Article

Exogenous Salicylic Acid Alleviates Physiological Stress in *Salix matsudana* Seedlings and Increases 2,4-Dinitrophenol Removal

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Abstract: 2,4-dinitrophenol (2,4-DNP) is a new kind of pollutant that is highly toxic and difficult to be biodegraded. In this study, the feasibility of using exogenous growth regulator salicylic acid (SA) to improve the purification ability of *Salix matsudana* (*S. matsudana*) seedlings to 2,4-DNP stress was investigated by a hydroponic simulation experiment. The main research results are as follows: (1) After adding exogenous SA, a high concentration of SA (1000 mg·L^{-1}) inhibited the photosynthetic process and the normal physiological process of the chlorophyll fluorescence system of *S. matsudana* seedlings to a certain extent. When adding a low concentration of SA (10 mg·L^{-1}) to treat *S. matsudana* seedlings, all exogenous treatment groups could alleviate the stress of 2,4-DNP on the photosynthetic system and chlorophyll fluorescence system of *S. matsudana* seedlings, and 10 mg·L^{-1} SA (DNP + S1) was the best. (2) The addition of exogenous SA could alleviate the damage of 2,4-DNP to *S. matsudana* seedlings by enhancing the activity of its antioxidant enzymes to remove excess reactive oxygen species (ROS) in the body and reducing the level of membrane lipid peroxidation and the size of membrane damage. The treatment with 10 mg·L^{-1} SA had the best effect. (3) Exogenous low concentration of SA (10 mg·L^{-1}) could alleviate the decline of biomass index of *S. matsudana* seedlings under 2,4-DNP stress, but a high concentration of SA (1000 mg·L^{-1}) could not alleviate the 2,4-DNP toxicity of *S. matsudana* seedlings leaves. Exogenous SA could effectively alleviate the growth damage caused by 2,4-DNP stress on *S. matsudana* seedlings and increase the tolerance threshold range of *S. matsudana* seedlings to 2,4-DNP (8.81–33.78 mg·L^{-1}). (4) Exogenous addition of SA could increase the removal percentage of 2,4-DNP in Salix matsudana seedlings. Among them, the removal percentage of Salix matsudana was the highest at 10 mg·L^{-1} SA, which was 1.46 times (5 days) and 1.19 times (10 days) higher than that of the DNP treatment group, respectively. Overall, when SA reached 10 mg·L^{-1}, the photosynthetic productivity of *S. matsudana* was the highest, and *S. matsudana* had the best purification effect on 2,4-DNP in wastewater.

Keywords: 2,4-dinitrophenol; physiological; phytoremediation; salicylic acid; *Salix matsudana*

1. Introduction

Environmental pollution has become one of the serious challenges facing humankind. Every year, tens of thousands of pollutants enter the environment in various ways, thus causing great harm to the ecological environment and human health [1]. Nitrophenol,

an important chemical raw material in phenolic compounds, is widely used in rubber, medicine, and other fields [2]. It has certain chemical toxicity and often pollutes the surrounding environment with the discharge of wastewater in the process of use and production [3]. 2,4-dinitrophenol (2,4-DNP), as a new pollutant, has high toxicity and is difficult to be biodegraded [4,5]. It is listed in the "Priority Control Pollutants List" because of its high toxicity [6]. At present, 2,4-DNP is mostly found in explosive, pesticide, and other industries [7], which often pollutes the environment through wastewater, thereby harming animals, plants, microorganisms, and human health [4]. Under normal natural conditions, 2,4-DNP has good stability and a slow degradation rate and can stay in the environment for a long time.

Salicylic acid (SA) is commonly present in plants as a phytohormone. It is a small molecule corresponding to phenolic compound, which can participate in regulating various physiological and biochemical processes such as stomatal closure and seed germination in plants, and effectively improve the ability of plants to cope with drought, salt damage, heavy metals, and other external stresses [8]. Wang et al. [9] found that the addition of exogenous SA could alleviate the toxic effect of ipratazine on corn (*Zea mays*), wheat (*Triticum aestivum*), and rape (*Brassica napus*). Guan et al. [10] found that the application of exogenous SA could significantly improve the photosynthesis and antioxidant enzyme activity of tobacco (*Nicotiana tabacum*), thereby improving the tolerance of tobacco to triclosan (TCS). Yusuf et al. [11] found that the application of exogenous SA can alleviate the oxidative stress damage caused by $NiCl_2$ and $NaCl$ on *Brassica juncea*, and significantly improve the physiological and biochemical parameters of mustard (*Brassica juncea*). Therefore, it has certain practical feasibility to apply SA to the research of 2,4-DNP pollution purification and removal and exogenous substances addition to alleviate the 2,4-DNP toxicity stress of *S. matsudana*.

The addition of plant growth regulators (PGRs) can improve the resistance of plants to abiotic and biotic stresses [12,13]. There have been many studies on exogenous SA in improving plant stress resistance [14]. *S. matsudana* is a type of deciduous tree. It can grow well in both wetlands and dry land and has the ability to degrade some toxic substances in wastewater. Therefore, in this paper, the feasibility of using exogenous SA to improve the purification ability of *S. matsudana* seedlings under 2,4-DNP stress was discussed by using a hydroponic simulation experiment. The growth physiological response, growth morphological characteristics, and purification effect of *S. matsudana* seedlings treated with specific 2,4-DNP concentration were determined by adding exogenous SA. The optimal concentration of SA to enhance the tolerance of *S. matsudana* seedlings to 2,4-DNP was determined. The purpose of this study was to explore the regulatory effect of exogenous growth substance SA on the purification of 2,4-dinitrophenol wastewater by *S. matsudana* seedlings in order to provide a theoretical reference for the phytoremediation of 2,4-DNP wastewater.

2. Materials and Methods

2.1. Overview of the Study Site

The experiment was set up in the forestry experimental station (36°11′ N, 116°08′ E) of the provincial agricultural high-tech park of Shandong Agricultural University. The region has a continental monsoon climate in the warm temperate zone. The average annual temperature is 13.2 °C, and the average annual precipitation is 683.2 mm. It is windy and dry in spring, rainy and hot in summer, cool in autumn, and cold in winter.

2.2. Test Materials

In the first ten days of March 2021, in Donghu Park, Taian City, Shandong Province, healthy willows will be cut from the middle and lower parts of the crown of willow. The damaged leaves were removed from the plant materials, washed with tap water, taken to the greenhouse, cut into 20 cm long cuttings, and put into a 500 mL conical flask for culture (25 °C). After rooting, the plants were cultured with 400 mL 1/2 Hoaglands nutrient solution, and the bottles were wrapped with black plastic bags to inhibit the growth of algae.

2.3. Experimental and Treatments

In late June, healthy and basically consistent seedlings of *S. matsudana* were selected for the experiment of exogenous SA (Macklin Biochemical Technology Co., Ltd., Shanghai, China) to alleviate the 2,4-DNP stress of *S. matsudana*. Two levels of 2,4-DNP (Jingchun Reagent Co., Ltd., Shanghai, China) were set: 0 (CK) and 15 (DNP) mg·L^{-1}. Four levels of exogenous SA were set: 0, 10 (S1), 100 (S2), 1000 (S3) mg·L^{-1}. A total of 8 processing groups were set, and they were CK, DNP, S1, DNP + S1, S2, DNP + S2, S3, and DNP + S3.

In the treatment group, each group was treated for four times, and nutrient solution was added to the marking line every 2 days and before each sampling. To prevent 2,4-DNP volatilization, the conical mouth is sealed with plastic film. The control group was set without 2,4-DNP (CK). At the 5th and 10th day of stress, chlorophyll fluorescence parameters, photosynthetic gas exchange parameters, enzyme activities (SOD, POD, CAT), chlorophyll content, H_2O_2 content, $O_2^{-·}$ content, 2,4-DNP removal percentage of leaves of each treatment group were measured respectively. At the end of the test, the malondialdehyde content (MDA), relative conductivity (REC), biomass, growth morphology, and other indicators of the leaves were measured. The leaves were dyed with DAB, NBT, and trypan blue and photographed.

2.4. Measurement of Superoxide Dismutase (SOD), Peroxidase (POD), Catalase (CAT), and MDA Levels

First, 0.2 g fresh weight leaves were weighed, 1 mL of phosphoric acid buffer (pH 7.8, Macklin Biochemical Technology Co., Ltd., Shanghai, China) was injected, and then 4 mL of phosphoric acid buffer was injected after ice bath grinding. Then the obtained grinding liquid was poured into the centrifuge tube. After centrifuge and 0–4 °C for 20 min [15], the supernatant was then used to determine the SOD, POD, MDA and CAT.

The SOD was determined by nitrogen blue tetrazole colorimetry [16], the POD was determined by the guaiacol method [16], the MDA was determined by the thiobarbituric acid method [17], the CAT was determined by the ultraviolet absorption method [18], respectively.

