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Protective Effect of Nitric Oxide (NO) against Oxidative Damage in *Larix gmelinii* Seedlings under Ultraviolet-B Irradiation

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Abstract: Ultraviolet-B (UV-B) stress appears to be more striking than other research works because of the thin ozone layer. The protective influence of an exogenous nitric oxide donor and sodium nitroprusside (SNP) on the growth properties of *Larix gmelinii* seedlings was investigated under ultraviolet-B radiation conditions. The results indicated that 0.1 mM SNP could effectively alleviate the damage caused by ultraviolet-B radiation, and improved the seedling growth properties, the relative water content, and photosynthetic pigment content in leaves. Additionally, the photosynthetic capacity and antioxidant enzyme activity were increased during the exposure. On the contrary, the damage caused by active oxygen was decreased in SNP-treated seedling leaves. The damage caused by ultraviolet-B radiation was slightly reduced after treating with 0.01 mM SNP. Nevertheless, treatment with 0.5 mM SNP had a negative effect under ultraviolet-B radiation. Furthermore, supplementing NO (nitric oxide) improved the photosynthetic capacity and antioxidant enzyme activity and alleviated the damage of caused by active oxygen. The best effective concentration of SNP was 0.1 mM. Therefore, a suitable amount of exogenous NO can protect the *Larix gmelinii* seedlings and increase their tolerance to ultraviolet-B radiation.

Keywords: Ultraviolet-B; sodium nitroprusside; *Larix gmelinii* seedlings; photosynthetic capacity; antioxidant enzyme activity

1. Introduction

Deterioration of the environment has become a serious issue because of rapid development of modern industries and agriculture. One of the consequences is the depletion of the ozone layer in the stratosphere, due to which earth is being irradiated with strong UV (Ultraviolet) radiation (UV-B, 280~320 nm). The enhanced UV-B radiation directly influences the biology and survival of many plants and animals by changing their morphological structure, physiological metabolism, and genetic characteristics. A comparison of the responses of 12 different varieties of *Triticum aestivum* to different doses of UV-B radiation showed that UV-B radiation inhibits plant height and fresh weight [1]. A study involving two varieties of the genus *Portulaca* to UV-B radiation showed that UV-B radiation had significant effects on seed germination, growth, protein content, and peroxidase and protease activities [2]. A study involving *Primula malacoides* revealed that UV-B radiation affected the production of flavonoids in different tissues and organs (leaves and flowers) [3]. Chloroplast peroxidase of *Brassica oleracea* participates in the degradation of chlorophyll, which is also affected by UV-B radiation [4]. After treatment with UV-B radiation, the chlorophyll, carotene, and protein

contents in desert plants were significantly decreased [5]. A high dose of UV-B radiation significantly inhibited the photosynthesis of wheat [6]. UV-B radiation can regulate and inhibit the cell cycle of *Arabidopsis thaliana* cells [7,8]. In leaves of *Arabidopsis thaliana*, UV-B radiation inhibits growth regulation and cell proliferation, which is not dependent on the UV-B receptor (UVR8), but is dependent on the MPK3 (Mitogen-activated protein kinase 3) signaling pathway [9]. UVR8 is a light receptor protein of UV-B, which was a breakthrough finding in recent years on the mechanism underlying UV-B radiation effect on plant growth and physiologic regulation [10]. Therefore, understanding the effect of UV-B radiation on biological systems and formulating effective protective measures are of worldwide importance.

Previous studies have shown that ultraviolet radiation can induce the generation of reactive oxygen species (ROS) [11]. As the ozone layer gets thinner, UV-B radiation increases, which is believed to be disrupting the balance between the generation and elimination of ROS in plants, leading to the peroxidation of membrane lipids. However, the UV-B induced peroxidation of membrane lipids is a very complex process, which is a combination of enzymatic and non-enzymatic reactions. Furthermore, ROS also induces various signals, thus maintaining the ROS at a certain level.

NO (nitric oxide) is one of the substances that can adjust plant growth and transfer signals, thus playing an important role in the plant development process. Abundant evidence has revealed that low concentrations of NO can rapidly eliminate superoxide anions ($O^{2\cdot-}$) and lipid free radicals (R·). It reduces the harmful effects of ROS, such as membrane lipid peroxidation, and induces antioxidant enzyme expression. Thus, the protective effect of NO on cells is based on adjusting the levels of ROS and regulating its toxicity. Nitric oxide (NO) has anti-adversity efficacy under stress, such as drought and heavy metal exposure. Exogenous NO can resist Mn stress in rice by regulating the metabolic enzymes of *Medicago truncatula* [12] and increasing the antioxidant activity of clover to reduce the toxicity of the heavy metal cadmium [13,14]. By enhancing the expression of alternative oxidase (Aox1), NO can also relieve the toxicity of As (Arsenic) in barley [15]. NO participates in the regulation of ABA (Abscisic acid) and JA (Jasmonic acid) on the physiologic metabolism of plants under drought stress and increases the drought resistance of barley, rice, and wheat [16–18].

