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Effects of Foliage Spraying with Sodium Bisulfite on the Photosynthesis of *Orychophragmus violaceus*

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Abstract: Sulphurous acid derived from sulfur dioxide (SO_2) emission leads to the pollution of irrigation water and the inhibition of plant growth. The safe concentration threshold of $NaHSO_3$ in plants should be clarified to promote agricultural production. In this study, *Orychophragmus violaceus* seedlings were used as experimental materials and five $NaHSO_3$ concentrations (i.e., 0, 1, 2, 5, 10 mmol·L^{-1}) were simultaneously sprayed on the leaf surface of different seedlings separately. Leaf physiology responses under different concentrations were analyzed. The $NaHSO_3$ did not promote photosynthesis in *O. violaceus* under the 1 and 2 mmol·L^{-1} treatments. It was conducive to the net photosynthetic rate (P_N), photorespiration rate (R_p), chlorophyll content, actual photochemical quantum yield (Y_{II}) and photochemical quenching (qP) under the 5 mmol·L^{-1} treatment. However, quantum yield of regulated energy dissipation (Y_{NPQ}) and nonphotochemical quenching (NPQ) were inhibited. Under the 10 mmol·L^{-1} treatment, P_N, chlorophyll content, Y_{II}, qP, dark respiration rate (R_d) and electron transport rate (ETR) showed significant decreases, while the photorespiration portion (S_p) significantly increased. Our results demonstrated that $NaHSO_3$ provided a sulfur source for plant growth and interfered with the redox reaction of the plant itself, and its role as a photorespiratory inhibitor might be masked.

Keywords: agricultural production; redox; photorespiration; chlorophyll fluorescence; dose effect

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1. Introduction

Orychophragmus violaceus is a member of the family *Brassicaceae* that is widely used for beautifying the city and ecological restoration [1]. *O. violaceus* is also a healthy seasonal vegetable that can be eaten year round and is widely distributed, especially in Yunnan, Guizhou and other southern cities [2]. The plant species has high economic and ornamental value. Sulfur dioxide (SO_2) is a widely diffused air pollutant, which is easily dissolved in the water of rivers or lakes and which forms sulfite and sulfuric acid. If the water source polluted by SO_2 is used for irrigation or spraying on greening plants, it may not be conducive to the plants' growth. Studies have shown that the toxicity of SO_2 to plants was mainly attributed to the highly active intermediate bisulfite [3]. Katainen et al. has also reported that the treatment of sphagnum moss with 0.1 mmol·L^{-1} of H_2SO_3 increased the net photosynthetic rate [4]. Therefore, HSO_3^- may have a two-way effect on the photosynthesis and growth of plants when it is used for irrigation.

$NaHSO_3$ is one of the most commonly used sulfites, which can be used as a photosynthetic accelerator in agricultural production [5–8]. However, the effect of $NaHSO_3$ on the photosynthetic growth of plants depends on its concentration. Studies have shown that 0.5 mmol·L^{-1} of $NaHSO_3$ is the best concentration to promote the photosynthetic oxygen release of Anabaena, while 1 mmol·L^{-1} of $NaHSO_3$ can increase the net photosynthetic rate of *Satsuma mandarin* by approximately 15% [9,10]. In general, low concentrations of $NaHSO_3$ (<1 mmol·L^{-1}) can significantly improve the photosynthetic oxygen release rate and dry matter accumulation of algae and other lower plants [6,10,11], while most higher plants after low concentrations of $NaHSO_3$ (<8 mmol·L^{-1}) spraying can significantly enhance the photosynthetic carbon assimilation ability [7–9,12]. Bisulfite can represent a sulfur source for plants. *Botryococcus braunii* reportedly stopped growing after surviving for 12 days in a sulfur-free medium, but grew well under a bisulfite treatment of 0.1 or 0.8 mmol·L^{-1} [6]. However, the promotion of plant growth by the addition of low concentrations of $NaHSO_3$ is not just attributed to the supply of sulfur nutrients. At present, the effect of $NaHSO_3$ on the photorespiration of plants is still controversial. Kang et al. [7] demonstrated that 5 mmol·L^{-1} of $NaHSO_3$ inhibited the photorespiration rate of *Caragana korshinskii*, and the content of glyoxylic acid decreased significantly. However, Chen et al. [8] found that photosynthetic and photorespiration rates increased simultaneously after soybean leaves were treated with 5 mmol·L^{-1} of $NaHSO_3$. Under normal conditions, photorespiration consumes approximately a quarter of the total output of photosynthesis and the portion of photorespiration will increase when the atmospheric carbon dioxide significantly affects the stomata [13]. In recent years, studies on the effects of foliar sprays of $NaHSO_3$ on plants have mainly focused on the response of the photorespiration rate to $NaHSO_3$ [7,8], whereas the proportion of photorespiration in total photosynthesis has not yet been reported. Therefore, variations in the portion of photorespiration must be determined when studying the photosynthetic physiological mechanism of $NaHSO_3$ in plants. In addition, high concentrations of $NaHSO_3$ (>8 mmol·L^{-1}) can cause certain toxicity to the photosynthetic physiology of plants. Ten mmol·L^{-1} of $NaHSO_3$ significantly decreased the net photosynthetic rate of strawberry leaves [9]. The photosynthetic electron transport of pea leaves was inhibited by high concentrations of sulfite [14]. It is interesting to note that $NaHSO_3$ is a chemical compound with both oxidizing and reducing properties. Sulfite in plants can be reduced to sulfide by sulfite reductase or oxidized to sulfate by sulfite oxidase [15]. During photosynthesis, plants produce and accumulate different forms of reactive oxygen species (i.e., ROS) and reducing agents (i.e., ascorbic acid, thioredoxin and reduced glutathione), which are important regulators of photosynthesis-related gene expression [16]. Wei et al. showed that HSO_3^- could react with superoxide anion to form SO_4^{2-} [17]. However, it has also been reported that $NaHSO_3$ oxidation destroys the structure of algae cell membranes [18]. When $NaHSO_3$, which has both oxidation and reduction properties, enters the plant, the normal redox reaction will be disturbed and indirectly affect photosynthesis. However, few reports have focused on the regulation of plant redox by $NaHSO_3$.