2.5. Determination of Relative Conductivity (REC)

The plant leaves were rinsed several times. The leaf discs were punched with a hole punch. Then, they were immersed in deionized water for about 10 minutes and shook several times. Ten leaf discs were added into each test tube, and then 20 mL of deionized water was injected. The initial conductivity S_1 of each test tube was measured, then they were sealed for 8 min in a boiling water bath, and the final conductivity S_2 of the conductivity of each test tube and the blank conductivity S_0 of distilled water after 3 min of cooling were measured, respectively.

$$REC(\%) = (S1 - S0) \times 100/(S2 - S0)$$

2.6. Dye the Leaves with 3,3-Diaminobenzidine Method (DAB) to Show the Accumulation of H_2O_2 in the Leaves

The DAB powder was dissolved in distilled water, and dilute hydrochloric acid (Macklin Biochemical Technology Co., Ltd., Shanghai, China) was used to adjust the pH to 3.8. DAB dye solution was prepared with a concentration of 1 mg·mL^{-1}. The decolorizing solutionwas used to decolorize the leaf chlorophyll. It was boiled in boiling water for 20–30 min, and the photos were taken on the copy board after decolorization.

2.7. Staining the Leaves with Plant Superoxide Anion Staining Solution Method (NBT) to Show the Accumulation of $O_2^{-·}$ in the Leaves

The NBT powder is dissolved in 50 mM sodium phosphate buffer (pH 7.5, Macklin Biochemical Technology Co., Ltd., Shanghai, China) to prepare 2 mg·mL^{-1} NBT dye solution. The decolorizing solution (lactic acid: glycerin: ethanol = 1:1:3 mixture) was used to decolorize the leaf chlorophyll. It was boiled in boiling water for 20–30 min, and the photos were taken on the copy board after decolorization.

2.8. Determination of Photosynthetic Parameters

The basic photosynthetic indexes were measured with the Li-6800 (LI-COR Inc., Lincoln, NE, USA) portable photosynthesis system. The net photosynthetic rate (P_n, $\mu mol \cdot m^{-2} \cdot s^{-1}$), transpiration rate ($T_r$, $mmol \cdot m^{-2} \cdot s^{-1}$), intercellular CO_2 concentration (C_i, $mol \cdot m^{-1}$), and stomatal conductance (G_s, $mmol \cdot m^{-2} \cdot s^{-1}$) of mature leaves were measured on a sunny morning (8:30–11:00). Three seedlings in each treatment were randomly selected to measure the parameters of basic photosynthetic indexes, and one upper mature leaf of each seedling was measured. The measurement was repeated three times. CO_2 concentration was 400 $\mu mol \cdot mol^{-1}$, the indoor air temperature of the leaves was set at 25 °C, the indoor relative humidity was 55%, and the photosynthetically active radiation (PAR) was set at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Water use efficiency (WUE) = P_n / T_r, light energy utilization efficiency (LUE) = P_n / PAR, and porosity limit value (L_s) = $1 - C_i / C_a$ were calculated, respectively [19,20].

2.9. Chlorophyll Fluorescence Determination

Chlorophyll fluorescence parameters and photosynthetic gas exchange parameters were measured in the morning of the same day. Three well-developed leaves were selected from the middle and upper parts of different treated plants. The chlorophyll fluorescence parameters, including initial fluorescence (F_o), maximum fluorescence (F_m), maximum fluorescence under light (F_m'), steady-state fluorescence (F_s), and minimum fluorescence under light (F_o'), were measured by FMS-2 pulse-modulated fluorometer (Hansatech, Norfolk, UK). PSII open actual photochemical efficiency(Φ_{PSII}), photochemical burst coefficient (qP), non-photochemical burst coefficient (NPQ), PSII regulated energy dissipation quantum yield (Y), PSI excitation energy partition coefficient (α), PSII excitation energy partition coefficient (β), photochemical dissipation (P), antenna thermal dissipation (D), non-photochemical energy dissipation (Ex) and other parameters were calculated, respectively [21].

2.10. Measurement of the Chlorophyll, H_2O_2, $O_2^{-\cdot}$ Levels

The chlorophyll content was determined by the ethanol extraction method [22]. The plant leaves were wiped and cut into pieces (remove the midrib), and mixed well. 0.1 g of plant leaves was taken and put into a test tube with a stopper (Sigma Aldrich Trading Co., Ltd., Shanghai, China). 95% ethanol was injected into the test tube and stored in a dark place with a closed light. When the leaves were soaked to a colorless state, the absorbance at 665, 649, and 470 nm was measured with 95% ethanol as a blank.

For H_2O_2, a series of 0, 0.05, 0.10, 0.15, 0.20, and 0.25 $\mu mol \cdot L$ H_2O_2 solution was made to determine a standard curve. The content of H_2O_2 was determined from the standard curve [23].

For $O_2^{-\cdot}$, about 0.1 g above the plant tissue was taken, 1.5 mL of 50 $mmol \cdot L^{-1}$ ophosphoric acid buffer (pH 7.8, Macklin Biochemical Technology Co., Ltd., Shanghai, China) was added, and the $O_2^{-\cdot}$ was determined by hydroxylamine oxidation method [24].

2.11. Root Vigor Determination

Root vigor was determined by α-naphthylamine oxidation method [25]. According to the standard curve, the α-naphthylamine content corresponding to the two absorbance values was compared to obtain the α-naphthylamine oxidation amount (i.e., the root vigor value).

2.12. Determination of the Root Morphology, Biomass, Tolerance Index (TI) and Toxicity Factor

Morphological parameters such as root length were measured using a root system analysis system (WinRHIZO, Pro) and then recorded and photographed.

The plants were uprooted, cleaned, and placed in a ventilated oven at a temperature of 85 °C until the weight was constant.

Healthy and moderately sized functional leaves were selected from the plants and blotted with filter paper, and the leaf area was calculated using a leaf area meter (CI-202, CID BioScience, Camas, WA, USA) and then dried to a constant weight. The specific leaf

weight was calculated using the same method of biomass determination. The tolerance index and toxicity factor were calculated based on the changes in plant biomass with reference to the calculation of tolerance index and toxicity factor of heavy metals and salt stresses [26], and the 2,4-DNP concentration at a 50% decrease in plant growth index was used as the tolerance threshold [27]. Meanwhile, a fitted curve was constructed with leaf P_n as the dependent variable and 2,4-DNP concentration (C) as the independent variable, and a regression equation was established [28].

$$\text{Specific leaf weight} = \text{dry weight/leaf area}$$

$$\text{R/S} = \text{belowground biomass/aboveground biomass}$$

$$\text{TI} = \text{fresh weight of test group/fresh weight of control group}$$

$$\text{Toxicity factor} = (\text{control value} - \text{treatment value})/\text{control value} \times 100\%$$

2.13. Percentage Removal of 2,4-DNP Was Determined

The measurement of 2,4-DNP removal was carried out using an enzyme marker (Epoch2T, Biotek, Winooski, VT, USA). After aspirating 1 mL of the treatment solution, the treatment solution was adjusted to alkaline (pH = 10.0) and centrifuged. The OD values were measured at 10 nm intervals in the 300–600 nm band and at 360 nm alone. The results were taken once every 5 d. Each treatment was repeated three times. The calculation equation is as follows.

$$R_T = \frac{(C_0 - C_r)}{C_0} \times 100\%$$

R_T: percentage of 2,4-DNP removal (%); C_0: initial 2,4-DNP concentration (mg·L^{-1}); C_r: final 2,4-DNP concentration (mg·L^{-1}).

2.14. Plant Growth and Morphological Index Records

The measurements of plant growth morphological indexes were observed and recorded daily, including plant appearance color, plant height, survival rate change leaves, and root growth status [29].

2.15. Data Analysis

The processing and analytical calculations of the experimental data obtained from this study were performed by Excel 2010 (Microsoft, Redmond, WA, USA) and SPSS 22.0 (IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA) was conducted, and means were compared using the minimum significant range method (Duncan) test at the 0.05 confidence level. Origin 9.0 (OriginLab, Northampton, MA, USA) was used for plotting.

3. Results

3.1. Photosynthetic Gas Exchange Parameters

Tables 1 and 2 show that under fixed light intensity conditions (1200 $\text{μmol·m}^{-2}\text{·s}^{-1}$), 2,4-DNP treatment alone significantly inhibited photosynthesis in *S. matsudana* seedlings, and its P_n was reduced by 48.66% at 5 d and 10 d, respectively, compared to the control (CK). The values were increased to different degrees after the exogenous addition of SA treatment. The best increase in the tolerance threshold concentration of 2,4-DNP in the DNP + S1, DNP + S2, and DNP + S3 treatment groups was 180.57% of that in the 2,4-DNP treatment alone. At 10 d, the 2,4-DNP concentrations at the time of P_n halving in the DNP, DNP + S1, DNP + S2, and DNP + S3 treatment groups were compared with 2,4-DNP treatment alone. DNP, DNP + S1, DNP + S2, and DNP + S3 treatment groups showed the best increase in the tolerance threshold concentration of 2,4-DNP, with DNP + S1 being the best compared to 2,4-DNP treatment alone. It is evident that the exogenous addition of SA treatment improved the ability of *S. matsudana* seedlings to tolerate 2,4-DNP stress

and reduced the inhibitory effect of 2,4-DNP stress on the photosynthesis of *S. matsudana* seedling leaves. Compared with other treatment groups, DNP + S1 had the best effect. The tolerance threshold of 2,4-DNP in *S. matsudana* seedlings was increased from 13.41 mg·L^{-1} to 13.87–24.21 mg·L^{-1} in 5 d and from 10.06 mg·L^{-1} to 10.03–19.62 mg·L^{-1} in 10 d. The tolerance threshold of 2,4-DNP in *S. matsudana* seedlings was increased from 13.41 mg·L^{-1} to 13.87–24.21 mg·L^{-1} in 5 d and from 10.06 mg·L^{-1} to 10.03–19.62 mg·L^{-1} in 10 d.