Recently, it was found that NO plays an important role in response of plants to UV-B radiation. Following exposure to UV-B radiation, the NO content in maize increases rapidly. In maize mutants, UV-B cannot induce an increase in NO. Compared with the wild type, mutants have higher lethality [19]. After exposure to UV-B radiation in corn and *Vitis vinifera*, the UV-B damage in cells is reduced through activation of NO. Moreover, as reported by Krasylenkoet et al. [20], the increase in the NO level in plant cells protects the microtubule organization and the growth process of the microtubule-dependent root from UV-B radiation damage. Tossi et al. has also found that UV-B induced stomatal closure in *Arabidopsis*, which was regulated by the UV resistance locus 8 photoreceptor in an NO-dependent mechanism [21].

Most papers have reported the effect of exogenous NO on plant growth during stress. Very few papers have reported the effect of exogenous NO on the seedling growth properties under UV-B radiation conditions. In this paper, we studied the effect of NO on *Larix gmelinii* seedlings under enhanced ultraviolet-B radiation conditions by supplying SNP at different concentrations as an exogenous NO donor. The results provided valuable theoretical evidence in plant resistance responses to severe conditions.

2. Materials and Methods

2.1. Plant Material, Growth Conditions, and Experimental Design

Dahurian Larch (*Larix gmelinii* Rupr.) seedlings were planted on 26 April 2014 in plastic pots (inner volume 50 × 50 × 50 cm^3) containing a mixture of soil (95%) and crushed peat (5%) in a greenhouse at the experimental field of the Urban Forestry Demonstration Base of Northeast Forestry University (45°4′10″ N, 128°42′12″ E), China. Only one plant was plotted per pot. The pots were divided into

five groups: control group (with natural UV-B radiation and normal water status, CK), UV-B treatment group (with enhanced UV-B radiation and normal water status, T_0), and the combination group (supplementary UV-B radiation with the nitric oxide donor sodium nitroprusside (SNP), T_1, T_2, T_3). UV-B radiation was supplied by UV-B fluorescent tubes (UVB-303, Beijing Electric light Source Research institute, 340 Lux, China). UV-B lamps were filtered with 0.1 mm thick cellulose acetate film to eliminate UV-C and UV-A radiation. The cellulose-diacetate films were regularly replaced every five days to avoid aging effects on the filters. The plants received only ambient levels of solar UV-B radiation (3.34 $kJ \cdot m^{-2} \cdot day^{-1}$ under clear-sky conditions), whereas plants beneath the cellulose-acetate-filtered lamps received ambient plus supplemental UV-B radiation (1.67 $kJ \cdot m^{-2} \cdot day^{-1}$). The desired spectral irradiance (5.02 $kJ \cdot m^{-2} \cdot day^{-1}$) was obtained by carefully adjusting the distance between the lamps and the top of the plants. The spectral irradiance from the lamps was determined with an AvaSpec 2048-2 (Avantes BV, Tilburg, The Netherlands) spectroradiometer. The supplemental UV-B (5.02 $kJ \cdot m^{-2} \cdot day^{-1}$) dose was equivalent to that anticipated with a 25% stratospheric ozone decrease at Harbin, China, on a clear solstice summer day. The lighting time was between 8:30–16:30 every day, which was slightly adjusted according to sunny or cloudy conditions.

SNP at different concentrations, 0.01 mM (T_1), 0.1 mM (T_2) and 0.5 mM (T_3), was sprayed on the seedlings with enhanced UV-B radiation every day. The seedlings of the CK and UV-B treatment were sprayed with water. After four weeks, the leaves were collected and immediately stored at −70 °C. For each treatment, three plants were repeatedly measured and three pots of the leaves of nursery stocks were collected. Each physiological index was then measured in triplicate.

2.2. Methods

2.2.1. Measurement of Gas Exchange Parameters and Pigments

Fully expanded young leaves were selected to detect gas exchange parameters. For each measurement, three replicates were performed with a portable photosynthesis system (Li-6400, Li-COR Inc., Lincoln, NE, USA). Values for maximum net photosynthetic rate (Amax, μmols (CO_2) $m^{-2} \cdot s^{-1}$), transpiration rate (Tr, μmol (H_2O) $m^{-2} \cdot s^{-1}$), intercellular CO_2 concentration (Ci, $\mu mol \cdot mol^{-1}$), apparent quantum yield (AQY), compensation irradiance (Ic, $\mu mol \cdot m^{-2} \cdot s^{-1}$), and saturation irradiance (Im, $\mu mol \cdot m^{-2} \cdot s^{-1}$) were calculated by using the equations of von Caemmerer and Farquhar (1981) [22]. The plant leaves were placed in 6 cm^2 chambers and the photon flux density was set at 1000 $mol \cdot m^{-2} \cdot s^{-1}$. The flow rate in the chamber was 500 $mL \cdot s^{-1}$ and the leaf temperature was 28 °C. Pigment analysis was performed according to the methods described by Chappelle and Wellburn [23,24]. Briefly, 10 mg (FW) of tissue was extracted with 2 mL of dimethyl sulfoxide for 12 h in the dark at 45 °C. Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids contents were calculated from the absorbance at 665, 649, and 480 nm, respectively.

