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Effects of exogenous NO on the growth and photosynthetic fluorescence characteristics of ryegrass seedlings under B[a]P stress

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Abstract –Benzoapyrene (B[a]P) pollution poses a threat to the environment and the food chain and consequently to human health. However, the alleviation of the harmful effects of B[a]P pollution in perennial ryegrass (*Lolium perenne* L.) by the application of exogenous nitric oxide (NO) has been ignored. Thus, in this paper the effects of exogenous sodium nitroprusside (SNP, a NO donor) on the growth, photosynthetic fluorescence characteristics, and antioxidant enzyme activity of ryegrass exposed to B[a]P stress are investigated. B[a]P stress induced the reduction of the aboveground and belowground dry weights, chlorophyll (*a*, *b*), the total chlorophyll contents, the carotenoid content, the net photosynthetic rate (Pn), the intercellular carbon dioxide concentration (Ci), the water use efficiency (WUE), the photosystem II (PSII) potential activity (Fv/F0), the maximum quantum yield of PSII photochemistry (Fv/Fm), the steady-state fluorescence yield (Fs), and the non-photochemical quenching (qN), while enhancement was recorded in response to the foliar spray of SNP at 200 and 300 μmol L^{-1} under B[a]P stress. Gray correlation and principal component analyses show that 200 μmol L^{-1} of SNP more drastically alleviated the damage caused by B[a]P stress than 300 μmol L^{-1} of SNP. The exogenous NO-mediated alleviation of B[a]P toxicity in ryegrass was associated with preserved photosynthetic characteristics and activation of antioxidant enzymes.

Keywords: NO, B[a]P stress, ryegrass, growth, photosynthesis, chlorophyll fluorescence parameters

Introduction

Benzo(a)pyrene (B[a]P), a typical polycyclic aromatic hydrocarbon (PAH) organic compound commonly found in the natural environment, has been recognized as one of the three major carcinogens by the World Health Organization and is often used as a representative indicator for determining PAHs (Ye et al. 2019). B[a]P has a high octanol-water partition coefficient and high vapor pressure, so it is difficult to degrade in the natural environment and can easily accumulate in the atmosphere, water bodies, and soil and cause serious environmental pollution (Ncube et al. 2017). B[a]P contaminated soil, which is primarily distributed in industrially contaminated sites, such as in Northeast and North China, is a major concern in several regions in China, with the B[a]P content of the soils of industrial areas peaking at over 1500 μg kg^{-1}. The average B[a]P content of the soil in the Yangtze River delta region of China exceeds 200 μg kg^{-1} (Fismes et al. 2002).

Nitric oxide (NO), which is a reactive nitrogen species, is recognized to play a very important signaling role in plants and has been reported to be involved in plant growth processes (Dai et al. 2020) and responses to various environmental stresses, including salinity (Ali et al. 2017), UV light (Yan et al. 2016), water deficit (Silveira et al. 2016), heat (Song et al. 2013), and heavy metals (He et al. 2014). Reportedly, exogenous NO application is involved in various physiological mechanisms that improve plant tolerance to various stresses, including metal toxicity, by increasing the activity of antioxidant enzymes and subsequently reducing the accumulation of reactive oxygen species (ROS) (Nagel et al. 2019). Foliar spray of SNP can enhance metal transporters and reduce As uptake while inducing new adventitious root formation and enhancing antioxidant and defense capacities (Souri et al. 2020), indicating the positive role of exogenous NO in As detoxification. NO also plays a key role in regulating plant stomatal movement and maintaining chlorophyll content under environmental stress. The application of NO synthesis promotes the production of plant carotenoids and enhances photosynthetic capacity by increasing the quantum production of photosystem II (PSII) in stressed plants (Tiwari et al. 2019). However, very

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few studies have focused on the influence of exogenous NO on a series of physiological changes and growth abnormalities in plants under B[a]P toxicity.

Perennial ryegrass (*Lolium perenne* L.) is a commonly used turfgrass species in the urban areas of Central and Western Europe and China and has a very wide scientific value (Ding et al. 2002). Given its high biomass production rates, ryegrass has been selected as a ditch plant for domestic wastewater treatment and plays an important role in nitrogen removal from wastewater (Duan et al. 2017). Ryegrass is also used as a forage crop and exhibits easy germination (Dąbrowski et al. 2015). Recent research confirms ryegrass' potential in phytoextraction for single metal Cd pollution or combined Cd and Zn pollution (Zhang et al. 2019). Ryegrass can be used as a material for the remediation of contaminated soil and is widely used for phytoremediation (Xie et al. 2021). However, little information is available regarding the alleviation of the adverse effects of B[a]P stress in ryegrass through seeding treatment with exogenous NO. Therefore, the present study aims to explore (1) whether exogenous sodium nitroprusside (SNP, an exogenous NO donor) treatment ameliorates B[a]P toxicity in ryegrass and (2) the mechanisms by which NO improves B[a]P tolerance, by investigating the growth parameters, photosynthetic characteristics, chlorophyll fluorescence properties, and antioxidant enzyme activities.