Table 1. Effects of SA on the net photosynthetic rate (P_n), transpiration rate (T_r), intercellular CO_2 concentration (C_i), stomatal conductance (G_s), porosity limit value (L_s), water use efficiency (WUE), and light energy utilization efficiency (LUE) in leaves of *S. matsudana* seedlings under stress of 2,4-DNP.

Time (d)	Treatment	P_n (mmol·m^{-2}·s^{-1})	T_r (mmol·m^{-2}·s^{-1})	C_i (μmol·mol^{-1})	G_s (mmol·m^{-2}·s^{-1})	L_s (%)	WUE	LUE
5	CK	13.1 ± 0.15 a	4.5 ± 0.07 a	237.5 ± 2.78 d	219.1 ± 0.17 a	0.41 ± 0.007 a	2.9 ± 0.06 a	1.09 ± 0.012 a
	DNP	6.6 ± 0.11 e	3.0 ± 0.05 c	329.1 ± 1.97 a	114.8 ± 0.32 d	0.18 ± 0.005 d	2.3 ± 0.04 d	0.55 ± 0.009 e
	S1	12.9 ± 0.07 a	4.6 ± 0.06 a	244.7 ± 3.13 d	212.2 ± 2.99 a	0.39 ± 0.008 a	2.8 ± 0.05 a	1.08 ± 0.006 a
	DNP + S1	12.4 ± 0.07 a	4.7 ± 0.06 a	247.1 ± 6.02 d	207.30 ± 3.04 a	0.38 ± 0.015 a	2.7 ± 0.02 b	1.04 ± 0.005 b
	S2	10.0 ± 0.04 c	4.1 ± 0.09 b	261.4 ± 2.95 c	193.8 ± 6.05 b	0.35 ± 0.007 b	2.4 ± 0.05 c	0.83 ± 0.003 c
	DNP + S2	9.9 ± 0.07 c	4.1 ± 0.06 b	263.6 ± 3.93 c	190.4 ± 5.67 b	0.34 ± 0.010 b	2.4 ± 0.02 c	0.82 ± 0.006 c
	S3	7.2 ± 0.11 d	2.9 ± 0.06 c	298.0 ± 2.78 b	168.8 ± 5.78 c	0.25 ± 0.007 c	2.5 ± 0.04 c	0.61 ± 0.009 d
	DNP + S3	7.2 ± 0.09 d	3.0 ± 0.06 c	303.4 ± 5.82 b	160.0 ± 5.75 c	0.24 ± 0.015 c	2.5 ± 0.03 c	0.60 ± 0.007 d
10	CK	10.9 ± 0.11 a	2.8 ± 0.01 a	233.5 ± 7.02 e	147.1 ± 5.40 b	0.42 ± 0.018 a	4.0 ± 0.05 a	0.91 ± 0.009 a
	DNP	4.6 ± 0.05 d	1.4 ± 0.02 d	338.5 ± 5.66 a	53.4 ± 3.97 e	0.15 ± 0.014 e	3.4 ± 0.07 d	0.39 ± 0.004 d
	S1	10.9 ± 0.10 a	2.8 ± 0.02 a	238.2 ± 6.01 e	165.4 ± 7.05 a	0.40 ± 0.015 a	3.9 ± 0.03 ab	0.91 ± 0.008 a
	DNP + S1	10.7 ± 0.06 a	2.8 ± 0.05 a	257.2 ± 5.60 d	140.0 ± 4.78 b	0.36 ± 0.014 b	3.9 ± 0.05 ab	0.89 ± 0.005 a
	S2	7.7 ± 0.08 b	2.1 ± 0.02 b	279.8 ± 4.13 c	120.8 ± 3.21 c	0.30 ± 0.010 c	3.6 ± 0.03 c	0.64 ± 0.008 b
	DNP + S2	7.5 ± 0.13 b	2.1 ± 0.05 b	288.5 ± 3.31 c	115.8 ± 8.13 c	0.28 ± 0.008 c	3.6 ± 0.06 c	0.63 ± 0.007 b
	S3	7.0 ± 0.03 c	1.9 ± 0.03 c	315.7 ± 5.53 b	91.1 ± 3.21 d	0.21 ± 0.014 d	3.7 ± 0.07 bc	0.58 ± 0.003 c
	DNP + S3	7.0 ± 0.07 c	1.8 ± 0.03 c	318.1 ± 5.49 b	88.6 ± 4.35 d	0.20 ± 0.013 d	3.9 ± 0.08 ab	0.89 ± 0.006 c

Note: The same columns within the different treatments followed by different letters are significantly different at the same time ($p < 0.05$).

Table 2. One-dimensional linear equation simulation of leaf net photosynthetic rate (Y = P_n) and 2,4-DNP concentration (X = C) of *S. matsudana* seedlings under exogenous application of SA and 2,4-DNP alone.

Time (d)	Treatment	One-Dimensional Linear Equation	2,4-DNP Concentration When P_n Is Halved (mg·L^{-1})
5	DNP	Y = −0.6293X + 15	13.41
	DNP + S1	Y = −0.3485X + 15	24.21
	DNP + S2	Y = −0.3881X + 15	21.74
	DNP + S3	Y = −0.5934X + 15	14.22
10	DNP	Y = −0.9484X + 15	10.06
	DNP + S1	Y = −0.4862X + 15	19.62
	DNP + S2	Y = −0.6855X + 15	13.91
	DNP + S3	Y = −0.7366X + 15	12.95

When exogenous SA was added to 2,4-DNP to treat *S. matsudana* seedlings, each exogenous addition treatment group alleviated the stress of 2,4-DNP on the photosynthetic system of *S. matsudana* seedlings to some extent (Table 1). Among the SA-added treatment groups, DNP + S1 had the best effect compared with the other treatment groups. At 5 d, P_n increased by 57.02%, T_r increased by 47.74%, G_s increased by 80.60%, L_s increased by 115.54%, C_i decreased by 24.90%, LUE increased by 57.02%, and WUE increased by 6.30%, compared to the 2,4-DNP treatment group alone. While, at 10 d, P_n increased by 108.78%, T_r increased by 99.63%, G_s increased by 162.08%, L_s increased by 132.13%, C_i decreased by 24.02%, LUE increased by 108.78%, and WUE increased by 4.57%, compared to the 2,4-DNP treatment group alone.

Under fixed light intensity conditions (1200 μmol·m^{-2}·s^{-1}), 2,4-DNP treatment alone significantly reduced P_n, T_r, G_s, L_s, LUE, and WUE while it significantly increased C_i ($p < 0.05$) in leaves of *S. matsudana* seedlings. When SA was added alone, the P_n, T_r, G_s, L_s, C_i, LUE, and WUE of the leaves of *S. matsudana* seedlings changed significantly ($p < 0.05$) with the increase of its concentration compared with the control (CK), indicating that

the high concentration of SA (1000 mg·L^{-1}) inhibited the photosynthetic process of the photosynthetic system of *S. matsudana* seedlings.