2.2.2. Antioxidant System

Freshly expanded leaves (0.5 g) without major veins were ground with a mortar in 5.0 mL of ice-cold extraction buffer containing 1% polyvinylpyrrolidone (PVP) in 50 $mM \cdot L^{-1}$ phosphate buffer (pH 7.0). The homogenate was centrifuged (Sigma3K30, Sigma Inc., Cologne, Germany) at 12,000 *g* for 15 min. The supernatant was used for the enzyme assay. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were measured according to Jiang et al. [25].

2.2.3. Flavonoids

First, 0.5 g of fresh sample was homogenized with 10 mL of acidified methanol (methanol:water:HCl, 79:20:1) for 30 min and filtered. The residue was ultrasonically extracted 3 times with 30 mL of acidified methanol (methanol:water:HCl, 79:20:1) for 30 min. Then, it was combined with the supernatant and concentrated to dryness, and then dissolved in 2 mL methanol.

Finally, the flavonoid content was measured by a spectrophotometer (Shimadzu UV-2550, Shimadzu Inc., Tokyo, Japan) at 305 nm.

2.2.4. Hydrogen Peroxide Content

The H_2O_2 concentration was measured as described by Bernt and Bergmeyer [26]. Briefly, 50 mg leaves were homogenized with 1.5 mL 100 mM phosphate buffer (pH = 6.8) and centrifuged at 18,000 *g* for 20 min. The supernatant (0.5 mL) and 2.5 mL peroxidase solution (comprising 83 mM phosphate buffer, pH 7.0; 0.005% (*w*/*v*) dianisidine dichloride; 40 μg/ mL horseradish peroxidase, (Sigma product No. P6782, Sigma Inc., Cologne, Germany) were mixed and incubated for 10 min at 30 °C. The reaction was stopped by adding 0.5 mL 1 Mol·L^{-1} perchloric acid, and the absorbance was measured at 436 nm. The H_2O_2 concentration was calculated from a standard curve and presented as μmol·g^{-1} FW.

2.2.5. Peroxidation of Leaf Membrane Lipid

Lipid peroxidation in leaf tissues was determined as 2-thiobarbituric acid (TBA) reactive metabolites, chiefly malondialdehyde (MDA). Fresh leaves samples (0.5 g) were immediately crushed in 10% (*w*/*v*) trichloroacetic acid (TCA) at 4 °C. The homogenate was centrifuged at 15,000 *g* for 15 min. An aliquot (2 mL) of the supernatant was added to 2 mL of 0.5% thiobarbituric acid (TBA, *w*/*v*) in 20% TCA. The mixture was heated at 95 °C for 30 min and then cooled in an ice bath. The samples were centrifuged at 10,000 *g* for 10 min, and the absorbance of the supernatant was read at 532 nm and 600 nm. The level of lipid peroxidation was expressed as mmol of MDA.

2.2.6. Statistical Analyses

All the data were evaluated by SPSS 16.0 (IBM SPSS Inc., Chicago, IL, USA) and significance was accepted at $p < 0.05$.

3. Results

3.1. Effect of SNP on Biological Characteristics

After a 28-day treatment, the height, base diameter, root shoot ratio, and relative water content of CK, T_0 and T_2 showed similar trends: CK > T_2 > T_0 (Table 1). The height, root shoot ratio, and relative water content of T_1 were also higher than those of T_0, while the height, base diameter, and relative water content of T_3 were the lowest. These results indicated that the increase in UV-B radiation reduced the height of the *Larix gmelinii* seedlings and decreased their growth. SNP at T_2 concentration had the best inhibition effect of the plant damage induced by enhanced ultraviolet-B radiation, whereas the treatment with T_3 concentration had a negative effect on the *Larix gmelinii* seedlings.

Table 1. Effect of the NO (nitric oxide) donor on the height of *Larix gmelinii* seedlings under enhanced UV-B (Ultraviolet-B) radiation.