Materials and methods

Plant material and treatment

Seeds of ryegrass were available from Liaoning Fuyou Seed Co. Ltd. in Shenyang, China. SNP and B[a]P were purchased from Sigma-Aldrich. All reagents used in this study were of analytical grade.

Ryegrass seeds of uniform size were selected and surface sterilized with 5% H_2O_2 for 3 min. After soaking in distilled water for 12 h, the seeds of uniform size and shape were pregerminated on a double layer of moist filter paper for 48 h. The germinated seeds were transferred to plastic pots (22 cm in height, 20 cm in diameter) with 2 kg of nutrient soil. The measured soil parameters were as follows: soil pH 6.25, organic matter 20.27 g kg^{-1}, and cation exchange capacity 20.54 cmol kg^{-1}, total nitrogen and total phosphorus 3.66 and 3.79 g kg^{-1}, respectively. The soil was sieved through a 3-mm sieve, and the uncontaminated soil was air-dried and mixed thoroughly with the base fertilizer. The soil pH, organic matter, alkaline soluble nitrogen, available phosphorus, and available potassium after mixing were 5.56, 25.3 g kg^{-1}, 5.37 g kg^{-1}, 6.22 g kg^{-1} and 5.93 g kg^{-1}, respectively. The pots were placed in an experimental field in the Ecological Research Center of Liaoning University in a controlled environment, with an environmental temperature of 29 ± 5 °C/22 ± 3 °C (12 h day/ 12 h night), relative humidity of 62%~76%, and a photosynthetic photon flux density (PPFD) of 625 µmol m^{-2} s^{-1} provided by the LED light source. Appropriate soil moisture was maintained by watering every four days throughout the experimental duration. Excess seedlings were removed, leaving thirty ryegrass seedlings for growth in each pot.

Pre-experiments were conducted to analyze the inhibition rate and growth of ryegrass seedlings at different concentrations of B[a]P applied foliarly, and 30 µmol L^{-1} was identified as the B[a]P stress concentration (data not shown). In this experiment, these treatments were applied via leaf spraying: the control was sprayed with deionized water; B[a]P 30 was sprayed with a solution with 30 µmol L^{-1} B[a]P; SNP 100 + B[a]P 30, SNP 200 + B[a]P 30, SNP 300 + B[a]P 30, and SNP 400 + B[a]P 30 were sprayed with solutions with 30 µmol L^{-1} B[a]P plus 100, 200, 300, and 400 µmol L^{-1} SNP, respectively. When the ryegrass plants reached approximately 15 cm in height, 100 mL of the prepared treatment solution was sprayed uniformly per pot every other day by foliar spraying at 16:00. These treatments were arranged in a randomized complete block design with at least three pots per treatment. The investigated parameters were measured and analyzed when the plants reached approximately 22 cm in height after 14 days of SNP treatments under B[a]P stress.

Growth measurements

Ten seedlings were harvested and divided into roots and leaves. Root length and aboveground plant height of ryegrass seedlings (five plants of each treatment) were measured with a sliding caliper. Then they were rinsed with tap water and distilled water three times, blotted with filter paper to dry the surface water, weighed immediately for belowground and aboveground fresh weight. The fresh sample materials were dried at 85 °C for 60 hours and weighed for aboveground dry weight and belowground dry weight.

Photosynthetic pigment content measurements

Photosynthetic pigments contents were determined using fresh leaves according to the method described by Lichtenthaler (1987). Ryegrass leaves were accurately weighed to 0.5 g, and soaked in extraction solution for 24 h in the dark. The extraction solution was composed of 10 mL of 80% acetone and 5 mL of 95% ethyl alcohol. The absorbance of the extract was recorded at 663, 645 and 470 nm, and the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid were obtained using the equations described by Arnon (1949).

Photosynthetic parameters measurements

Five leaves of similar height and shape were selected from each pot to measure net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular carbon dioxide concentration (Ci), transpiration rate (Tr) and water use efficiency (WUE) measurements were made using a LI-6400XT (LI-COR, USA) portable photosynthesis meter in each application. Measurements were performed from 9:00 to 12:30 in sunny weather conditions. To ensure that the measurements were carried out under approximately ideal photosynthetic conditions, the leaf surface temperature was controlled at 25 °C, the relative humidity was controlled at about 60%, the photosynthetically active radiation (PAR) was set to 1400 mol m^{-2} s^{-1}, the anaerobic conditions were set to T = 27 ± 2 °C, and airborne CO_2 concentration was 430 ± 20 μmol mol^{-1}.