3.2. Chlorophyll Fluorescence Parameters

Figures 1 and 2 showed that 2,4-DNP treatment alone significantly reduced qP, Φ_{PSII}, F_V/F_m, α, and P in leaves of *S. matsudana* seedlings while it significantly increased F_O, F_m, NPQ, Y, β, D, and Ex ($p < 0.05$). When SA was added alone, the chlorophyll fluorescence parameters of the *S. matsudana* seedlings changed significantly ($p < 0.05$) with the increase of its concentration compared with the control (CK), indicating that the high concentration of SA (1000 mg·L^{-1}) inhibited the normal physiological processes of the chlorophyll fluorescence system of the *S. matsudana* seedlings to some extent. When exogenous SA was added on top of 2,4-DNP to treat *S. matsudana* seedlings, the stress of 2,4-DNP on the chlorophyll fluorescence system of willow seedlings was alleviated to some extent in all exogenous addition treatment groups.
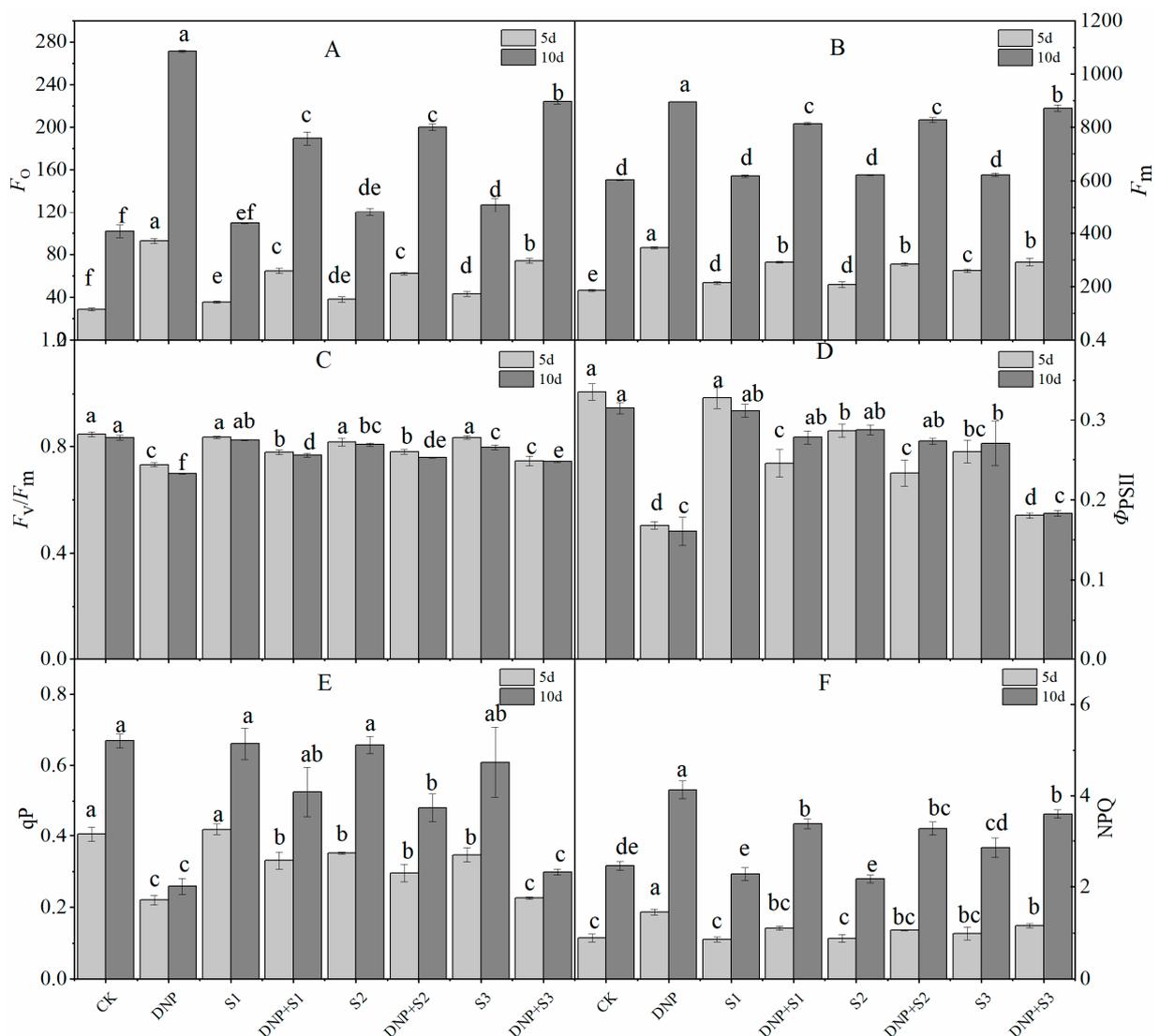

Figure 1. Effects of SA on the F_O, F_m, F_V/F_m, Φ_{PSII}, qP, and NPQ in leaves of *S. matsudana* seedlings under stress of 2,4-DNP. (**A**): Including initial fluorescence (F_O); (**B**): Maximum fluorescence(F_m); (**C**): maximal quantum yield of PSII photochemistry (F_V/F_m); (**D**): Effective quantum yield of PSII photochemistry (Φ_{PSII}); (**E**): Photochemical quenching coefficient (qP); (**F**): Nonphotochemical quenching (NPQ). Note: Different lowercase letters within different treatments are significantly different at the same time ($p < 0.05$).
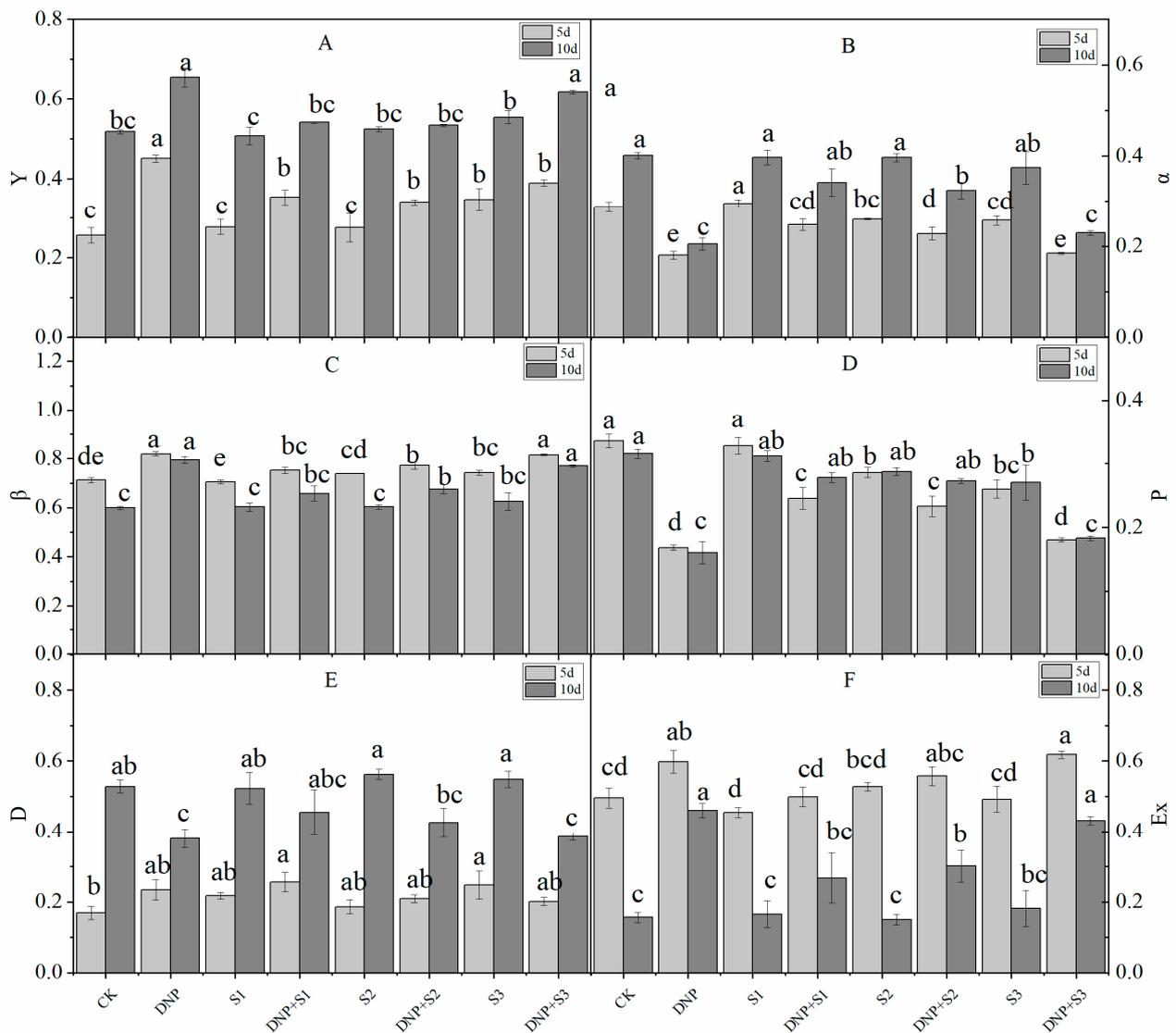
Figure 2. Effects of SA on Y, α, β, P, D, and Ex in leaves of *S. matsudana* seedlings under stress of 2,4-DNP. (**A**): PSII regulated energy dissipation quantum yield (Y); (**B**): PSI excitation energy partition coefficient (α); (**C**): PSII excitation energy partition coefficient (β); (**D**): Photochemical dissipation (P); (**E**): Antenna thermal dissipation (D); (**F**): Non-photochemical energy dissipation (Ex). Note: Different lowercase letters within different treatments are significantly different at the same time ($p < 0.05$).

As with the photosynthetic gas exchange parameters, the result of DNP + S1 was better in each treatment group added with SA compared to the other treatment groups. At 5 d and 10 d, the qP changed by 149.96% and 203.50%, respectively, compared to the 2,4-DNP treatment group alone, Φ_{PSII} changed by 150.00% and 173.09%, F_v/F_m changed by 106.38% and 110.02%, α changed by 137.43% and 166.88%, P changed by 150.00% and 173.10%, F_o changed by 69.78% and 69.90%, F_m changed by 84.44% and 90.84%, NPQ changed by 75.86% and 81.95%, Y changed by 78.25% and 82.78%, β changed by 91.76% and 82.78%, D changed by 109.55% and 119.70%, and Ex changed by 83.34% and 58.19%, respectively (Figure 2). This indicated that the addition of 10 mg·L^{-1} SA maintained the chlorophyll fluorescence utilization capacity of the leaves of *S. matsudana* seedlings at a higher level compared with the 2,4-DNP treatment group alone, which enhanced the tolerance of *S. matsudana* seedlings to 2,4-DNP by increasing D and decreasing P in the leaves of willow seedlings, and by adjusting Ex to stabilize its own allocation of light energy utilization.