Treatments	Height (cm)	Base Diameter (cm)	Root Shoot Ratio	Relative Water Content (%)
CK	74.2 ± 1.825 ^{a}	0.759 ± 0.025 ^{a}	0.49 ± 0.017 ^{a}	68.72 ± 1.656 ^{a}
T_0	71.9 ± 0.891 ^{c}	0.751 ± 0.014 ^{b}	0.40 ± 0.011 ^{d}	58.48 ± 1.824 ^{c}
T_1	72.1 ± 1.182 ^{c}	0.744 ± 0.015 ^{c}	0.47 ± 0.006 ^{b}	63.27 ± 2.031 ^{b}
T_2	73.6 ± 2.049 ^{b}	0.757 ± 0.016 ^{a}	0.43 ± 0.007 ^{c}	62.58 ± 1.583 ^{b}
T_3	70.3 ± 2.801 ^{d}	0.740 ± 0.007 ^{d}	0.41 ± 0.018 ^{c}	56.91 ± 1.354 ^{d}

Values are expressed as the mean ± SE, $n = 9$. The significance of each group is represented by the superscript letters. Mean values within a column with different lower case letters are significantly different ($p < 0.05$). Mean values within a column with the same lowercase letters are not significantly different ($p > 0.05$); CK, control check.

3.2. Adjustment of NO Levels in Leaves

An increase in UV-B radiation led to a higher NO content, and thus higher activity of NOS (Nitic oxide synthase), while NR (Nitrate reductase) activity decreased (Table 2). In the absence of SNP, the concentration of NO and the activity of NOS were obviously lower than those in T_1, T_2 and T_3, but higher than that of CK. Meanwhile, the changing trend of NR activity was the opposite.

Table 2. Effect of the NO donor on NO, NOS, and NR content under enhanced UV-B radiation in *Larix gmelinii* seedlings.

Treatments	NO (ng·g^{-1} FW)	NOS (μmol·g^{-1})	NR (μg·g^{-1}·h^{-1})
CK	24.310 ^{c}	0.436 ^{d}	25.556 ^{c}
T_0	28.056 ^{a}	0.614 ^{a}	22.401 ^{d}
T_1	26.413 ^{b}	0.530 ^{b}	31.032 ^{a}
T_2	25.810 ^{b}	0.5410 ^{b}	28.902 ^{b}
T_3	27.444 ^{a}	0.504 ^{c}	27.444 ^{b}

The significance of each group is represented by the superscript letters. Mean values within a column with different lowercase letters are significantly different ($p < 0.05$). Mean values within a column with the same lowercase letter are not significantly different ($p > 0.05$); FW, Fresh weight; NOS, Nitic oxide synthase; NR, Nitrate reductase.

3.3. Photosynthetic Characteristics

After a 28-day exposure to the enhanced UV-B radiation, the photosynthetic pigment content in the leaves of *Larix gmelinii* seedlings was significantly decreased after treatment with SNP. The photosynthetic pigment content was obviously increased under the same condition. As shown in Table 3, after UV-B radiation treatment, the total chlorophyll and carotenoid contents exhibited the same changing trend (CK > T_0) while the ratios of chlorophyll a/b and Car/CHl (a + b) exhibited the same changing trend (CK < T_0). After treatment with SNP, the total chlorophyll content, carotenoid content and chlorophyll a/b ratio of the T_1, T_2 and T_3 treatments were higher than those of T_0, and the chlorophyll content of T_2 was the highest. After the three treatments, the CAR/CHl (a + b) ratios were all lower than those of CK and T_0, and the lowest value was measured in T_1.

Table 3. Effect of the NO donor on pigment concentrations under enhanced UV-B radiation in *Larix gmelinii* seedlings.

Treatments	CHl (a + b) (mg·g^{-1})	Car (mg·g^{-1})	Chlorophyll a/b	Car/CHl (a + b)
CK	1.933 ± 0.069 ^{a}	0.230 ± 0.008 ^{a}	2.707 ± 0.087 ^{d}	0.119 ^{b}
T_0	1.366 ± 0.032 ^{e}	0.171 ± 0.005 ^{d}	2.791 ± 0.067 ^{c}	0.125 ^{a}
T_1	1.643 ± 0.059 ^{c}	0.172 ± 0.006 ^{d}	2.885 ± 0.102 ^{b}	0.105 ^{d}
T_2	1.762 ± 0.057 ^{b}	0.195 ± 0.004 ^{b}	2.808 ± 0.091 ^{c}	0.111 ^{c}
T_3	1.498 ± 0.047 ^{d}	0.178 ± 0.003 ^{c}	3.089 ± 0.039 ^{a}	0.119 ^{b}

Values are expressed as the mean ± SE, $n = 9$. The significance of each group is represented by the superscript letters. Mean values within a column with different lower case letters are significantly different ($p < 0.05$). Mean values within a column with the same lowercase letter are not significantly different ($p > 0.05$); CHL, Chlorophyll; Car, Carotenoid.

Additional UV-B radiation inhibited the photosynthesis of *Larix gmelinii* seedlings (Table 4). Compared to the control sample, the maximum net photosynthetic rate, transpiration rate, intercellular CO_2 concentration, apparent quantum efficiency, light compensation point, and light saturation point of T_0 decreased after exposure to the enhanced UV-B radiation for 28 days. After application of SNP, the maximum net photosynthetic rate, transpiration rate, and intercellular CO_2 concentration increased. The maximum net photosynthetic rate, transpiration rate, apparent quantum efficiency, and light saturation point of T_3 all had the lowest values. The photosynthetic parameters showed that, when the SNP concentration was low, with increasing SNP concentration, its inhibition effect on the

photosynthetic stress induced by ultraviolet-B radiation was increased, while at high concentration, SNP had a negative effect with respect to protecting against UV-B radiation stress.

