.10 mmol·L⁻¹ and 0.50 mmol·L⁻¹, the leaf POD activity in stressed *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* plants, respectively, increased significantly ($p < 0.05$). When SNP solutions were sprayed at 0.50 mmol·L⁻¹, 0.25 mmol·L⁻¹ and 0.75 mmol·L⁻¹, the leaf CAT activity in stressed *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* plants, respectively, could be significantly increased ($p < 0.05$).

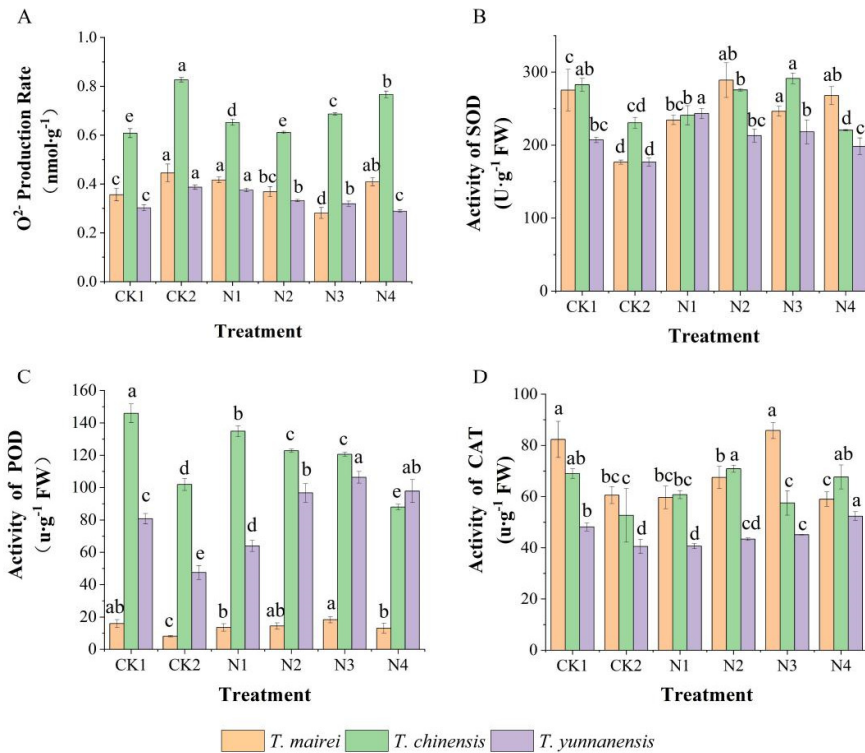


Figure 1. Effects of different sodium nitroprusside (SNP) treatments on the O²⁻ production rate (A) and activities of SOD (B), POD (C) and CAT (D) in the leaves of *Taxus* seedlings under simulated acid rain stress

Different lowercase letters indicate a significant difference ($p < 0.05$), same the below. CK1 represents a control group (simulated acid rain at a pH of 5.6), as does CK2 (simulated acid rain at a pH of 3.0). The other treatments involved simulated acid rain (pH = 3.0) with 0.10 mmol·L⁻¹ (N1), 0.25 mmol·L⁻¹ (N2), 0.50 mmol·L⁻¹ (N3) and 0.75 mmol·L⁻¹ (N4) SNP solution concentrations.

Effects of SNP on the ASA and GSH contents in leaves of Taxus spp. under acid rain stress