3.3. Chlorophyll Content

Figure 3 showed that 2,4-DNP treatment alone significantly reduced Chla (chlorophyll a), Chlb, c (carotenoid), Chla/Chlb, and Chla + Chlb in the leaves of *S. matsudana* seedlings. However, the result of c/(Chla + Chlb) (total chlorophyll content) was the opposite ($p < 0.05$). When SA was added alone, with the increase of the concentration, there was no significant differences ($p > 0.05$), except c/(Chla + Chlb). Chla, Chlb, c, Chla/Chlb, and Chla + Chlb in the leaves of *S. matsudana* seedlings showed a downward trend and were smaller than those in the control group (CK). These results indicated that a high concentration of SA (1000 mg·L^{-1}) damaged the synthesis of chlorophyll in *S. matsudana* seedling leaves to some extent or accelerated its degradation, resulting in a significant decline in chlorophyll content. When exogenous SA was added on the basis of 2,4-DNP, all exogenous treatment groups alleviated the reduction of 2,4-DNP on the chlorophyll content in the leaves of *S. matsudana* seedlings to some extent. Among the treatment groups added with SA, the result of DNP + S1 was better than other treatment groups. Compared with the 2,4-DNP treatment group alone, Chla increased by 130.51%, Chlb increased by 57.23%, c increased by 55.53%, Chla/Chlb increased by 46.67%, Chla + Chlb increased by 100.74%, and c/(Chla + Chlb) decreased by 22.49%.
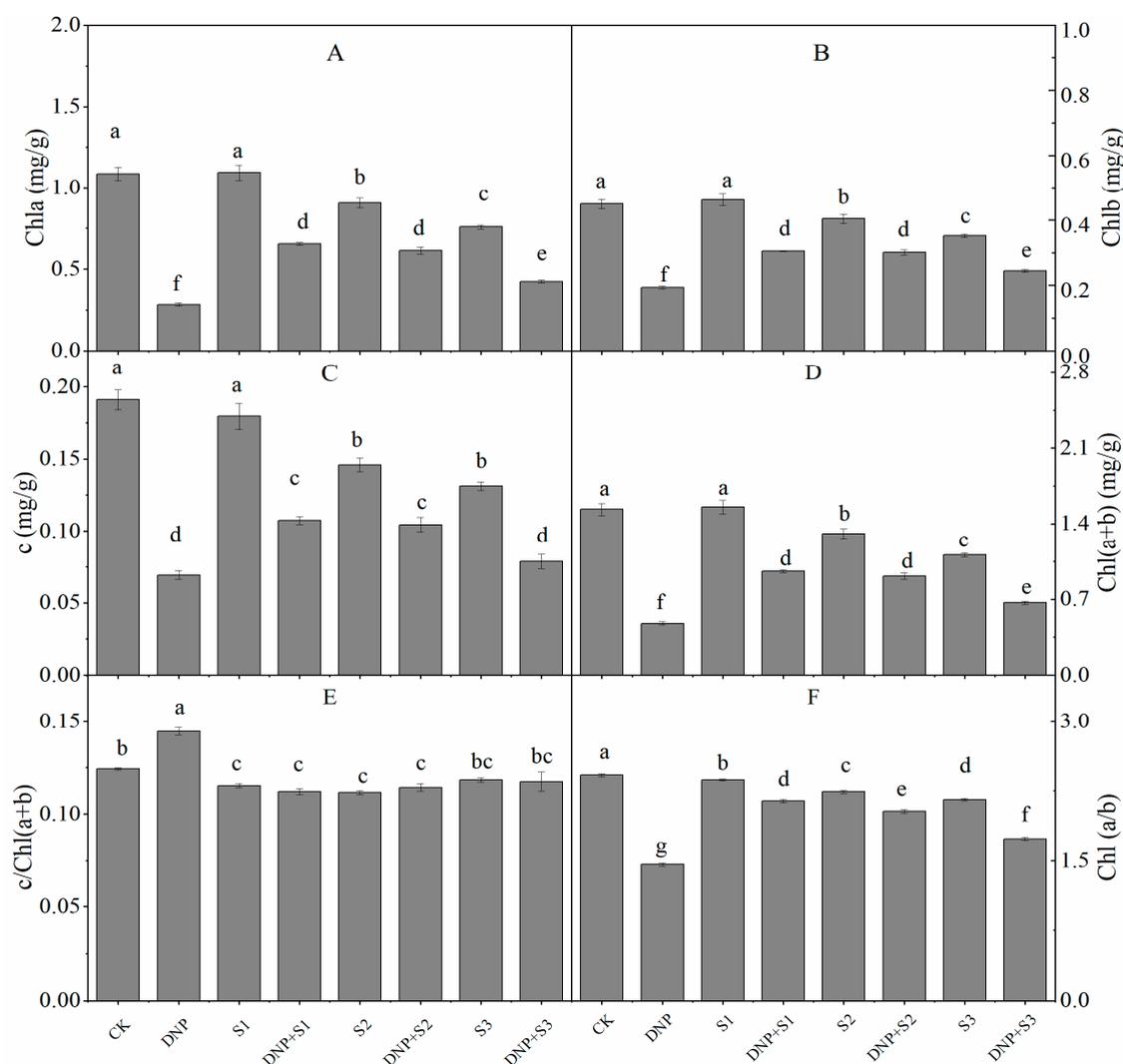

Figure 3. Effects of SA on chlorophyll content in leaves of *S. matsudana* seedlings under stress of 2,4-DNP. (**A**): chlorophyll a (Chla); (**B**): chlorophyll b (Chlb); (**C**): carotenoid (c); (**D**): chlorophyll a + chlorophyll b (Chla + Chlb); (**E**): carotenoid/(chlorophyll a + chlorophyll b) (c/(Chla + Chlb)); (**F**): chlorophyll a/chlorophyll b (Chla/Chlb)Note: Different lowercase letters within different treatments are significantly different ($p < 0.05$).

3.4. Cell Membrane Damage Level

Table 3 showed that 2,4-DNP treatment alone significantly increased MDA, REC, and leaf injury degree (LD) of *S. matsudana* seedling leaves ($p < 0.05$). When SA was added alone, MDA and REC were significantly different from the control group (CK) at the concentration of 1000 mg·L^{-1} SA ($p < 0.05$), which were 1.93 times and 2.92 times that of the control group (CK), respectively. At this time, LD was also significantly different from other concentrations of SA ($p < 0.05$). This showed that the high concentration of SA (1000 mg·L^{-1}) had damaged the cell membrane structure of the leaves of *S. matsudana* seedlings to a certain extent, leading to electrolyte leakage and REC reduction.

Table 3. Effects of exogenous application of SA on Malonicdialdehyde (MDA),Relative conductivity (REC), and Leaf damage (LD) in leaves of *S. matsudana* seedlings under stress of 2,4-DNP.

Treatment	MDA (mmol·g^{-1})	REC	LD
CK	13.8 ± 0.66 d	7.4 ± 0.47 d	-
DNP	38.2 ± 1.40 a	48.6 ± 0.63 a	44.5 ± 0.95 a
S1	14.0 ± 1.05 d	8.1 ± 0.46 d	0.7 ± 0.13 d
DNP + S1	32.6 ± 0.31 b	40.1 ± 0.57 b	35.3 ± 0.94 b
S2	16.4 ± 1.25 d	8.5 ± 0.19 d	1.2 ± 0.32 d
DNP + S2	35.7 ± 0.70 a	41.0 ± 0.98 b	36.3 ± 1.35 b
S3	29.6 ± 1.30 c	21.7 ± 0.47 c	15.4 ± 0.32 c
DNP + S3	38.7 ± 0.39 a	47.3 ± 0.64 a	43.1 ± 0.90 a

Note: The same columns within the different treatments followed by different letters are significantly different ($p < 0.05$).

When exogenous SA was added on the basis of 2,4-DNP to treat *S. matsudana* seedlings respectively, each exogenous treatment group alleviated the damage of 2,4-DNP to the leaves of *S. matsudana* seedlings to a certain extent (Table 3). Among the treatment groups added with SA, the result of DNP + S1 was better than that of other treatment groups. Compared with the 2,4-DNP treatment group alone, the MDA, REC, and LD decreased by 12.08%, 17.42%, and 20.57%, respectively.

3.5. Antioxidant Enzyme Activity and Active Oxygen Level

As shown in Table 4, 2,4-DNP treatment alone significantly increased the activities of SOD, POD, and CAT in the leaves of *S. matsudana* seedlings ($p < 0.05$). When SA was added alone, after 5 and 10 days of treatment, when the concentration of SA was 1000 mg·L^{-1}, SOD, POD, and CAT were significantly different from the control group (CK) ($p < 0.05$).

Table 4. Effects of SA on the superoxide dismutase (SOD) activity, peroxidase (POD) activity, Catalase (CAT) activity, and ROS levels in leaves of *S. matsudana* seedlings under stress of 2,4-DNP.