Table 4. Effect of the NO donor on the photosynthetic parameters of *Larix gmelinii* seedlings under enhanced UV-B radiation.

Treatments	Amax ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Tr ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Ci ($\mu mol \cdot mol^{-1}$)	AQY	Ic ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Im ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
CK	6.012 ± 0.180^{a}	1.533 ± 0.046^{a}	300.919 ± 15.046^{d}	0.032 ± 0.0009^{a}	42.951 ± 2.148^{a}	1261.737 ± 63.087^{a}
T_0	4.824 ± 0.145^{d}	1.112 ± 0.033^{d}	290.161 ± 14.508^{e}	0.021 ± 0.0006^{d}	19.628 ± 0.981^{e}	934.596 ± 46.730^{d}
T_1	4.876 ± 0.146^{c}	1.285 ± 0.039^{c}	308.806 ± 15.440^{b}	0.024 ± 0.0007^{c}	31.643 ± 1.582^{c}	1194.757 ± 59.738^{b}
T_2	5.947 ± 0.178^{b}	1.461 ± 0.044^{b}	309.711 ± 15.486^{a}	0.025 ± 0.0007^{b}	30.424 ± 1.521^{d}	1092.483 ± 54.624^{c}
T_3	4.271 ± 0.128^{e}	0.923 ± 0.028^{e}	306.724 ± 15.336^{c}	0.019 ± 0.0006^{e}	36.554 ± 1.828^{b}	868.484 ± 53.424^{e}

Amax, Maximum net photosynthetic rate; Tr, Transpiration rate; Ci, Intercellular carbon dioxide concentration; AQY, Apparent quantum yield; Ic, Compensation irradiance; Im, Saturation irradiance.

3.4. Peroxidation of the Leaf Membrane Lipid

MDA is the final product of membrane lipid peroxidation. It is one of the most important signals indicating the injury of the membrane system. As shown in Figure 1, enhanced UV-B radiation induced an increase in the peroxide content in *Larix gmelinii* seedlings, resulting in an increase in MDA and the flavonoid content, and enhancement of lipid peroxidation, which destroyed the membrane structure. The addition of SNP significantly reduced hydrogen peroxide, thereby reducing the peroxidation injury severity of the cell membrane lipids and increased *Larix gmelinii* seedlings antioxidant capacity. Among the treatments with three different concentrations of SNP, T_2 resulted in the best effect. Compared with the control sample, the MDA, flavonoid, and hydrogen peroxide contents were significantly increased under UV-B radiation. After applying SNP, the MDA, flavonoid and hydrogen peroxide contents of T_1, T_2 and T_3 were obviously higher than those of T_0 but lower than those of CK. The MDA content of T_2 was remarkably different from the control sample, indicating that the T_2 treatment effectively alleviated the damage induced by UV-B radiation. The MDA and hydrogen peroxide contents of T_3 were not significantly different from those of T_0, illustrating that the T_3 treatment could not significantly relieve the stress caused by UV-B radiation. The flavonoid contents of treatments T_1 and T_2 were not significantly different. T_1 had the smallest increasing degree of hydrogen peroxide content relative to T_2 and T_3.

Antioxidant enzymes, such as SOD, POD, and CAT, are the main active oxygen scavengers in plants. The major function of SOD is to alleviate the cell damage caused by H_2O_2, which is generated by the superoxide radicals. POD and CAT can remove H_2O_2 and prevent the generation of more oxygen free radicals to maintain the active oxygen balance [27]. Under UV-B radiation, the activities of SOD, POD, and CAT were significantly reduced compared to the control sample ($p < 0.05$) (Table 4). Various concentrations of SNP significantly elevated the activity of SOD and POD under UV-B radiation. The activities of SOD, POD, and CAT in T_2 were significantly higher than those of T_0 but nearly equal to those of CK. The SOD activities of T_3 and T_2 were not significantly different while the CAT activity of T_3 was obviously lower than that of T_0. Similarly, the CAT activity of T_1 was obviously lower than that of T_0, indicating that under the T_1 and T_3 treatments, the hydrogen peroxide removal effects were reduced under UV-B radiation while the T_2 concentration had a positive effect, i.e., a reduction in active oxygen accumulation under UV-B radiation. Finally, T_2 kept the active oxygen metabolism balance of *Larix gmelinii* seedlings from being destroyed and maintained oxygen metabolism at the control level even under ultraviolet-B radiation.