After simulated acid rain treatment (pH = 3.0), the contents of ASA (Figure 2A) and GSH (Figure 2B) in leaves of the three species were significantly lower than after CK1 treatment ($p < 0.05$). The ASA content of *Taxus mairei* leaves showed an upward trend with increasing SNP concentration, the GSH content increased at first and then decreased. As the SNP concentration increased, the contents of ASA and GSH in the leaves of *Taxus chinensis* increased first and then decreased. The content of ASA in the leaves of *Taxus yunnanensis* generally increased first and then decreased, and the content of GSH showed an upward trend. The ASA and GSH contents in the leaves of the three *Taxus* species were significantly higher ($p < 0.05$) after SNP treatment than after the CK2 treatment. For *Taxus mairei*, the ASA and GSH contents were most significantly increased in the N3 treatment ($p < 0.05$), at 27.52% and 108.77% higher than in the CK2 group, respectively. The contents of ASA (N1, N2 and N3) and GSH in the leaves of *Taxus chinensis* were the most significantly increased after N2 treatment ($p < 0.05$), with increases of 80.94% and 91.45%, respectively. For *Taxus yunnanensis*, the most significant increase in ASA content was observed in the N3 group ($p < 0.05$), with an increase of 37.88%. Meanwhile, the *Taxus yunnanensis* leaf GSH content was most significantly increased by the N4 treatment ($p < 0.05$), with an increase of 62.85%. The results show that the application of simulated acid rain with a pH of 3.0 can significantly ($p < 0.05$) reduce the leaf ASA and GSH contents in the three *Taxus* species. After spraying the stressed *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* plants with SNP concentrations of 0.50 mmol·L⁻¹, 0.25 mmol·L⁻¹ and 0.50 mmol·L⁻¹, respectively, the leaf ASA content increased significantly ($p < 0.05$). When SNP concentrations of 0.50 mmol·L⁻¹, 0.25 mmol·L⁻¹ and 0.75 mmol·L⁻¹ were applied to stressed *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* plants, respectively, the leaf GSH content could also be increased significantly ($p < 0.05$).

Effects of SNP on APX and GR activities in leaves of Taxus spp. under acid rain stress

As shown in Figure 2C and 2D, after simulated acid rain treatment (pH = 3.0), the leaf APX and GR activities of the three *Taxus* species were significantly lower than after CK1 treatment ($p < 0.05$). Upon application of SNP, the leaf APX activity in the three *Taxus* species first increased and then decreased with increasing SNP concentration. For *Taxus mairei*, the leaf APX activity was significantly higher in the N1, N2 and N3 groups than in CK2 ($p < 0.05$), the most significant increase (42.94%) was observed after the N3 treatment ($p < 0.05$). The leaf APX activity of *Taxus chinensis* was significantly higher (26.13%) in the N3 group than in the CK2 group ($p < 0.05$). The APX activity in the leaves of *Taxus yunnanensis* was significantly higher in the N3 and N4 groups than in CK2 ($p < 0.05$), the N4 treatment had the most significant effect, leading to an increase in APX activity of 53.45%. With increasing SNP solution concentration, the GR activity of *Taxus mairei* and *Taxus yunnanensis* increased, while that of *Taxus chinensis* showed a downward trend. The leaf GR activity of *Taxus mairei* was significantly higher in the N1, N2 and N3 groups than in CK2 ($p < 0.05$), the N3 treatment had the most significant effect, leading to an increase in GR activity of 43.56%. The GR activity of the leaves of *Taxus chinensis* was significantly higher in the N1, N2, N3 and N4 groups than in CK2 ($p < 0.05$). The N1 treatment had the most significant effect, leading to an increase in GR activity of 56.45%. For *Taxus yunnanensis*, the leaf GR activity was significantly higher in the N2, N3 and N4 groups than in CK2 ($p < 0.05$), the N4 treatment had the most significant effect, leading to an increase in GR activity of 129.15%. The results show that the application of simulated acid rain with a pH of 3.0 can significantly ($p < 0.05$) reduce the APX and GR activities of the leaves in all three *Taxus* species. When SNP solutions were sprayed on stressed *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* plants at concentrations of 0.50 mmol·L⁻¹, 0.50 mmol L⁻¹ and 0.75 mmol·L⁻¹, respectively, the leaf APX activity of the significantly increased ($p < 0.05$). When SNP solutions were sprayed on stressed *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis* plants at concentrations of 0.50 mmol L⁻¹, 0.10 mmol L⁻¹ and 0.75 mmol·L⁻¹, respectively, the leaf GR activity significantly increased ($p < 0.05$).