O. violaceus was used as experimental material in this study, the mechanisms of different concentrations of $NaHSO_3$ on photosynthesis were investigated, the safe concentration threshold of $NaHSO_3$ in plant leaves was clarified and the theoretical basis for promoting agricultural production and reducing agricultural ecological environment pollution could be provided.

2. Materials and Methods

2.1. Plant Culture and Treatment

The experiment was carried out in the Key Laboratory of Modern Agricultural Equipment and Technology of the Ministry of Education, College of Agricultural Engineering, Jiangsu University (N 32°11′ and E 119°27′). The seeds of *O. violaceus* were placed on wet gauze and germinated in a light incubator with a light intensity of 40 μmol·m^{-2}·s^{-1}. Water was sprayed every day to keep the gauze moist. The seeds were seeded in a 12-hole seedling tray with perlite and exposed to white light. Seedlings were cultivated in the tray with a small amount of 1/4-strength Hoagland solution until the 2 leaf stage. The culture conditions were as follows: photoperiod of 12 h, CO_2 concentration of 390 ± 10 μmol·mol^{-1}, relative humidity of air of 60 ± 5%, day/night cycle temperature of 28 °C/20 °C and light intensity of 280 ± 20 μmol·m^{-2}·s^{-1}.

After 45 days of growth, the leaves of different seedlings were sprayed with 0 (CK), 1 (NS_1), 2 (NS_2), 5 (NS_3) and 10 (NS_4) mmol·L^{-1} of $NaHSO_3$ solutions. The spraying was conducted from 9:00 to 10:00 in the morning. The 50 mL $NaHSO_3$ solution was sprayed on plants in each pot every 5 days, and the seedlings were sprayed 5 cm from the top in all directions. During the treatment period, the leaves of the seedlings were sprayed every 5 days for a total of 5 times, and the experiment was carried out 25 days after the spray treatment.

2.2. Gas Exchange Measurements

The third fully expanded leaves from the top were chosen for the gas exchange measurement at 9:00–12:00 a.m. on a sunny day. A portable LI-6400XT photosynthesis measurement system (LI-COR Inc., Lincoln, NE, USA) was used. The flow rate was set to 500 μmol·s^{-1}, and the leaf temperature was 30 ± 2 °C. The net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular carbon dioxide (C_i), transpiration rate (E) and other photosynthetic parameters were selected from the two response curves under a light intensity of 800 μmol·s^{-1} and a CO_2 concentration of 400 μmol·mol^{-1}. The P_N-PAR response curves were always fitted using the nonrectangular hyperbola equation [19], which is expressed as follows:

$$P_N = \frac{\alpha\, I + A\mathrm{max} - \sqrt{(\alpha\, I + A\mathrm{max})^2 - 4\,\mathrm{k}\,\alpha\, I\, A\mathrm{max}}}{2\mathrm{k}} - R_d \tag{1}$$

where P_N is the net photosynthetic rate (μmol·m^{-2}·s^{-1}); I is the photosynthetically active radiation (μmol·m^{-2}·s^{-1}); α (apparent quantum efficiency) is the initial slope of the P_N-PAR curves (μmol·μmol^{-1}); Amax is the net photosynthetic rate at light saturation (μmol·m^{-2}·s^{-1}); k is the curve representing the degree of curvature of the curve angle, the value of which is [0,1]; and R_d is the dark respiration rate (μmol·m^{-2}·s^{-1}). The atmospheric CO_2 concentration during the measurement was 400 μmol·mol^{-1}. For every measurement, the PAR was set at 800, 600, 400, 300, 250, 200, 150, 100, and 50 μmol·m^{-2}·s^{-1}. After those photosynthetic parameters were acquired, the light saturation point (LSP) and light compensation point (LCP) for the photosynthetic capacity were obtained.

The P_N-C_i response curves were always fitted using the rectangular hyperbola equation [19], which is expressed as follows:

$$P_N = \frac{CE\ \mathrm{Bmax}\ Ci}{CE\ Ci + B\mathrm{max}} - R_t \tag{2}$$

where P_N is the net photosynthetic rate (μmol·m^{-2}·s^{-1}); CE (carboxylation efficiency) is the initial slope of the P_N-PAR curves (mol·m^{-2}·s^{-1}); C_i is the intercellular CO_2 concentration (μmol·mol^{-1}); Bmax is the net photosynthetic rate at CO_2 saturation (μmol·m^{-2}·s^{-1}); and R_t is the total respiratory rate (μmol·m^{-2}·s^{-1}). The photosynthetically active radiation during the measurement was 800 μmol·mol^{-1}. For every measurement, the CO_2 concentration was set at 1500, 1200, 1000, 800, 600, 400, 350, 300, 250, 200, 100, and 50 μmol·mol^{-1}. After those photosynthetic parameters were acquired, the CO_2 saturation point (CSP) and CO_2 compensation point (CCP) for the photosynthetic capacity were obtained.

The plant photorespiration portion was calculated as follows [20]:

$$R_p = R_t - R_d;\ P_t = P_N + R_t;\ \text{and}\ \mathrm{S}_p = R_p/P_t \tag{3}$$

where the definitions of R_d and R_t are the same as those in Formulas (1) and (2); R_p was the photorespiration rate (μmol·m^{-2}·s^{-1}); P_N and P_t are the net photosynthesis rate (μmol·m^{-2}·s^{-1}) and total photosynthetic rate (μmol·m^{-2}·s^{-1}) under specific CO_2 concentrations and light intensities, respectively; and S_p is the photorespiratory portion.