Chlorophyll fluorescence measurements

Five leaves of similar height and shape were selected from each pot to measure chlorophyll fluorescence parameters using a Li-6400 portable photo synthesizer equipped with a pulse-modulated-fluorescent leaf chamber (6400-30, LI-COR Inc., USA). Before fluorometer measurements, plants were dark-adapted for 6 hours with leaf clips. The dark-adapted minimal fluorescence (F_0) and maximal fluorescence (F_m) were measured by applying a saturating actinic pulse of 8000 μmol m^{-2} s^{-1} for 1 s. The variable fluorescence ($F_v = F_m - F_0$), PSII potential activity (F_v/F_0) and maximum quantum yield of PSII photochemistry (F_v / F_m) were calculated from F_m, F_v and F_0. Steady-state fluorescence yield (F_s) was recorded in the light. A saturating actinic pulse of 8000 μmol m^{-2} s^{-1} for 1 s was applied to produce maximum fluorescence yield in the light-adapted state (F'_m). The actual quantum yield of PSII photochemistry (Φ PSII), minimum fluorescence value in the light (F'_0) (Murchie and Lawson 2013), photochemical quenching (qP) and non-photochemical quenching (qN) were calculated as follows (Schreiber et al. 1995):

$F_v/F_0 = (F_m - F_0) / F_0$,

$F_v/F_m = (F_m - F_0) / F_m$,

$\Phi\ PSII = (F'_m - F_s) / F'_m$,

$qP = (F'_m - F_s) / (F'_m - F'_0)$,

$qN = 1 - (F'_m - F'_0) / (F_m - F_0)$.

Determination of antioxidant enzyme activity

Leaf samples (0.6 g) were ground with 10 mL 50 mmol L^{-1} phosphate buffer (pH 7.0). Then the homogenate was centrifuged (13,000 *g*, 20 min), and the supernatant was used to determine antioxidant enzyme activity. SOD activity was defined by measuring the inhibition of nitro blue tetrazolium (NBT) photochemical reduction (Tandy et al. 1989). POD activity was determined by monitoring guaiacol oxidation using the method described by Pinhero et al. (1997). CAT activity was assayed in a reaction mixture containing 50 mmol L^{-1} sodium phosphate buffer (pH 7.0), 0.2 μmol L^{-1} H_2O_2 and a suitable aliquot of enzyme extract (Dai et al. 2020).

Data analysis

All experimental data were expressed as mean ± SD of at least three replicates. All figures were plotted by using Origin PRO 8.5. Statistical significance analysis was performed by Duncan's multiple range test at 0.05 probability level using computer Software SPSS 24.0 (SPSS Inc, Chicago, IL, USA). The value of $P < 0.01$ or $P < 0.05$ represented a very significant difference or remarkable variance, respectively. Significant differences at the $P < 0.05$ level were indicated by different lower-case letters.

As a systemic analysis method, gray correlation degree theory is often used to measure the correlation degree between each factor according to the similar degree or different degree of their development situation. The comprehensive evaluation of evaluated parameters of ryegrass under B[a]P stress after SNP application was carried out according to the equations of grey correlation degree and entropy weight method using SPSS 24.0.

Principal component analysis (PCA) using Origin PRO 8.5 and SPSS 24.0 was performed to further evaluate the responses of growth, photosynthetic characteristics and chlorophyll fluorescence parameters and antioxidant enzymes activities of ryegrass to different SNP treatments under B[a]P stress. The PCA allowed the ordination of the parameters to discover potential groupings within the parameters. Plots were generated using principal components (PC) 1, 2 and 3 as axes. Therefore, PCA can be used to determine the most appropriate exogenous NO concentration which can alleviate B[a]P stress of ryegrass.

Results

Effect of exogenous NO on the growth characters of ryegrass under B[a]P stress

B[a]P stress significantly reduced the underground dry weights ($P < 0.05$) of the ryegrass plants compared with the control (Fig. 1). In contrast, the aboveground fresh weight and the belowground root length significantly increased by 14.16% and 26.17%, respectively, in the B[a]P treatment compared with the control. B[a]P stress did not affect the aboveground dry weight and plant height and the belowground fresh weight. The exogenous application of 200 μmol L^{-1} SNP considerably enhanced the belowground dry and fresh weights, the root length, and the aboveground plant height by 78.67%, 86.07%, 7.78%, and 14.38% compared with those under B[a]P stress, respectively. The foliar application of 200 μmol L^{-1} SNP showed more pronounced results than that of the other three concentrations of SNP treatments on ryegrass plants under B[a]P stress.