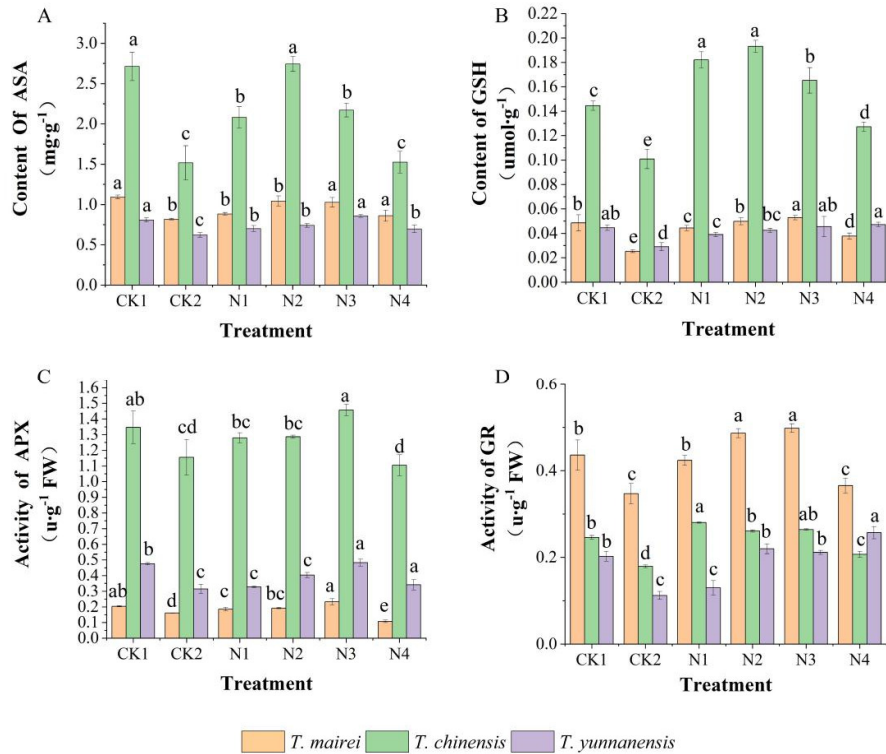


Figure 2. Effects of different sodium nitroprusside (SNP) treatments on the content of ASA (A) and GSH (B), the activity of APX (C) and GR (D) in the leaves of *Taxus* seedlings under simulated acid rain stress.

Comprehensive evaluation of plant resistance to acid rain stress in different treatment groups

As shown in Table 2, the resistance coefficient (α) differed for the different indicators of the same treatment and for the same indicators of different treatments. Therefore, it is difficult to reasonably compare the resistance to acid rain stress under different treatments and to draw a conclusion with high reliability. The results of evaluating acid rain resistance using a single index are different, which shows that the acid rain resistance of the plants is not determined by a character index. It is thus one-sided to evaluate the acid rain resistance using a certain index, so principal component analysis (PCA) was used to evaluate the acid rain resistance.

Table 2. The resistance coefficient (α) of each treatment

Species	Treatment	Resistance coefficient (α)							
		POD	O ²⁻	APX	ASA	SOD	CAT	GR	GSH
<i>Taxus mairei</i>	CK2	0.506	1.252	0.79	0.748	0.642	0.736	0.796	0.521
	N1	0.846	1.171	0.909	0.808	0.851	0.725	0.972	0.913
	N2	0.904	1.036	0.945	0.954	1.049	0.82	1.116	1.026
	N3	1.145	0.790	1.14	0.94	0.894	1.042	1.143	1.088
	N4	0.815	1.149	0.526	0.787	0.973	0.717	0.839	0.778
<i>Taxus chinensis</i>	CK2	0.698	1.359	0.858	0.559	0.816	0.764	0.729	0.698
	N1	0.924	1.073	0.95	0.767	0.852	0.881	1.139	1.26
	N2	0.841	1.006	0.955	1.012	0.974	1.028	1.059	1.336
	N3	0.826	1.130	1.082	0.801	1.030	0.834	1.073	1.144
	N4	0.603	1.261	0.82	0.562	0.779	0.981	0.841	0.881

<i>Taxus yunnanensis</i>	CK2	0.588	1.280	0.661	0.77	0.853	0.842	0.554	0.651
	N1	0.792	1.240	0.689	0.867	1.175	0.845	0.641	0.874
	N2	1.197	1.097	0.847	0.916	1.029	0.901	1.087	0.953
	N3	1.317	1.055	1.014	1.061	1.053	0.937	1.046	1.021
	N4	1.211	0.954	0.718	0.862	0.958	1.088	1.27	1.06