2.3. Chlorophyll-A Fluorescence (ChlF) Measurement

The ChlF parameters were measured on the third fully expanded leaves from the top, which were the same leaves used for gas exchange measurements. Before the measurements, the leaves were dark-adapted for 30 min to ensure complete relaxation of all reaction centers. ChlF under dark adaptation was measured using a modulated chlorophyll fluorescence imaging system (IMAGING-PAM, Heinz Walz Gmbh) from 19:00 to 21:00. The minimum chlorophyll fluorescence (F_o) was determined using a measuring beam, whereas the maximum chlorophyll fluorescence (F_m) was recorded after a 0.8 s saturating light pulse (2800 μmol·m^{-2}·s^{-1}). Actinic light (340 μmol·m^{-2}·s^{-1}) was then applied for 3 min to drive photosynthesis. Maximum fluorescence in the light-saturated stage (F'_m), basic fluorescence after induction (F'_o) and fluorescence yield in the steady state (F_s) were determined. The actual photochemical quantum yield (Y_{II}) was calculated as (F_m'-F)/F_m'. The quantum yield of regulated energy dissipation (Y_{NPQ}) was calculated as 1-Y_{II}-1/(NPQ + 1 + qL($F_m/F_o - 1$)). The quantum yield of nonregulated energy dissipation (Y_{NO}) was calculated as 1/(NPQ + 1 + qL(F_m/F_o-1)). The photochemical quenching coefficient (qP) was calculated as ($F'_m - F_s$)/($F'_m - F'_o$), while the nonphotochemical quenching coefficient (NPQ) was calculated as ($F_m - F'_m$)/F'_m = F_m/$F'_m - 1$. Subsequently, the photosynthetic electron transport rate (ETR) was calculated as PAR × Y_{II} × 0.85 × 0.5, where 0.5 and 0.85 are the fractions of the excitation energy distributed to PSII and the fractional light absorbance, respectively, PAR is the photosynthetically active radiation, and PSII is photosystem II.

2.4. Chlorophyll and Carotene Content

The third fully expanded fresh leaves from the top were picked and immediately ground and extracted with 95% ethanol under dark conditions until the leaves turned white. The absorbance of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotene was measured with a 7230 G spectrophotometer at 665 nm (OD_{665}), 649 nm (OD_{649}) and 470 nm (OD_{470}), respectively. The corresponding chlorophyll concentration was calculated from the measured optical density values, and the chlorophyll content was determined by using the following formula [21].

$$\text{chlorophyll content}\left(\text{mg}\cdot\text{g}^{-1}\ \text{FW}\right) = \frac{C \times V \times A}{W \times 1000} \quad (4)$$

where C is the chlorophyll concentration (mg·L^{-1}); V is the the amount applied for the extraction (mL); A is the dilution ratio; W is the fresh weight of the sample (g).

2.5. Statistical Analysis

All measurements were based on 3 replicate plants. The statistical analysis included a 1-way analysis of variance (ANOVA), and significant differences between the means were tested using Duncan's multiple range test at 95% confidence.

3. Results

3.1. Effects of Foliage Spraying of $NaHSO_3$ on Gas Exchange of O. violaceus

The values of P_N, g_s and E in the NS_3 treatment were significantly higher than those in the CK (Figure 1A,B,D). However, the values of P_N, g_s and E in the NS_1, NS_2, NS_4 and CK treatments showed no significant difference. The values of P_N, g_s and E in the NS_4 treatment were significantly lower than those in the NS_3 treatment (Figure 1A,B).