Effect of exogenous NO on photosynthetic pigment contents of ryegrass under B[a]P stress

The chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of the ryegrass under 30 μmol L^{-1} B[a]P stress drastically decreased ($P < 0.05$) and the carotenoid content declined but not radically compared with the control (Fig. 2). Compared with the sample under 30 μmol L^{-1} B[a]P stress, the chlorophyll *b* contents of ryegrass under the 100, 200, 300, and 400 μmol L^{-1} SNP treatments were notably boosted by 60.92%, 72.73%, 161.11%, and 110.16%, respectively. Similarly, the carotenoid contents dramatically increased by 71.29%, 91.72%, 94.24%, and 71.16% compared with those under B[a]P stress. The total chlorophyll content also increased following the 200 μmol L^{-1} SNP treatment.

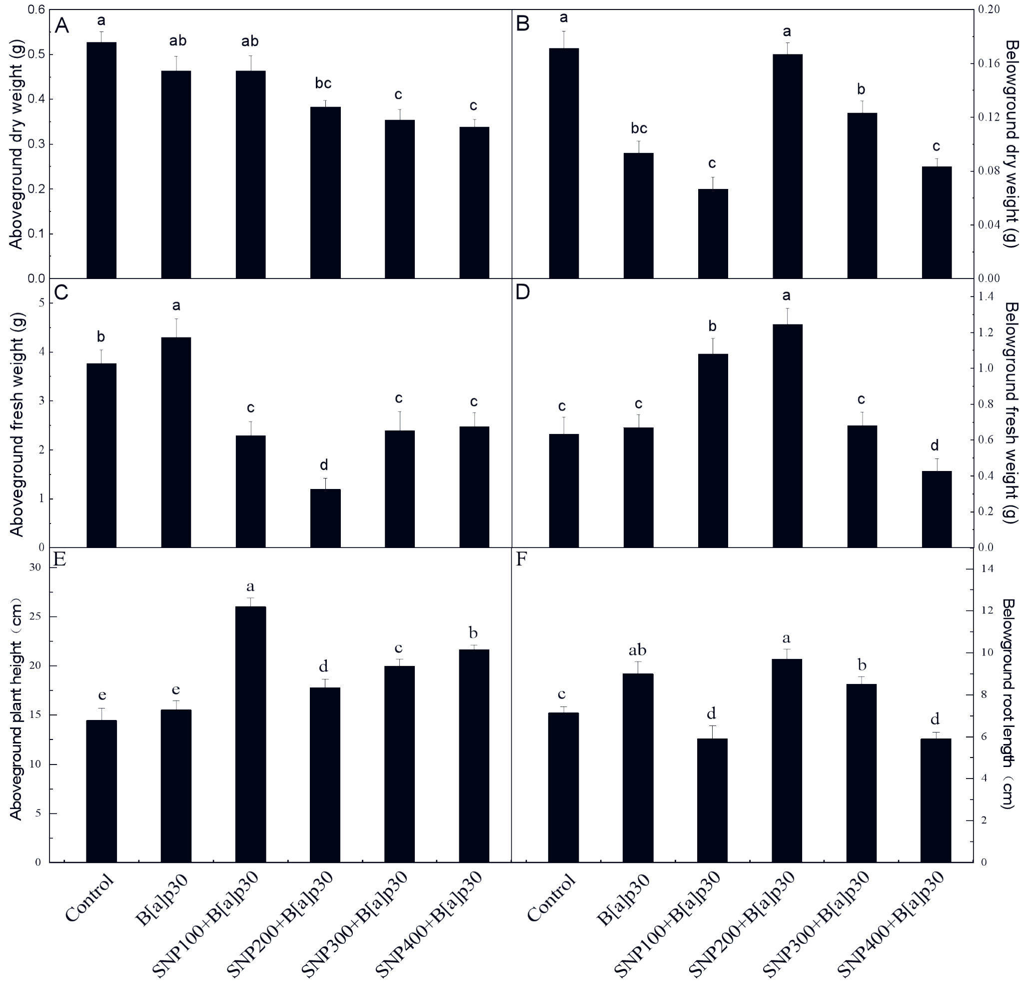

Fig. 1. Effect of different concentrations (100, 200, 300 and 400 μmol L^{-1}) of sodium nitroprusside (SNP), a NO donor, on the biomass of ryegrass seedlings under benzoapyrene (B[a]P) stress. (A) Aboveground dry weight, (B) Belowground dry weight, (C) Aboveground fresh weight, (D) Belowground fresh weight, (E) Aboveground plant height, (F) Belowground root length. Data are mean ± standard deviation, n = 3. Different letters indicate significant differences at P < 0.05.