The PCA was carried out for the resistance coefficient (α) of different treatments (Figure 3). Then, the comprehensive evaluation (D) of the acid rain resistance of each treatment was calculated using PC1 and PC2. The D value was then used to rank the acid rain resistance of the different treatments (Table 3). The results showed that the best SNP solution concentrations for improving the stress resistance of *Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis*, were 0.50 mmol·L⁻¹, 0.25 mmol·L⁻¹ and 0.75 mmol·L⁻¹, respectively. As shown in Figure 3D, the results of PCA analysis on the resistance coefficients of different *Taxus* plants under CK2 treatment were carried out. As above, we can also get the ranking of the resistance of different *Taxus* plants to simulated acid rain (Table 4). The comprehensive evaluation results showed that *Taxus mairei* and *Taxus yunnanensis* had the strongest resistance to simulated acid rain under N3 treatment, and *Taxus chinensis* had the strongest resistance to simulated acid rain under N2 treatment. Meanwhile, *Taxus chinensis* had the strongest resistance to simulated acid rain, followed by *Taxus mairei* and *Taxus yunnanensis*.

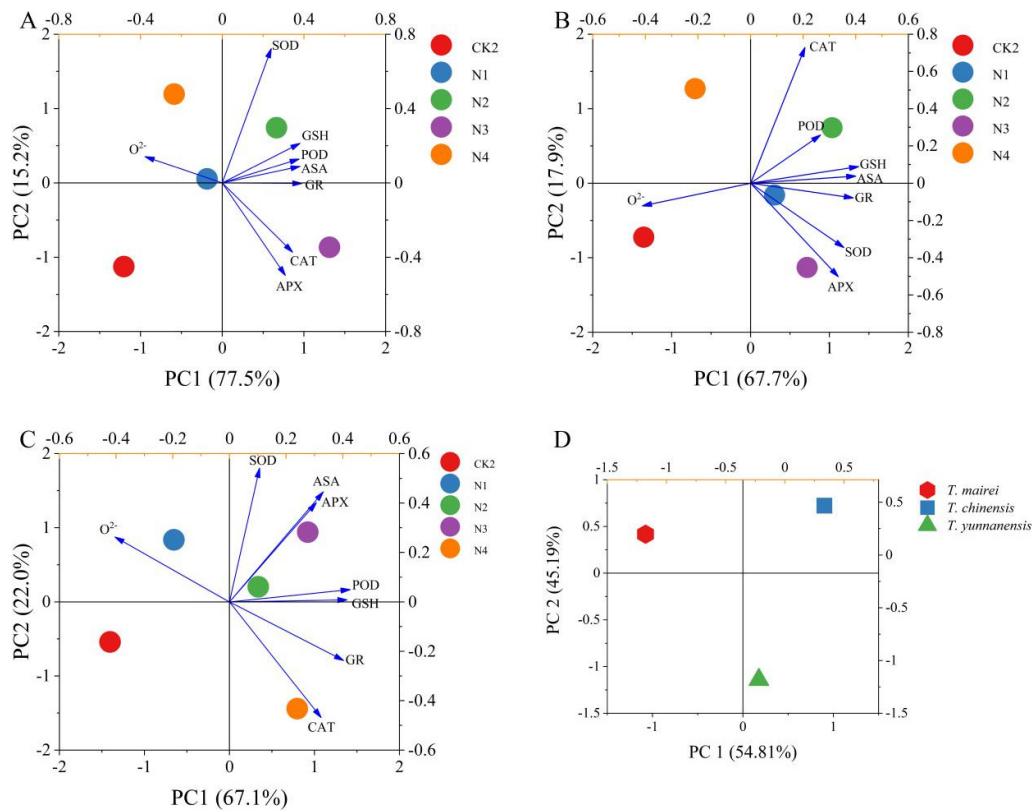


Figure 3. Results of the principal component analysis. (A) PCA analysis of *Taxus mairei*, (B) PCA analysis of *Taxus chinensis*, (C) PCA analysis of *Taxus yunnanensis*, (D) PCA analysis of tolerance of different *Taxus* plants to simulated acid rain

In figures A, B, and C, the lower abscissa and the left ordinate are the scores of the 5 treatments (CK2, N1, N2, N3 and N4) on PC1 and PC2 respectively. The upper abscissa and right ordinate are the loadings (indicated by a blue arrow) of 8 indexes (SOD, POD, O²⁻, ASA, CAT, APX, GSH and GR) on PC1 and PC2 respectively. In figures D, the abscissa and ordinate are the scores of the 3 species (*Taxus mairei*, *Taxus chinensis* and *Taxus yunnanensis*) on PC1 and PC2 respectively.