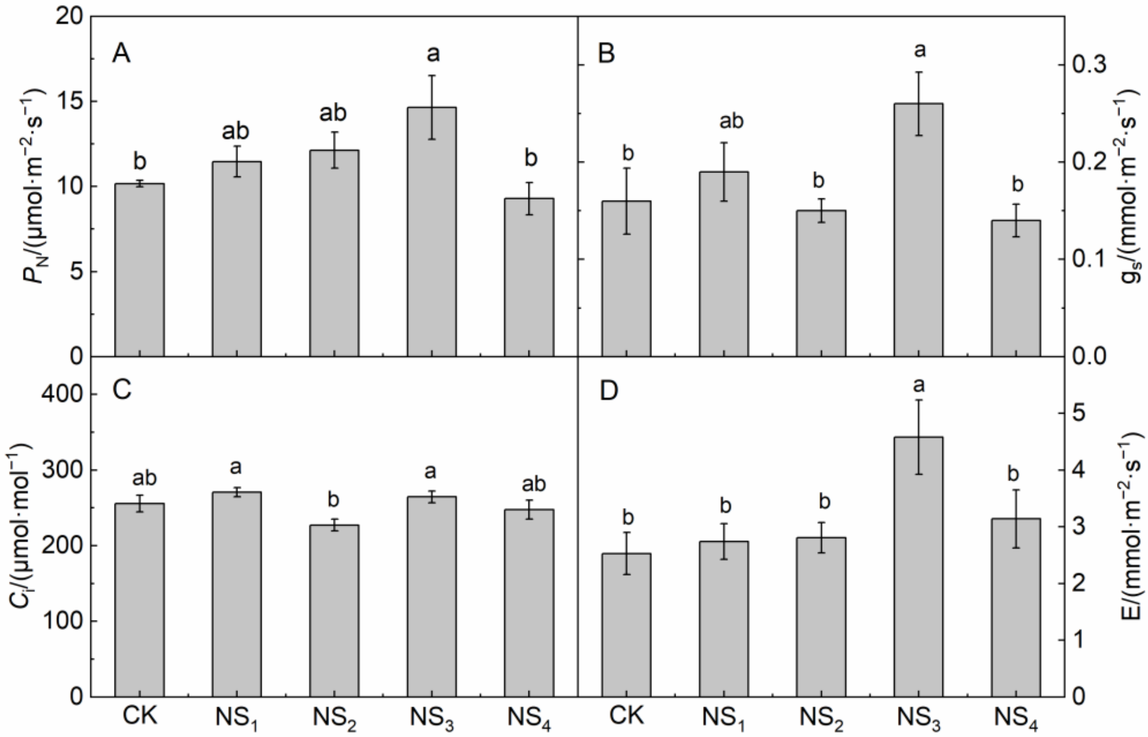

Figure 1. Effects of foliage spraying of $NaHSO_3$ on et photosynthetic rate (P_N) (**A**), stomatal conductance (g_s) (**B**), intercellular carbon dioxide concentration (C_i) (**C**), and transpiration rate(E) (**D**) of *O. violaceus*. Values are the means of five repetitions ± SE. Bars with different letters show significant differences at $p < 0.05$ (Duncan).

3.2. Responses of Net Photosynthetic Rate of O. violaceus to Photosynthetically Active Radiation (PAR) and Intracellular CO_2 Concentration (C_i) under Foliage Spraying of $NaHSO_3$

The correlation coefficients (R^2) of the P_N-PAR curve fitted by the nonrectangular hyperbolic model and the P_N-C_i curve fitted by the rectangular hyperbolic model were all higher than 0.98, which indicated that the two models fit the curves mentioned above well.

Different concentrations of $NaHSO_3$ affected the light response process differently. When the PAR was less than 200 μmol·m^{-2}·s^{-1}, the P_N increased rapidly as the PAR increased, but significant differences were not observed between the values of P_N in different treatments (Figure 2A). When the PAR was greater than 200 μmol·m^{-2}·s^{-1}, the P_N value in the NS_4 treatment increased more slowly than that in the CK as PAR increased. The P_N value was significantly lower than that in the CK when the PAR reached the light saturation point (LSP). The foliar application of 10 mmol·L^{-1} of $NaHSO_3$ decreased the LSP and inhibited the photosynthetic efficiency (Figure 2A). The P_N in the NS_3 treatment exhibited a clearer increase than that in the CK as the PAR increased. The foliar application of 5 mmol·L^{-1} of $NaHSO_3$ promoted the photosynthetic capacity of *O. violaceus* (Figure 2A).

Different concentrations of $NaHSO_3$ affected the CO_2 response process differently (Figure 2B). When the CO_2 concentration was less than 400 μmol·mol^{-1}, the values of P_N clearly increased as the CO_2 concentration increased but slowed down when the CO_2 concentration was greater than 600 μmol·mol^{-1} (Figure 2B). The values of P_N in the NS_2 and NS_3 treatments were higher than those in the CK, and the P_N value in the NS_3 treatment was the highest. The values of P_N in the NS_1 and NS_4 treatments exhibited no significant difference compared to those in the CK under different CO_2 concentrations (Figure 2B). The photosynthetic capacity of *O. violaceus* was the highest under the foliar application of 5 mmol·L^{-1} of $NaHSO_3$, which was the optimal concentration.

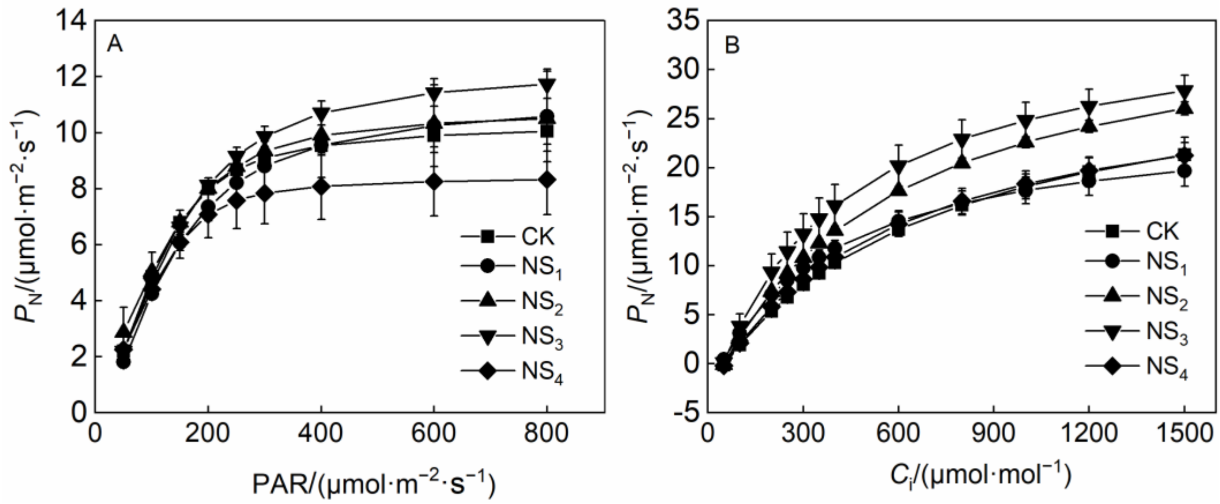

Figure 2. Net photosynthetic rate (P_N) −photosynthetically active radiation (PAR) curve (**A**) and net photosynthetic rate-intercellular carbon dioxide (C_i) curve (**B**) of *O. violaceus* under different concentrations of $NaHSO_3$. Values are the means ± SE. Symbols with different letters show significant differences at $p < 0.05$ (Duncan).