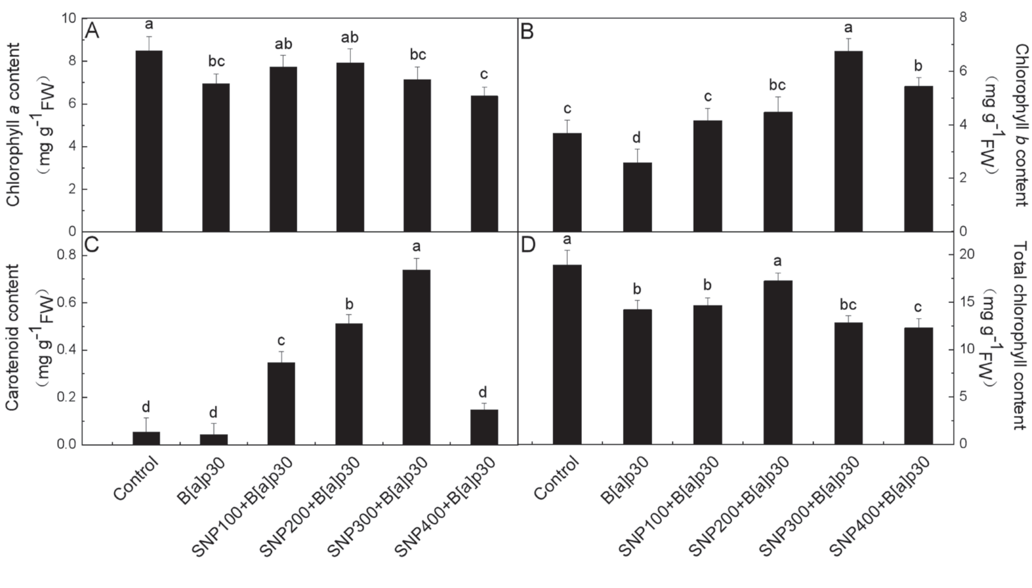

Fig. 2. Effect of different concentrations (100, 200, 300 and 400 μmol L^{-1}) of sodium nitroprusside (SNP), a NO donor, on the photosynthetic pigment content of ryegrass seedlings under benzoapyrene (B[a]P) stress. (A) Chlorophyll *a* content, (B) Chlorophyll *b* content, (C) Carotenoid content, (D) Total chlorophyll content. FW – fresh weight. Data are mean ± standard deviation, n = 3. Different letters indicate significant differences at P < 0.05.