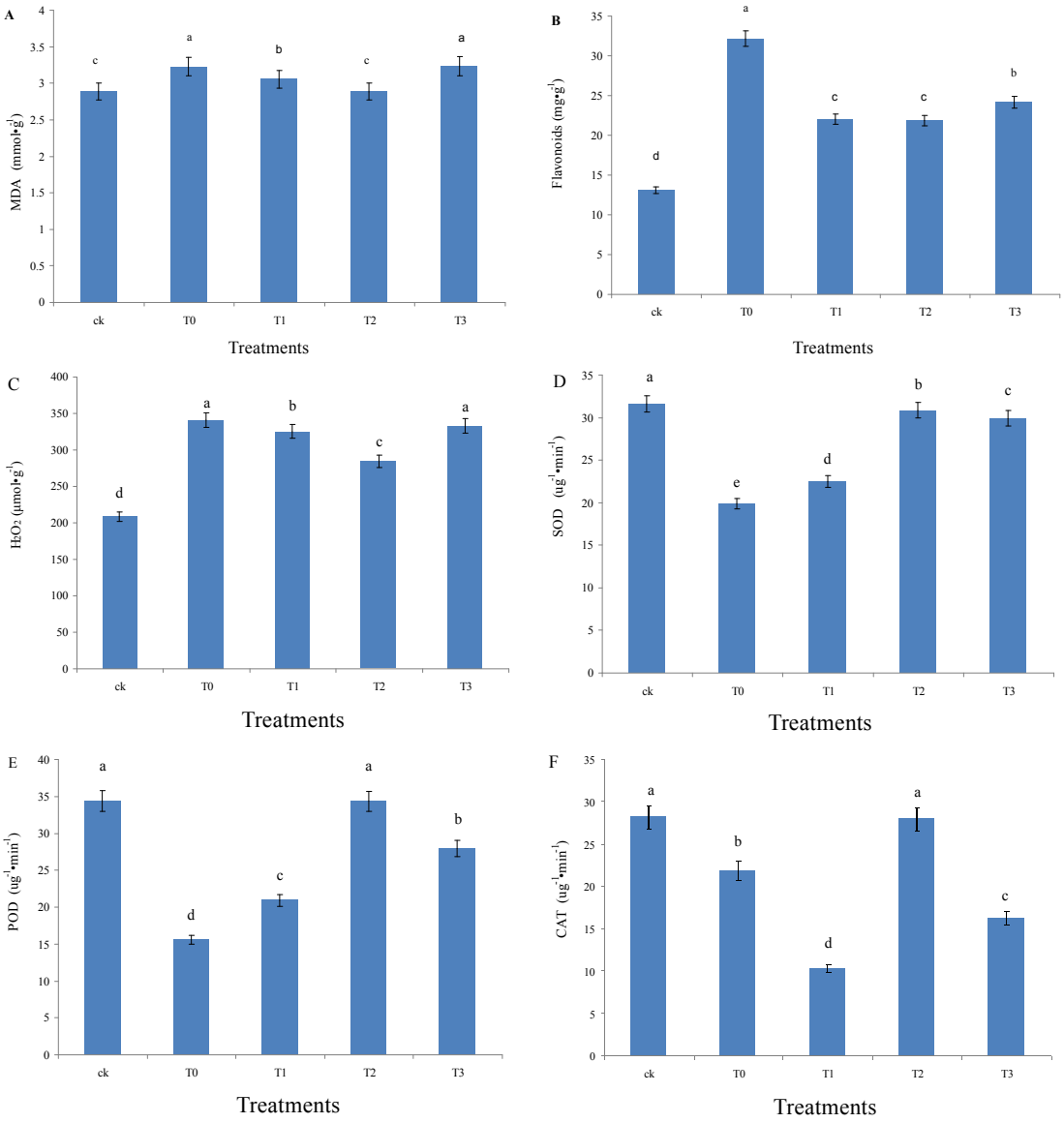

Figure 1. Effect of the NO donor on MDA (**A**), Flavonoids (**B**). H_2O_2 (**C**), SOD (**D**), POD (**E**) and CAT (**F**) in *Larix gmelinii* seedlings under enhanced UV-B radiation. Different lower case letters within a line indicate a significant difference ($p < 0.05$) between different treatments. The values are the means ± SD of three replicate samples per treatment. CK: natural UV-B radiation and normal water status; T_0: enhanced UV-B radiation and normal water status; T_1, T_2, T_3: the combination group (supplementary UV-B radiation with the nitric oxide donor sodium nitroprusside (SNP) at different concentrations, i.e., 0.01 mM (T_1), 0.1 mM (T_2) and 0.5 mM (T_3).

4. Discussion

SNP is one of the most important NO donors. In this study, the growth rate of *Larix gmelinii* seedlings was significantly reduced by UV-B ultraviolet radiation. A low concentration (0.01–0.1 mM) SNP alleviated the growth inhibition while a high concentration (0.5 mM) SNP enhanced the toxicity of UV-B radiation. Our results demonstrated that exogenous SNP (0.1 mM) at a suitable concentration alleviated the stress effect of UV-B radiation on *Larix gmelinii* seedlings.