Significant differences were not observed between the values of Amax and Bmax in all treatments (Table 1). The apparent quantum efficiency (α) is an important index that reflects the light energy utilization rate of plants [22]. The light compensation point (LCP) reflects the ability of plants to overcome their own assimilation resistance. The lower the LCP, the less the consumption of photosynthetic products and the stronger the ability to use low light intensity [23]. In this study, the values of α and LCP in the NS_4 treatment decreased by 20.97% and 76.08% of those in the CK, respectively (Table 1). The *O. violaceus* treated with 10 mmol·L^{-1} of $NaHSO_3$ showed improvement in the ability to use weak light and lower consumption of photosynthetic products to resist the stress of high concentrations of sulfite. The initial carboxylation efficiency (CE) can reflect the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the ability of plants to utilize CO_2 [24]. In this study, the value of CE in the NS_3 treatment increased by 105.77% relative to that in the CK, and the values in the other treatments exhibited no significant difference compared to those in the CK (Table 1).

Table 1. Photosynthetic parameters under different concentrations of $NaHSO_3$.

	CK	**NS_1**	**NS_2**	**NS_3**	**NS_4**
Amax	11.33 ± 0.26 a	12.53 ± 2.27 a	11.66 ± 1.46 a	13.06 ± 0.57 a	8.61 ± 1.05 a
α	0.062 ± 0.00 a	0.055 ± 0.01 a	0.058 ± 0.01 b	0.058 ± 0.00 ab	0.049 ± 0.00 b
CE	0.052 ± 0.00 b	0.078 ± 0.00 ab	0.0745 ± 0.00 ab	0.107 ± 0.02 a	0.059 ± 0.01 b
Bmax	34.92 ± 4.03 ab	28.14 ± 2.35 b	40.19 ± 1.28 a	41.02 ± 0.51 a	33.31 ± 1.46 ab
LSP	314.28 ± 8.87 a	370.24 ± 35.24 a	327.22 ± 35.43 a	344.00 ± 7.09 a	287.31 ± 8.75 a
LCP	14.09 ± 0.59 ab	17.78 ± 2.74 a	14.12 ± 1.62 ab	9.88 ± 0.41 b	3.37 ± 0.54 c
CSP	1410.10 ± 75.77 a	919.69 ± 12.95 b	1203.71 ± 27.46 ab	1110.06 ± 249.37 ab	1292.54 ± 109.82 ab
CCP	52.97 ± 7.49 a	42.63 ± 0.23 a	51.64 ± 3.32 a	49.83 ± 8.29 a	54.78 ± 5.08 a

Note: Amax: maximum photosynthetic value of P_N-PAR curve; α: apparent quantum efficiency; CE: initial carboxylation efficiency; Bmax: maximum photosynthetic value of P_N-C_i curve; LSP: light saturation point; LCP: light compensation point; CSP: CO_2 saturation point; CCP: CO_2 compensation point; Values are the means ± SE. Bars with different letters show significant differences at $p < 0.05$ (Duncan).

As the $NaHSO_3$ concentration increased, the values of the total respiratory rate (R_t) initially increased and then decreased in the NS_4 treatment and the value of R_t in the NS_3 treatment increased by 80.63% compared with that in the CK (Figure 3B). The values of the photorespiration rate (R_p) and R_t in each treatment showed the same change trends as follows: $NS_3 > NS_2 > NS_4 > NS_1 >$ CK (Figure 3A,B). The values of the photorespiration rate (R_d) gradually decreased as the $NaHSO_3$ concentration increased, and the value in the NS_4 treatment decreased by 85.56% of that in the CK (Figure 3A). The values of the photorespiratory portion (S_p) gradually increased, and the value in the NS_4 treatment increased by 53.85% of that in the CK (Figure 3A).

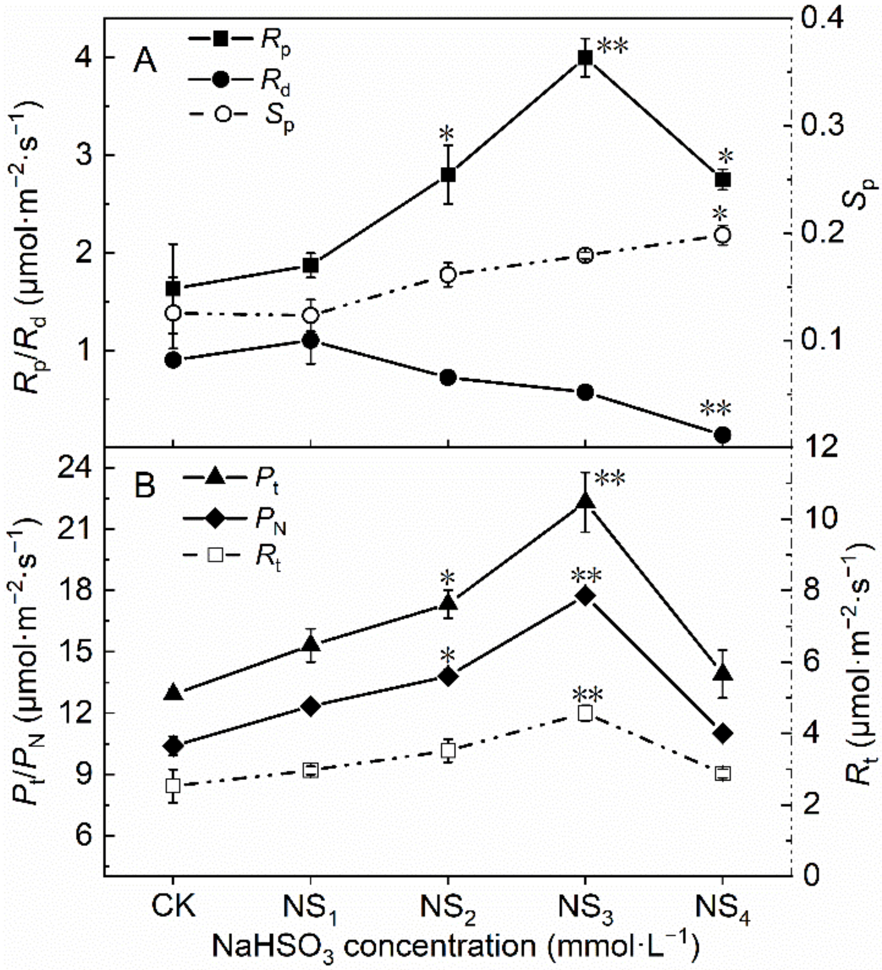

Figure 3. Photorespiration related parameters ((**A**): R_p, R_d and S_p; (**B**):P_t, P_N and R_t) under different concentrations of $NaHSO_3$. Significant differences between the control and treatment groups are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$). R_d: the dark respiration rate; R_p: the photorespiration rate; S_p: the photorespiratory portion; P_N: the net photosynthetic rate; P_t: the total photosynthetic rate; R_t: the total respiratory rate.