Time (d)	Treatment	SOD (U·g^{-1} FW)	POD (U·g^{-1}·min^{-1})	CAT (U·g^{-1}·min^{-1})	H$_2$O$_2$ (mmol·g^{-1})	O$_2^{-\cdot}$ (mmol·min^{-1}·g^{-1})
5	CK	336.6 ± 28.67 c	1133.3 ± 120.19 c	23.3 ± 3.33 d	1.4 ± 0.02 b	2.8 ± 0.20 b
	DNP	846.6 ± 14.08 a	3166.7 ± 120.19 a	73.3 ± 3.33 b	3.0 ± 0.10 a	6.8 ± 0.40 a
	S1	359.0 ± 52.57 c	1200.0 ± 57.73 c	26.7 ± 3.33 d	1.5 ± 0.15 b	2.9 ± 0.13 b
	DNP + S1	920.8 ± 10.09 a	3400.0 ± 152.75 a	76.7 ± 3.33 b	2.9 ± 0.05 a	6.8 ± 0.15 a
	S2	399.1 ± 5.31 c	1400.0 ± 57.74 c	33.3 ± 3.33 d	1.5 ± 0.08 b	2.9 ± 0.28 b
	DNP + S2	929.1 ± 6.36 a	3333.3 ± 145.30 a	86.7 ± 3.33 a	2.9 ± 0.06 a	6.7 ± 0.18 a
	S3	634.5 ± 62.09 b	2433.3 ± 88.19 b	46.7 ± 3.33 c	1.5 ± 0.24 b	2.7 ± 0.07 b
	DNP + S3	927.6 ± 34.02 a	3100.0 ± 115.47 a	66.7 ± 3.33 b	2.8 ± 0.05 a	6.9 ± 0.27 a

Table 4. *Cont.*

Time (d)	Treatment	SOD (U·g^{-1} FW)	POD (U·g^{-1}·min^{-1})	CAT (U·g^{-1}·min^{-1})	H$_2$O$_2$ (mmol·g^{-1})	O$_2^{-}$ (mmol·min^{-1}·g^{-1})
10	CK	319.7 ± 26.47 c	1066.7 ± 66.67 e	26.7 ± 3.33 d	1.6 ± 0.12 b	2.8 ± 0.52 b
	DNP	827.7 ± 32.94 a	2766.7 ± 88.19 b	66.7 ± 3.33 b	3.0 ± 0.12 a	6.8 ± 0.15 a
	S1	352.5 ± 27.58 c	1133.3 ± 88.19 de	33.3 ± 3.33 cd	1.4 ± 0.08 b	3.1 ± 0.22 b
	DNP + S1	864.1 ± 5.31 a	3133.3 ± 88.19 a	80.0 ± 5.77 a	2.9 ± 0.09 a	6.9 ± 0.13 a
	S2	375.7 ± 21.60 c	1333.3 ± 33.33 d	36.7 ± 3.33 cd	1.5 ± 0.09 b	2.8 ± 0.24 b
	DNP + S2	876.0 ± 10.01 a	3200 ± 57.74 a	90.0 ± 5.77 a	3.0 ± 0.05 a	6.9 ± 0.24 a
	S3	585.0 ± 43.21 b	2033.3 ± 88.19 c	43.3 ± 3.33 c	1.6 ± 0.11 b	3.1 ± 0.36 b
	DNP + S3	804.2 ± 15.90 a	2833.3 ± 88.19 b	63.3 ± 3.33 b	2.7 ± 0.06 a	6.8 ± 0.39 a

Note: The same columns within the different treatments followed by different letters are significantly different at the same time ($p < 0.05$).

When exogenous SA was added on the basis of 2,4-DNP to treat *S. matsudana* seedlings, each exogenous treatment group alleviated the damage of 2,4-DNP to the leaves of *S. matsudana* seedlings to a certain extent by enhancing their own antioxidant system activities (Table 4). In the 10-day experiment, the leaves of *S. matsudana* seedlings treated with exogenous SA were able to adjust the activities of SOD, POD, and CAT, so that the antioxidant system was always in a dynamic equilibrium change process to weaken the oxidative damage caused by 2,4-DNP stress, thereby maximizing their own stress resistance.

As shown in Table 4, histochemical staining of O$_2^{-}$· and H$_2$O$_2$ in leaves of *S. matsudana* seedlings after SA and 2,4-DNP addition revealed the accumulation of O$_2^{-}$· and H$_2$O$_2$ in leaves. During the experiment, when SA was added alone, there was no significant difference ($p > 0.05$) between the content of O$_2^{-}$· and H$_2$O$_2$ in the leaves of *S. matsudana* seedlings and the control group (CK). When treated with 2,4-DNP alone, the leaves of *S. matsudana* seedlings showed obvious dark blue and dark brown spots. At this time, the contents of O$_2^{-}$· and H$_2$O$_2$ in the leaves of *S. matsudana* seedlings were significantly different from those of the control group (CK) ($p < 0.05$). When exogenous SA was added on the basis of 2,4-DNP, the dark blue and dark brown spots of the leaves of *S. matsudana* seedlings decreased obviously.

3.6. Biomass

As shown in Table 5, when treated with 2,4-DNP alone, the total biomass, leaf area, and plant height of *S. matsudana* seedlings were significantly different from those of the CK ($p < 0.05$). When exogenous SA was added on the basis of 2,4-DNP, compared with 2,4-DNP alone, the leaf area of *S. matsudana* seedlings was significantly increased. The total biomass and plant height were significantly increased when SA concentration was 10 mg·L^{-1} SA alone decreased significantly compared with the CK, which were 49.58%, 60.97% and 86.16% of the CK respectively; When treated with 2,4-DNP alone (DNP), the total biomass, leaf area and plant height of *S. matsudana* seedlings were significantly different from those of the CK ($p < 0.05$); When exogenous SA was added on the basis of 2,4-DNP, compared with 2,4-DNP alone, the leaf area of *S. matsudana* seedlings was significantly increased, and the total biomass and plant height were significantly increased when SA concentration was 10 mg·L^{-1}.

As shown in Table 6, compared with 2,4-DNP treatment alone, the addition of exogenous SA increased the TI of *S. matsudana* seedlings and reduced the toxicity coefficient of *S. matsudana* seedlings. The TI of *S. matsudana* seedlings in DNP + S1, DNP + S2, and DNP + S3 treatment groups increased by 158.11%, 26.49%, and 11.37%, respectively, compared with 2,4-DNP treatment alone. The toxicity coefficients decreased by 61.40%, 21.08%, and 10.15%, respectively.

Table 5. Effects of SA on the total biomass, leaf area, and plant height in leaves of *S. matsudana* seedlings under stress of 2,4-DNP.

Treatment	Total Biomass (g)	Leaf Area (cm^2)	Plant Height (cm)
CK	52.1 ± 0.79 a	43.2 ± 2.63 a	71.5 ± 1.67 a
DNP	21.7 ± 0.46 f	18.6 ± 0.50 e	55.4 ± 1.75 d
S1	44.4 ± 0.55 b	33.9 ± 0.92 b	66.0 ± 2.00 b
DNP + S1	40.5 ± 1.21 c	30.5 ± 0.65 bc	65.6 ± 1.73 b
S2	38.5 ± 0.91 c	33.3 ± 0.24 b	62.6 ± 1.38 bc
DNP + S2	28.4 ± 0.61 d	27.9 ± 0.96 cd	61.7 ± 1.25 bc
S3	25.8 ± 0.47 e	26.2 ± 0.68 d	61.1 ± 2.00 bc
DNP + S3	25.2 ± 0.60 e	25.8 ± 1.11 d	58.0 ± 1.66 cd

Note: The same columns and within the different treatments followed by different letters are significantly different ($p < 0.05$).

Table 6. Effects of SA and 2,4-DNP alone on Tolerability Index (TI) and toxicity coefficient of *S. matsudana* seedlings.

Treatment	Tolerability Index	Toxicity Coefficient
CK	-	-
DNP	0.43 ± 0.02 d	0.57 ± 0.02 a
DNP + S1	0.78 ± 0.02 a	0.22 ± 0.02 d
DNP + S2	0.55 ± 0.01 b	0.45 ± 0.01 c
DNP + S3	0.48 ± 0.01 c	0.52 ± 0.01 b

Note: The same columns and within the different treatments followed by different letters are significantly different ($p < 0.05$).

As shown in Table 7, the tolerance index of the total biomass of *S. matsudana* seedlings was taken as 50% of the biomass of the control group (CK) as the threshold value of the tolerance of *S. matsudana* seedlings to 2,4-DNP, and the equation was obtained by fitting it with the unary linear equation, as shown in Table 7. The 2,4-DNP concentrations in DNP, DNP + S1, DNP + S2, and DNP + S3 treatment groups were 13.09, 33.78, 16.56, and 14.58 mg·L^{-1}, respectively, when the tolerance index of total biomass was halved. It could be seen that exogenous SA treatment improved the tolerance of *S. matsudana* seedlings to 2,4-DNP stress and reduced the inhibition of 2,4-DNP stress on the total biomass of *S. matsudana* seedlings. The tolerance threshold of *S. matsudana* seedlings to 2,4-DNP increased from 13.09 mg·L^{-1} to 14.58–33.78 mg·L^{-1}.