Many studies have reported the role of NO in the plant growth process [28]. It was reported that under adverse stress, NO regulated the growth of plant leaves and roots [29]. Studies have shown that NO donor treatment can promote the growth of pea leaves. NO regulates root growth, and the

effect is positively related to the NO donor concentration [30]. Other studies have suggested that NO donor treatment of seeds can promote the growth of the roots and aerial parts of seedlings [31]. Beligni et al. [32] have shown that NO under dark conditions significantly inhibited the hypocotyl and internode elongation. Giba et al. [33] also indicated that NO had an inhibitory effect on plant hypocotyl growth. Liu Kaili et al. [34] showed that SNP alleviated the inhibitory effect on the growth of rice seedlings. Many studies have shown that NO can affect the components of the cell wall via the apoplast, causing the cell wall to loosen, and affecting the membrane lipid bilayer, enhancing the fluidity of the membrane, as well as facilitating cell expansion and promoting plant growth. In this study, we found that 0.1 mM SNP significantly reduced the growth inhibitory effect of UV-B radiation on *Larix gmelinii* seedlings, increased the plant height, basal diameter, and root shoot ratio, and significantly increased the relative water content. Our results are in agreement with those presented by Carlos [35].

NOS is a critical enzyme in the synthesis of NO. When plants are exposed to environmental stresses, NOS is activated to produce NO. Then, NO acts as a signal substance in plants and induces a resistance response [36,37]. This study indicated that enhanced UV-B radiation led to an increase in NOS activity in leaves and the NO level in the *Larix gmelinii* seedlings. This suggested that under the UV-B radiation stress, NOS in *Larix gmelinii* seedlings leaves could promote the synthesis of NO. This result is in agreement with the results of Ming Zhang et al. [38]. SNP treatments were applied under enhanced UV-B radiation. SNP at three different concentrations led to a decrease in the activity of NOS, which may be because NOS is the stress response protein of UV-B, and the activity of NOS decreases with leaf stress.

NR is one of the nitrogen metabolism enzymes in plants. Related studies reported that an increase in UV-B radiation could significantly reduce the NR activity of soybean and present a breed differences effect [39]. In our study, UV-B radiation decreased the NR activity and affected the nitrogen metabolism regulation of *Larix gmelinii* seedlings.

In plants, decreased NR activity mainly affects the NO^{3-} absorption ability of plant tissues, which results in a decrease in the conversion of NO^{3-} to NO^{2-}. The NR activity reducing also influences nitrogen metabolism. NR activity is related to the peroxidation of plant membrane lipids [40]. After the SNP treatment at different concentrations, the NR activity was significantly elevated under enhanced UV-B radiation. This may indicate that the generation of NO in *Larix gmelinii* seedlings mainly depends on NOS rather than NR. Thus, it is deduced that NOS is the main factor for the synthesis of NO in *Larix gmelinii* seedlings under ultraviolet-B radiation. NR is an inducible enzyme; its activity is mainly induced by NO^{3-}. NR is affected by light, temperature, enzyme synthesis, and the NO^{3-} content. It is not clear why UV-B radiation leads to a decrease in NR activity in *Larix gmelinii* seedlings. We hypothesized that it might be because the metabolism of some important substances in the plant was influenced by enhanced UV-B radiation, and therefore, affected the NR stability.

Chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car) are the main plant photosynthetic pigments. To a certain extent, their contents reflect the capability to transfer solar energy into organic compounds. Recently, Lizana et al. found that artificial supplementary UV-B radiation led to a decrease in the photosynthetic pigments of wheat [27]. Strong UV-B radiation destroys the chloroplast structure of plants or inhibits its synthesis, and reduces the chlorophyll content. The decline in chlorophyll content reduces the absorption of light by leaves, so that the plant is protected from injury from strong sunlight. Usually, photosynthetic pigment degradation occurs more easily under strong UV-B radiation, which will reduce the plant chlorophyll content. However, it was also observed that supplementary UV-B radiation led to an increase in photosynthetic pigment content. These results explained why the net photosynthetic rate was increased under some conditions. However, it is not clear why the photosynthetic pigments increased. Carotenoid is also one of the major chloroplast photosynthetic pigments. It can absorb blue violet light and transfer it to the reaction center, i.e., a chlorophyll a molecule. Carotenoid can also scavenge reactive oxygen species and protect the membrane system from strong radiation. Experimental results showed that increasing UV-B radiation up-regulated the values of Chl/Car and Chl a/b, which is in agreement with studies

on soybean presented by Middl et al. [41]. The effects of Chl/Car and Chl a/b under increasing UV-B radiation will be further studied in our future work. In this paper, exogenous NO effectively inhibited a decrease in the photosynthetic pigment content in the leaves of *Larix gmelinii* seedlings and alleviated damage symptoms on the blades. The treatment with 0.1 mM SNP had the best effect.