3.3. Effects of Foliage Spraying of $NaHSO_3$ on Chlorophyll Content in Leaves of *O. violaceus*

Chlorophyll is a necessary molecule for the photosynthesis of plants. The NS_1, NS_2 and NS_3 treatments promoted the synthesis of chlorophyll a and b in *O. violaceus* (Table 2). The chlorophyll b contents in the NS_4 treatment had no significant difference compared with those in the CK (Table 2). The chlorophyll a, chlorophyll b and total chlorophyll contents in the NS_4 treatment were slightly lower than those in the NS_1, NS_2 and NS_3 treatments (Table 2). The chlorophyll a/b in each treatment had no significant difference compared with that in the CK (Table 2). The $NaHSO_3$ promoted the synthesis of chlorophyll in *O. violaceus* as a synchronous change of chlorophyll a and chlorophyll b. There was no significant difference in carotenoids between the CK and other treatments (Table 2).

Table 2. Effects of foliage spraying of $NaHSO_3$ on chlorophyll content in *O. violaceus* leaves.

Treatment	Chlorophyll a /(mg·g^{-1}FW)	Chlorophyll b /(mg·g^{-1}FW)	Chlorophyll(a + b)/(mg·g^{-1}FW)	Chlorophyll a/b	Carotenoid /(mg·g^{-1}FW)
CK	0.812 ± 0.026 b	0.323 ± 0.019 b	1.135 ± 0.041 b	2.525 ± 0.104 a	0.141 ± 0.008 a
NS_1	1.074 ± 0.046 a	0.445 ± 0.040 a	1.582 ± 0.074 a	2.459 ± 0.182 a	0.173 ± 0.018 a
NS_2	1.099 ± 0.069 a	0.440 ± 0.022 a	1.540 ± 0.120 a	2.526 ± 0.059 a	0.159 ± 0.019 a
NS_3	1.134 ± 0.076 a	0.440 ± 0.048 a	1.574 ± 0.261 a	2.648 ± 0.182 a	0.177 ± 0.014 a
NS_4	1.038 ± 0.049 a	0.394 ± 0.013 ab	1.432 ± 0.122 a	2.632 ± 0.061 a	0.157 ± 0.009 a

Note: Values are the means ± SE. Bars with different letters show significant differences at $p < 0.05$ (Duncan).

3.4. Effect of Foliage Spraying of $NaHSO_3$ on Chlorophyll a Fluorescence Parameters of *O. violaceus* Leaves

The light energy absorbed by the PSII reaction center is mainly distributed into three parts: photochemical pathway (Y_{II}), energy used for photoprotection mechanism (Y_{NPQ}) and other nonphotochemical energy (Y_{NO}) and $Y_{II} + Y_{NPQ} + Y_{NO} = 1$ [25]. With increasing $NaHSO_3$ concentrations, the value of Y_{II} gradually increased. The value in the NS_3 treatment increased by 17.95% of that in the CK, and thereafter, a decreasing trend was observed (Table 3). However, Y_{NPQ} is the opposite of Y_{II} and showed an initial decrease and then an increase. In the NS_3 treatment, Y_{NPQ} decreased by 16.28% relative to that in the CK, although the value of Y_{NO} in each treatment had no significant difference compared with that in the CK (Table 3). Photochemical quenching (qP) and nonphotochemical quenching (NPQ) are two forms of energy dissipation in chloroplasts [26]. qP is the part of light energy used for photochemical electron transfer, which reflects the utilization of light energy to a certain extent, while NPQ is the part where excess light energy is dissipated in the form of heat energy [26]. The values of NPQ in the NS_2 and NS_3 treatments decreased by 26.09% and 17.39% of those in the CK, respectively, while the values of qP in NS_2 and NS_3 increased by 18.57% and 14.29% of those in the CK, respectively (Table 3). The values of NPQ and qP in the NS_4 treatment exhibited no significant difference compared to those in the CK. The reduction in the photochemical reaction in the NS_4 treatment might be due to the excessive $NaHSO_3$ stress on *O. violaceus*, which would offset the appropriate amount of $NaHSO_3$ to promote the photochemical pathway. The apparent photosynthetic electron transport rate (ETR) mainly reflects the electron transport in the PS II reflection center [27]. The value of ETR in the NS_4 treatment decreased by 18.90% of that in the CK, while the values in other treatments showed no significant difference compared with those in the CK.

Table 3. Effects of foliage spraying of $NaHSO_3$ on chlorophyll *a* fluorescence parameters in *O. violaceus* leaves.