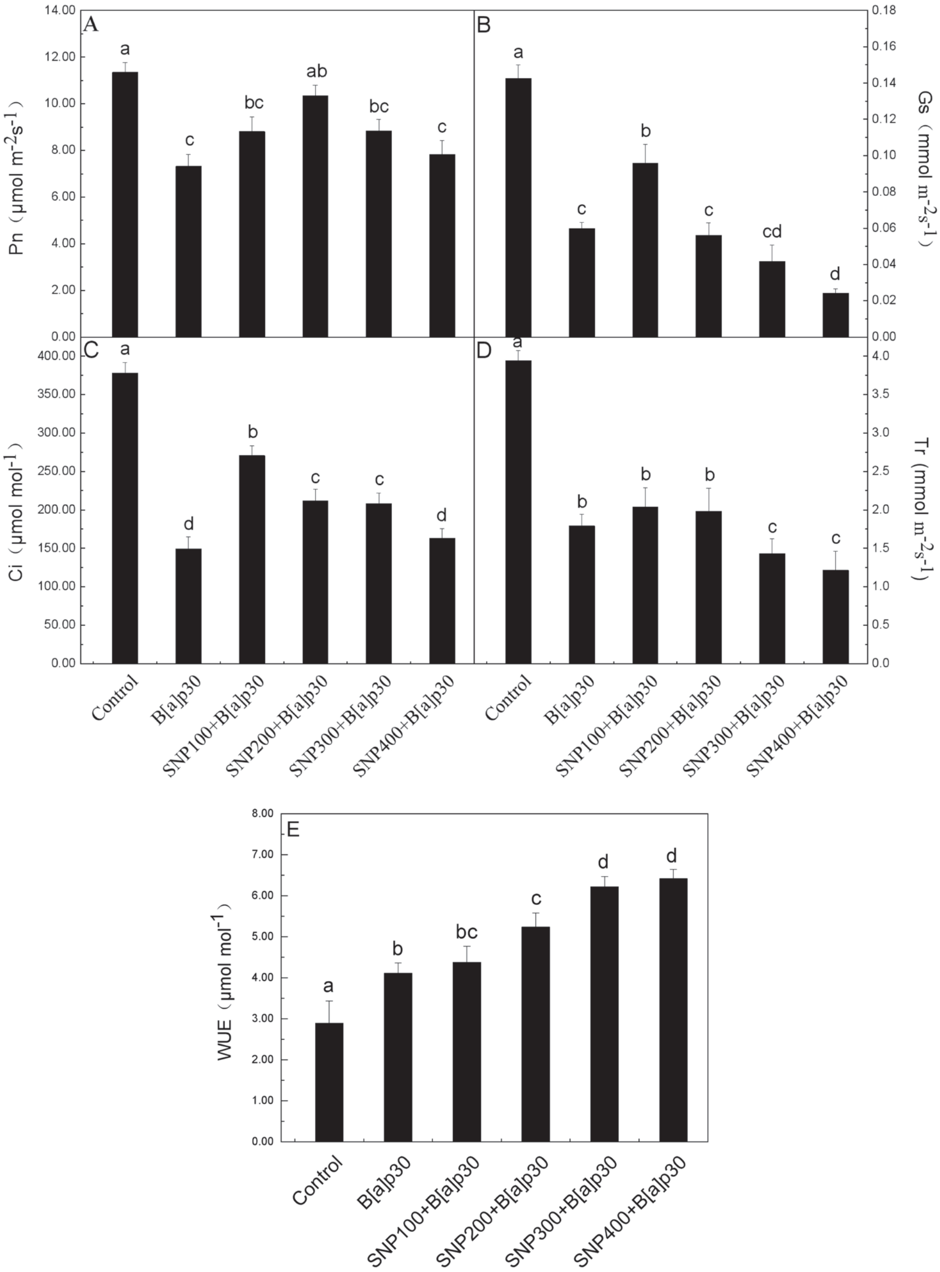

Fig. 3. Effect of different concentrations (100, 200, 300 and 400 μmol L^{-1}) of sodium nitroprusside (SNP), a NO donor, on photosynthetic gas exchange parameters of ryegrass seedlings under benzoapyrene (B[a]P) stress. (A) Net photosynthetic rate (Pn), (B) Stomatal conductance (Gs), (C) Intercellular carbon dioxide concentration (Ci), (D) Transpiration rate (Tr), (E) Water use efficiency (WUE). Data are mean ± standard deviation, n = 3. Different letters indicate significant differences at $P < 0.05$.

Effect of exogenous NO on photosynthetic gas exchange parameters in ryegrass under B[a]P stress

The Pn, Ci, and Tr in ryegrass radically decreased under 30 μmol L^{-1} B[a]P stress compared with the control, but WUE increased considerably by 42.21% (Fig. 3). The exogenous SNP application mostly alleviated the photosynthetic inhibition caused by B[a]P stress. The Pn significantly increased when the concentration of SNP was 200 μmol L^{-1} compared with that in the B[a]P treatment. Under 100 μmol L^{-1} SNP treatment, the Gs and the Ci significantly increased with the B[a]P treatment, indicating that 100 μmol L^{-1} SNP had the best effect in alleviating B[a]P stress on photosynthetic gas exchange parameters.

Effect of exogenous NO on chlorophyll fluorescence parameters of ryegrass under B[a]P stress

Compared with the control, the F_v/F_0 and ΦPSII decreased considerably due to B[a]P stress, whereas the qP and qN increased in ryegrass leaves under B[a]P stress conditions (Fig. 4). The F_v/F_0, Fs, and qN further increased in various degrees under low SNP concentrations compared with the B[a]P stress, and the most significant effects were observed in 200 μmol L^{-1} SNP treatment. After the application of 200 μmol L^{-1} SNP significantly improved the Fs and qN of ryegrass plants under 30 μmol L^{-1} B[a]P stress, the Fs and qN were at their highest, namely, 116.20% and 24.37%, respectively, higher than those under B[a]P treatment.