NO is a kind of reactive nitrogen with a strong oxidizing effect on the large chloroplast molecules. NO promotes the assembly and stabilization of thylakoid membrane protein complexes and enhances light energy absorption and the utilization ability of the chloroplast. Amax and AQY reflect the state of the photosynthetic apparatus [42]. This investigation showed that under UV-B radiation, Amax, AQY, and other photosynthetic parameters of *Larix gmelinii* seedlings decreased significantly. The addition of exogenous NO, however, significantly alleviated the decreased photosynthesis rate in *Larix gmelinii* seedlings caused by the UV-B radiation. Meanwhile, it promoted the transpiration rate and increased the water transport ability. NO also enhanced plant photosynthesis and the anti-stress ability of the seedlings. Therefore, the addition of NO is helpful to protect the chloroplast membrane, which maintains the structural integrity and a high photosynthetic pigment content to facilitate the photosynthetic reaction. NO enhances the anti-stress properties and alleviates ultraviolet-B radiation injury in *Larix gmelinii* seedlings.

The MDA content in cells reflects the degree of cellular oxidative damage. An increase in the ROS level induces the lipid peroxidation chain reaction, which decreases cell membrane integrity [43]. Mata et al. found that NO had an alleviation effect on the oxidative damage of wheat seedlings under drought stress. The effect might be associated with the peroxidation-free radical reaction of NO and ROS or lipids, which interrupted the oxidative stress and reduced the membrane injury [44].

Flavonoids act as plant barriers against UV-B radiation and are formed during UV-B radiation defense process. Flavonoids have been found in the vacuoles of leaf epidermal cells, mainly in the form of water-soluble, free, and lipophilic glycosides that are easily $O^{2\cdot-}$ methylated. The O-methylflavones are much more effective 280–320 nm spectral region (UV-B) absorbers. Second, plants undergoing UV-B radiation stress conditions preferentially accumulate dihydroxy B-ring-substituted flavonoids, which are effective scavengers of reactive oxygen species (ROS) [45]. The photomorphogenic effects of UV-B rely to a large extent on UVR8 [46]. Plants can perceive and distinguish UV-B in a direct way through the UVR8 photoreceptor, which is expressed throughout all plant tissues. When UV-B is absent, the main fraction of UVR8 is located in the cytoplasm in a dimeric state. Upon exposure to UV-B, nuclear accumulation of UVR8 monomers occurs within 5 min, causing downstream UV-B-induced signaling [47,48].

In this experiment, the MDA and flavonoids reached the highest values without SNP treatment under UV-B ultraviolet radiation. After treatment with a high concentration of SNP, the MDA and flavonoids were significantly increased, and the leaves treated with 0.5 mM SNP showed the highest amount of flavonoids and MDA. Compared with the stress of a single supplement of UV-B, the flavonoid content decreased under the combined action, which was mainly due to the exogenous NO-reduced membrane lipid peroxidation damage of the plants caused by UV-B radiation, and the excessive synthesis of flavonoids was inhibited. The response difference of flavonoids to single stress and the compound action was caused by differences in the genetic characteristics of the plant, and is related to the type of stress factor, stress intensity, and duration of stress [49]. Another possible reason might be that SNP changed the cell C flow direction and then affected the UVR8 protein synthesis, which eventually weakened the signal transduction of flavonoid metabolism. Our results indicated that SNP could effectively alleviate the oxidation injury caused by ultraviolet-B radiation.

Under UV-B radiation stress, SOD is transformed to generate H_2O_2 by a disproportionation process of $O^{2\cdot-}$, which is accompanied by an increase in the H_2O_2 content and the removal of POD and CAT as well as a decrease in the activity of the H_2O_2 decomposition enzyme [50]. Meanwhile, NO promotes the activities of SOD, POD, and CAT and leads to an increase in the defensive ability of the free radical defense system. Thus, the oxidative injury of *Larix gmelinii* seedlings caused by UV-B radiation was alleviated, the permeability of the membrane was decreased and the MDA content was

reduced. NO directly reacts with $O^{2\cdot-}$, which may also affect the endogenous $O^{2\cdot-}$ level of NO. NO has a high affinity for iron-containing enzymes. For example, NO can regulate the activity of iron enzymes, including CAT, and can inhibit the activity of the target enzymes with non-heme iron-like aconitase to participate in a series of physiological responses in plants [51]. Finally, NO increases the activity of the protective enzymes.

5. Conclusions

In conclusion, we found that SNP functioned as an exogenous nitric oxide donor. It could effectively alleviate the damage caused by ultraviolet-B radiation and improved the seedling growth properties. The most effective concentration of SNP was 0.1 mM, which protected the *Larix gmelinii* seedlings and increased their tolerance to ultraviolet-B radiation.

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