Table 7. One-dimensional linear equation simulation of the TI of total biomass (Y = B) and 2,4-DNP concentration (X = C) of *S. matsudana* seedlings under exogenous application of SA and 2,4-DNP alone.

Time (d)	One-Dimensional Linear Equation	2,4-DNP Concentration When the TI of Total Biomass Is Halved (mg·L^{-1})
DNP	Y = −0.0382X + 1	13.09
DNP + S1	Y = −0.0148X + 1	33.78
DNP + S2	Y = −0.0302X + 1	16.56
DNP + S3	Y = −0.0343X + 1	14.58

3.7. Growth Morphological Characteristics

As shown in Figure 4, when SA is added alone, with the increase of SA concentration, the edge of the leaves of *S. matsudana* seedlings gradually turns yellow, and the leaves fall off. More leaves fell along with higher concentration and deeper yellow. When 2,4-DNP was applied alone, the stem ends and some leaves of *S. matsudana* seedlings appeared obviously yellow. When exogenous SA was added on the basis of 2,4-DNP, the 2,4-DNP toxicity symptoms of *S. matsudana* seedlings were alleviated to varying degrees, but most of the leaves of *S. matsudana* seedlings were withered and yellow when treated with 1000 mg·L^{-1}

SA. It was speculated that a high concentration of SA (1000 mg·L^{-1}) had an inhibitory effect on the normal growth of *S. matsudana* seedlings.

Figure 4. Effects of SA on the percentage of 2,4-DNP removal and growth morphological characteristics of *S. matsudana* seedlings under stress of 2,4-DNP.

3.8. 2,4-DNP Removal Effect

As shown in Figure 5, the removal percentage of 2,4-DNP in *S. matsudana* seedlings remained at a low level (51.05% and 71.12%) when treated with 2,4-DNP alone. After SA addition, the removal percentage of 2,4-DNP increased significantly, and the color of 2,4-DNP solution gradually faded. Among them, there was no significant difference between SA treatment groups ($p > 0.05$), which increased by 19.24%, 18.89%, and 16.26%, respectively, compared with 2,4-DNP treatment alone at 10 days.
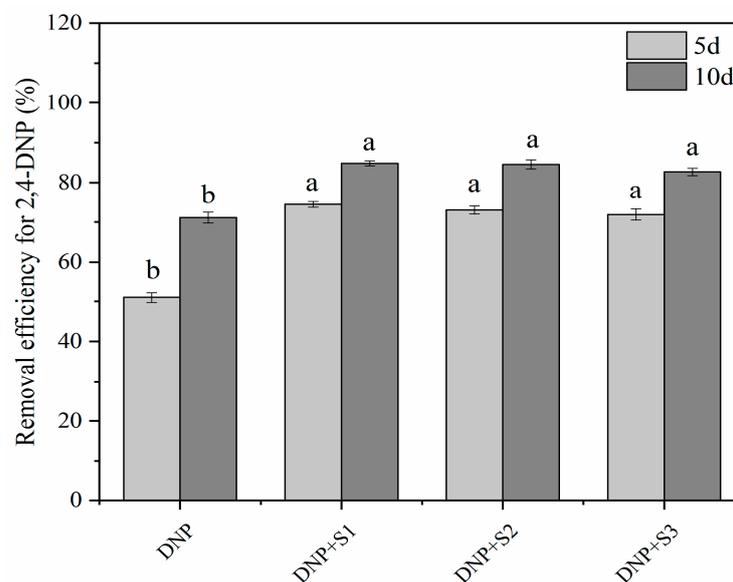
Figure 5. Effects of SA on the mitigation of the percentage of 2,4-DNP removal of *S. matsudana* seedlings under stress of 2,4-DNP. Note: Different lowercase letters within different treatments are significantly different at the same time ($p < 0.05$).

4. Discussion

Stress can affect the normal growth and physiological processes of plants. Photosynthesis is the basis for normal growth and development and physiological metabolism of plants [30]. Measuring the photosynthetic parameters of plant leaves can effectively measure the strength of their photosynthetic function [31]. The degree of tolerance to

stress can be effectively determined according to the extent of the change of photosynthetic parameters of plant leaves with the change of stress degree [32]. Zhang et al. [33] found that exogenous SA could increase the LUE of plant leaves under Cd stress and alleviate the inhibition of Cd stress on plant photosynthesis. In this study, when high-concentration SA (1000 mg·L^{-1}) was added to the seedlings of *S. matsudana*, each exogenous treatment group could significantly alleviate the stress of 2,4-DNP on the photosynthetic system of *S. matsudana* seedlings, and the effect of DNP + S1 was better. However, adding high-concentration SA (1000 mg·L^{-1}) inhibited the photosynthetic process of the photosynthetic system of *S. matsudana* seedlings. The reason might be that the high concentration of SA (1000 mg·L^{-1}) affected the absorption and utilization of light energy by *S. matsudana* seedlings, thereby inhibiting their normal photosynthetic process.

P_n can directly indicate the efficiency of plant photosynthesis under saturated light intensity [34]. The decline of P_n is often caused by stomatal and non-stomatal limitations [35]. Among them, stomatal restriction is often due to the decrease of G_s of plants caused by water stress, which could prevent CO_2 from entering the leaves. Non-stomal restriction is often due to the destruction of chloroplast structure in plants, which affects the photosynthetic activity of mesophyll cells [32]. In this study, exogenous SA treatment significantly improved the tolerance of *S. matsudana* seedlings to 2,4-DNP, and the tolerance threshold of 2,4-DNP increased from 13.41 mg·L^{-1} to 13.87–24.21 mg·L^{-1} within 5 days, and from 10.06 mg·L^{-1} to 10.03–19.62 mg·L^{-1} within 10 days.

The light energy absorbed by plant leaves can be divided into D, P, and Ex [36]. Investigating the distribution of absorbed light energy in plant leaves is convenient to explore the treatment strategy of absorbed light energy [37]. The low level of Y (NPQ) and the high level of Y (NO) mean that the plant's own defense system can no longer protect itself from damage under external stress [38]. In this study, after adding exogenous SA, a high concentration of SA (1000 mg·L^{-1}) inhibited the normal physiological process of the chlorophyll fluorescence system of *S. matsudana* seedlings to a certain extent, while adding exogenous low concentration SA (10 mg·L^{-1}) to treat *S. matsudana* seedlings, each exogenous treatment group could alleviate the stress of 2,4-DNP on the photosynthetic system of *S. matsudana* seedlings to a certain extent, and the effect of DNP + S1 was better. Compared with the 2,4-DNP treatment group alone, the addition of 10 mg·L^{-1} SA kept the chlorophyll fluorescence utilization capacity of the leaves of *S. matsudana* seedlings at a high level, which decreased P by increasing the D of the leaves of *S. matsudana* seedlings. It changed the NPQ dissipation while adjusting Ex to stabilize its own distribution and utilization of light energy, thus enhancing the tolerance of *S. matsudana* seedlings to 2,4-DNP.

Chloroplast pigment can transfer or convert light energy absorbed from the outside into chemical energy and store it. Its content represents the intensity of photosynthesis of plant leaves and can effectively reflect the strength of the photosynthetic capacity of leaves [39]. In this study, exogenous SA could enhance the photosynthetic rate of plant leaves by inhibiting the decrease of Chla + Chlb and c in plant leaves caused by Cd stress and increasing the value of Chla/Chlb [40]. Drazic et al. [41] found that exogenous SA could effectively alleviate the inhibition of Cd on soybean (*Glycine max*) seedlings and improve their chlorophyll contents. Mahmoud et al. found that exogenous SA treatment could promote the growth and chlorophyll content of citrus (*Citrus sinensis*) under salt stress [42]. In this study, when exogenous SA was added to *S. matsudana* seedlings on the basis of 2,4-DNP, each exogenous treatment group could alleviate the decrease of chlorophyll content in leaves of *S. matsudana* seedlings caused by 2,4-DNP, and the effect of DNP + S1 was better. When SA was added alone, with the increase of its concentration, except for c/(Chla + Chlb), there was no significant difference ($p > 0.05$). Chla, Chlb, c, Chla/Chlb, c/(Chla + Chlb), and Chla + Chlb in the leaves of *S. matsudana* seedlings showed a downward trend and were lower than those of the control group (CK), indicating that high concentration of SA (1000 mg·L^{-1}) destroyed the synthesis of chlorophyll in the leaves of *S. matsudana* seedlings or accelerated its degradation. Then, the content of

chlorophyll decreased significantly, but its decline was significantly smaller than that of 2,4-DNP alone.