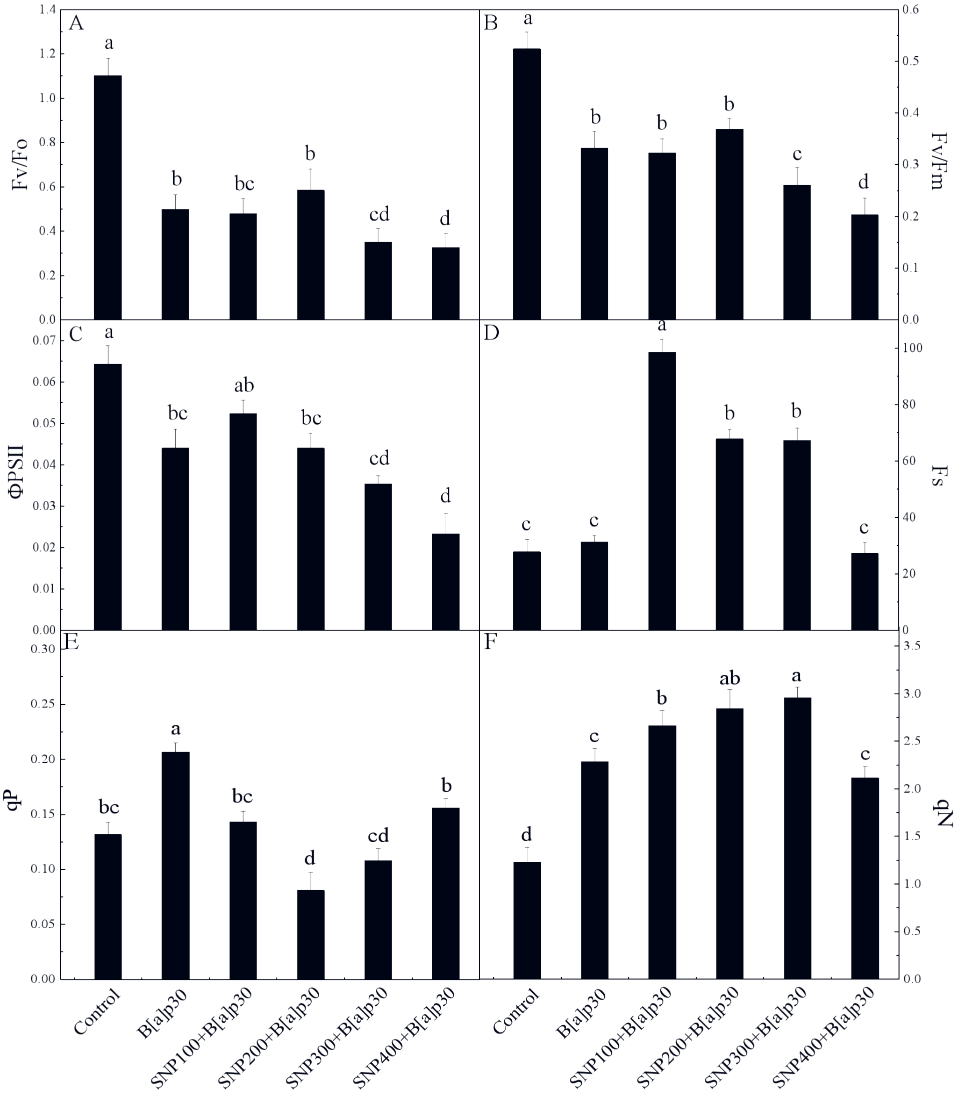

Fig. 4. Effect of different concentrations (100, 200, 300 and 400 µmol L^{-1}) of sodium nitroprusside (SNP), a NO donor, on chlorophyll fluorescence parameters of ryegrass seedlings under benzoapyrene (B[a]P) stress. (A) PSII potential activity (F_v/F_0), (B) PSII maximum light energy conversion efficiency (F_v/F_m), (C) Actual quantum yield of PSII photochemistry (ΦPSII), (D) Steady-state fluorescence (F_s), (E) Photochemical quenching coefficient (qP), (f) Non-photochemical quenching coefficient (qN). Data are mean ± standard deviation, n = 3. Different letters indicate significant differences at P < 0.05.

Effect of exogenous NO on antioxidant enzyme activity of ryegrass under B[a]P stress

The SOD, POD and CAT activities in the presence of 30 µmol L^{-1} B[a]P increased by 6.29%, 56.33% and 26.73%, respectively, compared with those in the untreated control plants (Fig. 5). However, no significant differences were found in the SOD between the 30 µmol L^{-1} B[a]P treatment and the control. Compared with the 30 µmol L^{-1} B[a]P treatment alone, the 100, 200, 300, and 400 µmol L^{-1} SNP treatments increased the SOD, POD and CAT activities. Among them, the 200 µmol L^{-1} SNP supplementation of the 30 µmol L^{-1} B[a]P stress boosted the SOD, POD and CAT activities by 17.85%, 14.74% and 53.58%, respectively. Meanwhile, the application of 300 µmol L^{-1} SNP under B[a]P stress further boosted the SOD, POD and CAT activities by 27.36%, 10.16% and 63.60%, respectively.

Gray correlation analysis and PCA

We analyzed the investigated parameters of ryegrass under B[a]P stress after SNP application using the gray correlation degree (Tab. 1). Our results show drastic differences in the 24 parameters among the different SNP treatments. The associative order of first six parameters is as follows: chlorophyll *a* content > belowground root length > aboveground dry weight > net photosynthetic rate > total chlorophyll content > qP.