Treatment	Y_{II}	Y_{NPQ}	Y_{NO}	NPQ	qP	ETR
CK	0.39 ± 0.015 bc	0.43 ± 0.014 a	0.18 ± 0.021 a	0.46 ± 0.021 a	0.70 ± 0.020 bc	23.49 ± 1.064 ab
NS_1	0.39 ± 0.033 bc	0.43 ± 0.038 a	0.18 ± 0.198 a	0.45 ± 0.057 a	0.72 ± 0.037 abc	21.91 ± 1.844 bc
NS_2	0.45 ± 0.021 ab	0.36 ± 0.017 b	0.19 ± 0.020 a	0.34 ± 0.020 b	0.83 ± 0.061 a	25.31 ± 1.165 ab
NS_3	0.46 ± 0.06 a	0.36 ± 0.01 b	0.18 ± 0.016 a	0.38 ± 0.016 ab	0.80 ± 0.010 ab	26.09 ± 0.337 a
NS_4	0.34 ± 0.008 c	0.47 ± 0.004 a	0.19 ± 0.024 a	0.48 ± 0.024 a	0.63 ± 0.030 c	19.05 ± 0.447 c

Note: Y_{II}: actual photochemical quantum yield; Y_{NPQ}: quantum yield of regulated energy dissipation; Y_{NO}: quantum yield of nonregulated energy dissipation; NPQ: nonphotochemical quenching; ETR: electron transport efficiency; Values are the means ± SE. Bars with different letters show significant differences at $p < 0.05$ (Duncan).

4. Discussion

Sulfur is an essential mineral element for plants, and it is fourth in the list of major plant nutrients after nitrogen, phosphorus and potassium [28]. Higher plants mainly uptake inorganic sulfate from the soil by their roots, and they can also absorb the atmospheric SO_2 and exogenous HSO_3^-, SO_3^- and S^{2-} through leaf stomata. During the process of sulfur metabolism, exogenous sulfur is first converted into the form of sulfate (SO_4^{2-}), which can be absorbed by plants. After activation and reduction, sulfite (SO_3^{2-}) can be produced, which has potential cytotoxicity [29]. Many metabolic pathways of SO_3^{2-}

are observed in plants, and their metabolites are closely related to chlorophyll synthesis. First, SO_3^{2-} is reduced to sulfide (S^{2-}) under the action of sulfite reductase and S^{2-} reacts with acetylserine (OAS) to form cysteine (Cys) [30]. As the precursor of sulfur-containing amino acids, Cys is further synthesized into various sulfur-containing proteins, thus guaranteeing the early synthesis of chlorophyll; then, SO_3^{2-} in chloroplasts could enter the thiolipid reduction pathway to synthesize sulfoquinovosyldiacylglycerol (SQDG) through two consecutive steps. SQDG is a sulfur-containing nonphosphorus glycerolipid that participates in the formation of the granum lamellae of chloroplasts, and its content is positively correlated with the chlorophyll concentration in the process of chloroplast dedifferentiation and regeneration [31]. Although sulfur is not the main component of chlorophyll, it obviously affects the synthesis of chlorophyll. It is noteworthy that the variation of chlorophyll content will directly affect the absorption, transformation and utilization of light energy by plants [32]. Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) catalyzes the first step of the reaction of CO_2 assimilation and photorespiration carbon oxidation in photosynthesis and is considered the main factor controlling the rate of photosynthesis. To ensure its catalytic ability, Rubisco must be activated by Rubisco activase (RCA). Studies have found that $NaHSO_3$ could promote the expression of RCA genes at the transcription and translation levels, thus enhancing the initial activity of Rubisco in plants [33]. RCA activity was sensitive to the ATP/ADP ratio observed in the chloroplast matrix, and the activation of RCA depended on the hydrolysis of ATP and was inhibited by ADP [34]. Wang et al. [35] reported that 1 mmol·L^{-1} of $NaHSO_3$ acted similarly as phenazine methyl sulfate (PMS), a cofactor that catalyzed cyclic photophosphorylation, by promoting photophosphorylation and increasing the ATP supply, thereby maintaining high levels of photosynthesis. Moreover, 5 mmol·L^{-1} of $NaHSO_3$, as a sulfur source absorbed and utilized by plants, may play an active role in *O. violaceus*. On the one hand, the increase in HSO_3^- in the leaves accelerated the metabolism of sulfate, and its metabolites directly or indirectly promoted the increase in chlorophyll content, which was conducive to the absorption of light energy by the treated leaves, which was consistent with the results of Li et al. [36]. Meanwhile, the ratio of light energy to the photochemical pathway and light protection mechanism was adjusted. As a result, more light energy was allocated to the photochemical pathway (Y_{II} increased significantly, while Y_{NPQ} decreased significantly), the light energy utilization rate increased and the final photosynthetic rate increased. On the other hand, $NaHSO_3$ may increase the expression of RCA genes by promoting photophosphorylation and increasing the supply of ATP, thus increasing the initial activity of Rubisco, which can catalyze the two reactions of RuBP carboxylation (photosynthesis) and oxidation (photorespiration) simultaneously, and the photosynthetic rate and photorespiration rate increase synchronously.