Table 3. Comprehensive evaluation of the acid rain resistance of the different treatments

Species	Treatment	PC1	PC2	D value	Ranking
<i>Taxus mairei</i>	CK2	-1.204	-1.126	-1.191	5
	N1	-0.186	0.054	-0.147	3
	N2	0.666	0.744	0.678	2
	N3	1.314	-0.866	0.956	1
	N4	-0.589	1.194	-0.296	4
	W	0.836	0.164		
<i>Taxus chinensis</i>	CK2	-1.354	-0.725	-1.222	5
	N1	0.305	-0.159	0.208	3
	N2	1.033	0.745	0.973	1
	N3	0.717	-1.132	0.331	2
	N4	-0.702	1.270	-0.289	4
	W	0.791	0.209		
<i>Taxus yunnanensis</i>	CK2	-1.405	-0.541	-1.191	5
	N1	-0.654	0.837	-0.286	4
	N2	0.339	0.205	0.306	2
	N3	0.921	0.941	0.926	1
	N4	0.798	-1.442	0.245	3
	W	0.753	0.247		

Table 4. Comprehensive evaluation of the acid rain resistance of the different *Taxus* plants

Species	Treatment	PC1	PC2	D value	Ranking
<i>Taxus mairei</i>	CK2	-1.077	0.418	-0.401	2
<i>Taxus chinensis</i>		0.900	0.723	0.820	1
<i>Taxus yunnanensis</i>		0.177	-1.141	-0.419	3
	W	0.548	0.452		

Discussion

When plants are subjected to external stress, many reactive oxygen species (ROS) are produced. These ROS accumulate in plant cells as the stress continues or increases, which causes damage to the cells (Wen *et al.*, 2020; Zhang *et al.*, 2020). The stress stimuli caused by unfavorable environmental factors can activate the antioxidant defense system within plant cells. The activation of the antioxidant defense system helps the plant to survive the unfavorable environment and alleviates the damage suffered under stress due to ROS (Anjaneyulu and Chopra, 2004). In this experiment, after the simulated acid rain stress (pH = 3.0) was applied to the leaves of the *Taxus* species, the O²- production rate in the leaf cells of all three *Taxus* species increased. This result is consistent with the results of Kamrun *et al.* (2016), who investigated the salt tolerance of mung bean seedlings. Furthermore, the simulated acid rain stress inhibited the metabolism of the protective antioxidant enzyme system of the leaves of three *Taxus* species. The activities of SOD, CAT, APX, GR and other protective enzymes were significantly reduced by the stress ($p < 0.05$). These results are consistent with those of the study by Seckin *et al.* (2009), in which wheat root antioxidant enzyme activity was investigated under stress. The reduced activities of CAT, APX and GR were significantly negatively correlated with the increase in O²- production rate ($p < 0.05$). This result was similar to the salt stress tolerance of maize (Kholova *et al.*, 2010). In the present study, it was also found that the contents of ASA and GSH in the cells of plants subjected to acid rain stress were significantly lower ($p < 0.05$) than in the CK1 treatment. This may have been due to the production rate of antioxidant substances in the plant being far less than the rate of oxidation.