Under normal conditions, the antioxidant enzyme systems, such as SOD and POD in plants, are in dynamic equilibrium with O_2^-· and H_2O_2 [43]. When a plant is under external stress, its ROS will be produced substantially to strengthen metabolism and self-resistance [44]. When a large amount of ROS cannot be cleared in time, it will cause metabolic disorder in the plant, weaken normal physiological activity, and even cause plant death. While ROS is produced in large quantities, MDA, as one of the important reaction products of membrane lipid peroxidation, will also change with the change of the stress degree of plants, reflecting the degree of damage of plants [28,45]. REC and LD can reflect the amount of electrolyte outflow and the degree of membrane structure damage in plant leaves, and their values gradually increase with the deepening of stress. In this study, when SA was added alone, CAT, POD, SOD, MDA, REC, and LD were significantly different from those of the control group (CK) at the concentration of 1000 mg·L^{-1} after 5 and 10 days of treatment ($p < 0.05$), respectively. This indicated that a high concentration of SA (1000 mg·L^{-1}) destroyed the cell membrane structure of *S. matsudana* seedlings leaves to a certain extent, resulting in electrolyte leakage and reduced REC. When *S. matsudana* seedlings were treated with exogenous SA on the basis of 2,4-DNP, each exogenous treatment group alleviated the damage of 2,4-DNP to *S. matsudana* seedlings leaves to a certain extent by enhancing the activity of its antioxidant system and reducing the size of MDA, REC, and LD. In the 10-day experiment, the leaves of *S. matsudana* seedlings treated with exogenous SA, by regulating the activities of SOD, POD, and CAT, and the sizes of MDA, REC, and LD, kept the antioxidant system in a dynamic balance process to reduce the oxidative damage caused by 2,4-DNP stress, thus maximized their resistance to stress. Adding exogenous SA on the basis of adding 2,4-DNP effectively alleviated the oxidative damage of *S. matsudana* seedlings, and DNP + S1 had the best effect among all the exogenous treatment groups.

Liang et al. [46] found that the exogenous addition of SA could effectively reduce the content of O_2^-· and H_2O_2 in wheat under the toxicity of isoproturon and increase the activity of antioxidant enzymes to improve the tolerance of wheat (*Triticum aestivum*) to isoproturon. Song et al. [47] found that spraying SA on the leaves of barley (*Hordeum uhulgare*) under Cd stress could promote the activities of CAT, and SOD in their bodies. Mahmoud et al. [42] found that exogenous SA treatment could promote the activities of POD, CAT, and other antioxidant enzymes of citrus (*Citrus sinensis*) under salt stress. In this study, there was no significant difference between the contents of O_2^-· and H_2O_2 in *S. matsudana* seedlings and the control group (CK) when SA was added alone ($p > 0.05$). Compared with 2,4-DNP alone, the content of O_2^-· and H_2O_2 in the leaves of *S. matsudana* seedlings treated with exogenous SA on the basis of 2,4-DNP significantly decreased. It could be seen that the addition of exogenous SA alleviated the stress of 2,4-DNP on the seedlings of *S. matsudana*, which reduced the content of O_2^-· and H_2O_2 in the leaves of *S. matsudana* seedlings under 2,4-DNP stress and the damage to the living cells of *S. matsudana* seedlings, thus enhancing its stress resistance.

When plant seedlings are used to repair organic pollutants, plants in the organic pollution environment are extremely sensitive to these pollutants. While repairing the environment of organic pollutants, because of the toxicity and hydrophobicity characteristics of organic pollutants, its ability to obtain the necessary substances for growth from the surrounding environment will be inhibited, and its biomass will also decline [48]. Usually, the deeper the degree of stress is, the greater the decline in biomass will be [49]. In this study, the addition of exogenous SA on the basis of 2,4-DNP significantly increased the leaf area of *S. matsudana* seedlings, and the total biomass and plant height were significantly increased when SA concentration was 10 mg·L^{-1}. The results showed that exogenous SA could alleviate the decrease of the biomass index of *S. matsudana* seedlings under 2,4-DNP stress. Some scholars chose to reduce plant biomass by about 50% as the upper limit of their tolerance to stress [50], while others chose to reduce plant P_n by about 50% as the

threshold of their tolerance to stress [51]. Mahmoud et al. [42] found that exogenous SA treatment could alleviate the inhibition of normal growth of citrus (*Citrus sinensis*) under salt stress. In this study, exogenous SA treatment improved the ability of *S. matsudana* seedlings to tolerate 2,4-DNP stress and reduced the inhibition of 2,4-DNP stress on the total biomass of *S. matsudana* seedlings. The tolerance threshold of *S. matsudana* seedlings to 2,4-DNP increased from 13.09 mg·L^{-1} to 14.58–33.78 mg·L^{-1}. Therefore, considering the changes in photosynthetic and biomass indexes of *S. matsudana* seedlings under 2,4-DNP stress, it is suggested that the range of 2,4-DNP tolerance threshold within 10 days can be 8.81–33.78 mg·L^{-1} as a reference when using exogenous SA to synergize *S. matsudana* seedlings for phytoremediation of 2,4-DNP polluted environment.

The change in plant growth morphology is the most obvious growth response change of plants under stress. When a plant is under stress, the root system is often the first to respond because of direct contact with the stress environment [52]. In this study, root morphological indexes such as root length and root activity of *S. matsudana* seedlings were significantly different ($p < 0.05$). When treated with exogenous SA on the basis of 2,4-DNP, the symptoms of 2,4-DNP toxicity of *S. matsudana* seedlings were alleviated to varying degrees, but the leaves of *S. matsudana* seedlings were mostly withered and yellow when treated with 1000 mg·L^{-1} SA. It was speculated that a high concentration of SA (1000 mg·L^{-1}) had an inhibitory effect on the normal growth of *S. matsudana* seedlings. In this study, only salicylic acid concentrations of 10, 100, and 1000 mg·L^{-1} were considered, and more salicylic acid levels will be conducted to ensure the concentration effects in future research, for example, 0, 10, 50, 100, 200, 400, 600, 800, and 1000 mg·L^{-1}.

Plants can enhance their tolerance to external pollution stress by enhancing the activity of the antioxidant system to better remove them and reduce the impact of toxicity [53]. Li et al. [54] found that exogenous Ca could alleviate the stress of Cd on *S. matsudana* seedlings by regulating the activity of the antioxidant system. In this study, the increased activities of CAT, POD, and SOD in *S. matsudana* seedlings under 2,4-DNP stress increased their tolerance to 2,4-DNP stress and then weakened their toxicities to themselves at low concentrations of 2,4-DNP (0–10 mg·L^{-1}). The reason might be that the enzymes such as CAT, POD, and SOD in their bodies converted 2,4-DNP into more water-soluble forms through degradation and binding process for transfer or carried out fixed accumulation to minimize the toxic effects of 2,4-DNP on themselves [55].

Some studies showed that exogenous SA could reduce the external oxidative stress on itself by combining with CAT, POD, SOD, and other enzymes in the plant and could also cause the expression of defense genes in the plant to reduce the damage of external stress on itself [56]. In this study, the percentage of 2,4-DNP removal by *S. matsudana* seedlings increased after exogenous SA was added, reaching the maximum value (84.80%) at 10 mg·L^{-1}, which was 19.24% higher than that of 2,4-DNP treatment alone. The reason might be that the addition of exogenous SA enhanced the tolerance of *S. matsudana* seedlings to 2,4-DNP, improved the antioxidant system activity of *S. matsudana* seedlings to a certain extent, and further promoted the removal of 2,4-DNP.

2,4-DNP is widely used in pesticides, which can enter the soil with rainwater, leading to surface runoff and groundwater pollution [3,7]. Farmland protection forests play an important role in improving microclimate environments, intercepting surface runoff, and regulating groundwater levels [57]. The author suggests planting a large number of *S. matsudana* on both sides of roads or ditches in farmland to form a willow farmland forest network to reduce pesticide residues in surface runoff or groundwater levels. 2,4-DNP is widely present in pollutants emitted from chemical plants [58]. For this type of point source pollutants, restoration can be achieved by establishing artificial wetland forests of *S. matsudana*. SA can enhance plant stress resistance and slow down the damage caused by pollutants to plant growth during plant remediation processes [59]. Therefore, when using *S. matsudana* for plant remediation, it is recommended to add exogenous salicylic acid, preferably at a concentration of 10 mg·L^{-1}, to enhance the carbon sequestration capacity of *S. matsudana* and absorb more pollutants.

In this study, the photosynthetic gas exchange parameters, chlorophyll fluorescence parameters, antioxidant enzyme activity, active oxygen levels, and biomass were analyzed to make clear the tolerance range, purification effect, and regulatory effect of *S. matsudana* under exogenous SA to 2,4-DNP in wastewater. However, the correlation and important values among these indicators need further research.

5. Conclusions

In this study, the feasibility of exogenous SA to improve the tolerance and purification ability of *S. matsudana* to 2,4-DNP stress was explored. It was found that SA treatment could effectively alleviate the stress of 2,4-DNP on the photosynthetic and chlorophyll fluorescence system of *S. matsudana* seedlings, and the best effect was DNP + S1. Exogenous SA could effectively alleviate the physiological damage caused by 2,4-DNP stress on *S. matsudana* seedlings, and DNP + S1 had the best effect. The exogenous addition of SA could effectively alleviate the growth damage caused by 2,4-DNP stress on *S. matsudana* seedlings and increase the tolerance threshold range of *S. matsudana* seedlings to 2,4-DNP. Exogenous SA could also promote the percentage of 2,4-DNP removal by *S. matsudana* seedlings. Overall, when SA reached 10 mg·L^{-1}, the photosynthetic productivity of S. *matsudana* was the highest, and S. *matsudana* had the best purification effect on 2,4-DNP in wastewater. The research results could provide a theoretical reference for wastewater remediation of S. *matsudana*.

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