Photorespiration is a process in which plants fix oxygen and release CO_2 under light conditions. Photorespiration can alleviate photoinhibition, eliminate toxic intermediate products and provide raw materials for other metabolic activities, and it plays an active role in photosynthesis [37]. Studies have suggested that the activities of RuBP carboxylase and RuBP oxygenase in high-yield genotype wheat were higher than those in low-yield genotype wheat. High photosynthesis and photorespiration intensities are important preconditions for ensuring high wheat yield [38]. The electron transport rate of PSII and ATP production increased when a low concentration of $NaHSO_3$ was sprayed on citrus leaves, which decreased photoinhibition and thereby increased the net photosynthetic rate [12]. Foliage sprayed with an appropriate concentration of $NaHSO_3$ increased the total photosynthetic and photorespiration rates, which was consistent with the results reported in the studies mentioned above. Photorespiration consumed excess light energy and protected the photosynthetic apparatus when the consumption ratio of photorespiration to photosynthate was maintained, which indirectly maintained photosynthesis. However, photorespiration consumes photosynthetic products without producing ATP, and it has also been considered a negative factor in photosynthesis. Stomata are important channels for CO_2 and water exchange between plants and the environment [39]. In this study, the stomatal conductance of *O. violaceus* leaves in the NS_4 treatment decreased, which may be due to the stress caused by higher concentrations of $NaHSO_3$. To respond to the deficiency of water and CO_2 caused by the decrease in stomatal conductance, the gene expression of carbonic anhydrase (CA) in leaves is upregulated, which catalyzes the conversion of intracellular HCO_3^- into H_2O and CO_2 [40]. The contents of chlorophyll a and b, the electron transport rate and the photosynthetic rate of soybean all reportedly increased when the leaves were sprayed with an appropriate concentration of HCO_3^- [41]. This demonstrated that the effect of HCO_3^- on plants was similar to that of HSO_3^- in this study. One possible hypothesis was that competition may occur between HCO_3^- and HSO_3^- in the process of photosynthesis due to their similar structure. Under high-concentration $NaHSO_3$ treatment, excessive HSO_3^- accumulated in the cell sap to compete with HCO_3^- for the active site of CA, thereby hindering the combination of HCO_3^- and CA. Plants could not offset the deficiency of H_2O and CO_2 in their leaves by converting intracellular HCO_3^- when stomatal conductance decreased. The carboxylation of RuBP was inhibited, and the total photosynthetic rate decreased. In addition, *O. violaceus* would suffer from stress when they were sprayed with high concentrations of $NaHSO_3$ (the reason for stress will be explained later). A high photorespiration rate and S_p in plants had a protective effect against photosynthetic apparatus damage in response to stress conditions, while a high proportion of photorespiration would also consume photosynthates and therefore decrease the net photosynthetic rate.

Among sulfites, the valence of sulfur is +4, which is both reducing and oxidizing. Sulfite dissolved in water can not only obtain electrons to form sulfur precipitates but also lose electrons to form sulfates: $SO_3^{2-} + 3H_2O + 4e^- \rightleftharpoons S + 6OH^-$ E = −0.66; $SO_3^{2-} + H_2O - 2e^- \rightleftharpoons SO_4^{2-} + 2H^+$ E = +0.2. Sulfite in plants has both reduction and oxidation properties, and it has dual effects on plant photosynthesis due to its concentration, which is protective or inhibitory. As a nucleophilic substance, sulfite can attack diverse substrates by splitting the disulfide bonds into peptides and cause inactivation of these compounds, which is called sulfitolysis. Sulfitolysis can lead to chlorophyll destruction, photosynthesis suppression, necrotic damage and growth retardation [42]. Therefore, if sulfite accumulates in plants and cannot be metabolized rapidly, it will cause serious damage at the cellular and even the entire plant level [30]. Sulfate oxidase (SO) plays a vital role in relieving this toxicity, and it can serve as a 'safety valve' to detoxify excess amounts of sulfite and protect the cells from sulfitolysis [43]. Wei et al. [44] found that an appropriate amount of $NaHSO_3$ could react with the superoxide anion produced by the PSI receptor of *Chlamydomonas reinhardtii*; as a result, an anaerobic environment was established, hydrogenase (H_2ase) was activated and the hydrogen production capacity was significantly improved. Golan and Whitaker [45] also proved that $NaHSO_3$ could be used as a reducing agent to inhibit the activity of mushroom polyphenol oxidase (PPO), thereby playing a certain role in preventing browning. Therefore, $NaHSO_3$ had a certain degree of reducibility. At appropriate concentrations, it oxidized into sulfate to enter sulfate metabolism, and it detoxified or reacted with active oxygen to reduce the damage of strong oxidizing substances to cells. However, when the concentration of $NaHSO_3$ increased to a certain extent, its oxidation led to adverse impacts on plants. Lüttge et al. [18] indicated that a certain concentration of bisulfite compounds interfered with membrane proteins and lipids, which impaired membrane integrity and inhibited photosynthetic CO_2 fixation and ion transport processes. Lin et al. [46] reported that the active oxygen content in the leaves of rice seedlings increased significantly as the $NaHSO_3$ concentration increased. Chlorophyll *a* fluorescence technology is often used to study photosynthesis under adversity [47,48]. In this study, the ETR and Y_{II} in the NS_4 treatment decreased significantly compared to those in the NS_3 treatment, while the Y_{NPQ} increased. The results demonstrated that the leaves of *O. violaceus* suffered from mild stress when they were sprayed with 10 mmol·L^{-1} of $NaHSO_3$. Excessive HSO_3^- not only had oxidative properties but also induced the production of active oxygen. These strong oxidizing substances attacked the cell biofilm system of plants, injured the photosynthetic apparatus and even a variety of organelles and affected the processes of photosynthetic CO_2 absorption and ion transport, thereby inhibiting the photosynthetic carbon assimilation and reducing the efficiency of photosynthetic electron transport. To avoid further damage to plants caused by excess light energy, plants need to convert part of the captured light energy into heat energy through a heat dissipation mechanism. Physiological activities, such as protein synthesis, nutrient absorption and transport were affected under stress, which reduced the dark respiration rate.

5. Conclusions

The 5 mmol·L^{-1} of $NaHSO_3$ was the appropriate concentration, which promoted the photosynthetic capacity and increased production, while a concentration of 10 mmol·L^{-1} inhibited the photosynthesis and caused pollution of *O. violaceus*. Photorespiration had a certain protective effect on plants that suffered from stress, but an excessive photorespiration portion consumed photosynthates and decreased the net photosynthetic rate. 5 mmol·L^{-1} of $NaHSO_3$ absorbed by plants could be considered a sulfur source. The results helped to better understand the dose effect of $HSO_3{}^-$ on plant photosynthetic physiology, which provided a theoretical basis for the reasonable utilization of $NaHSO_3$ and promotion of agricultural production.

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