After spraying different concentrations of SNP solution on the stressed plants, the activities of SOD, CAT, APX and GR in the *Taxus* plants significantly increased ($P < 0.05$). This demonstrated that exogenous

NO application can reactivate the activity of antioxidant enzymes in plant leaves. This result is in accordance with the research results of Egbichi *et al.* (2014), who investigated the effect of exogenous NO on the antioxidant capacity of soybeans treated with cadmium. In addition, NO can also be directly combined with O_2^- to produce ONOO⁻, which is less toxic to plant cells than O_2^- , thereby reducing the damage caused to plant cells by oxidative stress (Lehotai *et al.*, 2011; Long *et al.*, 2020; Piacentini *et al.*, 2020). In this study, it was found that treatment with specific concentrations of SNP solution significantly increased the antioxidant enzyme activity of the *Taxus* species ($p < 0.05$). This may have been related to the direct participation of NO in partially quenching O_2^- , which would eliminate the inhibition of antioxidant enzyme activity caused by high O_2^- concentrations.

NO also has a certain regulatory effect on the synthesis of some substances in plant leaves. For example, NO can promote the synthesis of GSH in plant leaves. As an antioxidant, GSH can reduce the amount of ROS within plant cells (Lin *et al.*, 2020; Sun *et al.*, 2015). In addition, NO can play a role in activating APX activity, which is essential for removing H_2O_2 (Egbichi *et al.*, 2014; Yun *et al.*, 2020). In the present study, after treatment with different concentrations of SNP solution, the content of GSH and ASA in the leaves of the *Taxus* plants subjected to acid rain stress increased significantly ($p < 0.05$). These results are consistent with those of the study by Gadelha CG *et al.* (2017), which focused on the salt tolerance of tree seedlings. Through further research, it was found that after spraying specific concentrations of SNP solution, the O_2^- production rate in the leaf cells of the *Taxus* species decreased to varying degrees, this decrease was significant at specific SNP concentrations ($p < 0.05$). Therefore, as the O_2^- production rate in the cells decreased significantly ($p < 0.05$), the content of ROS in the cells also decreased significantly, and the peroxidation of the cell membrane system was alleviated (Chen *et al.*, 2015).

A comprehensive analysis of the effect of different SNP concentrations on the antioxidant system indexes of *Taxus* species subjected to simulated acid rain was performed. The O_2^- generation rate in the leaf cells of *Taxus mairei* and *Taxus yunnanensis* was found to be significantly associated (negative correlation) with CAT activity, GR activity and GSH content ($p < 0.05$). This shows that antioxidant enzymes play a more important role in the antioxidant defense system of *Taxus mairei* and *Taxus yunnanensis* than antioxidant substances. It may be that these enzymes removed a large amount of H_2O_2 in the leaves, and accelerated the conversion rate of O_2^- to H_2O_2 , so that a large amount of O_2^- was converted into H_2O_2 and removed in the form of H_2O_2 . However, the rate of O_2^- production in the *Taxus chinensis* leaf cells was significantly negatively correlated with GR activity, and GSH and ASA content ($p < 0.05$). This indicated that compared with the antioxidant enzymes, the antioxidant substances played a more important role in *Taxus chinensis*. In the leaves of the *Taxus* species, the removal of antioxidants is the main reason. One possible mechanism underlying this effect is that O_2^- is converted into H_2O_2 under the action of antioxidants, and then under the combined action of SOD, GR, ASA and GSH, H_2O_2 is eradicated from the plant cells.

Conclusions

In this study, the effect of exogenous NO on the antioxidant system of *Taxus* seedling leaves under simulated acid rain stress was investigated. We explored the ability of exogenous NO to improve the tolerance of *Taxus* plants subjected to acid rain stress and identified *Taxus* species with strong acid rain tolerance. For *Taxus chinensis*, treatment with an SNP solution concentration of $0.25 \text{ mmol}\cdot\text{L}^{-1}$ led to optimal improvement of the antioxidant system. For *Taxus mairei* and *Taxus yunnanensis*, the treatment with $0.50 \text{ mmol}\cdot\text{L}^{-1}$ SNP was best for improving the antioxidant systems of the plants. Meanwhile, *Taxus chinensis* had the strongest resistance to simulated acid rain, followed by *Taxus mairei* and *Taxus yunnanensis*.

Authors' Contributions

BS, the first author, is responsible for determination of some indexes and the writing of this manuscript, ML, the co-first author, is responsible for the data arrangement and analysis, DH is responsible for providing suggestions on experimental methods, XP is responsible for the mapping and typesetting, and YF, the corresponding author, is responsible for the revision and quality control of the paper. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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