The PCA revealed that the first three components with eigenvalues could explain more than 70.4% of the total variation (Fig. 6). PC1, PC2, and PC3 respectively account for 46.2%, 24.2%, and 14.6% of the physiological indexes. The PCA provided a simplified classification of the growth, photosynthesis, fluorescence, and antioxidant enzyme activities of ryegrass for different SNP treatments under B[a]P stress. PC1 tended to separate the effects of B[a]P

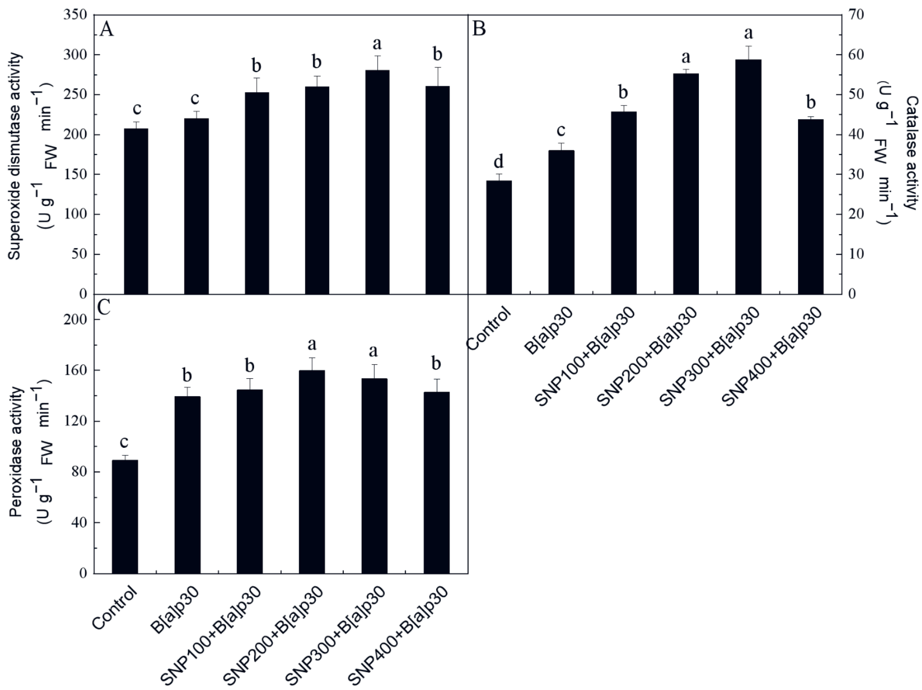

Fig. 5. Effect of different concentrations (100, 200, 300 and 400 μmol L^{-1}) of sodium nitroprusside (SNP), a NO donor, on antioxidant enzyme activity of ryegrass seedlings under benzoapyrene (B[a]P) stress. (A) Superoxide dismutase (SOD) activity. (B) Catalase (CAT) activity. (C) Peroxidase (POD) activity. Data are mean ± standard deviation, n = 3. U – unit of enzyme activity, FW – fresh weight. Different letters indicate significant differences at $P < 0.05$.

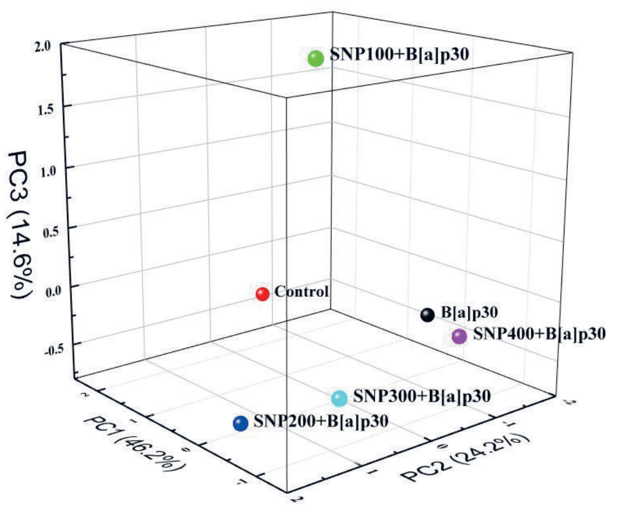

Fig. 6. Principal component analysis (PCA) plots of growth, photosynthetic characteristics and chlorophyll fluorescence parameters and antioxidant enzyme activity of ryegrass exposed to different SNP treatments (100, 200, 00 and 400 μmol L^{-1}) under benzoapyrene B[a]P stress.

stress and different concentrations of SNP, and PC2 further segregated the differences of SNP. According to the results, the application of 200 μmol L^{-1} SNP had a greater alleviative effect on B[a]P stress than the other three concentrations of SNP.

Discussion

B[a]P stress on plants causes reduced growth and photosynthesis, posing a threat to plant life due to metabolic

Tab. 1. Gray correlation degree and correlation sequence of indexes investigated in ryegrass under benzoapyrene stress after application of sodium nitroprusside (SNP) as a NO donor.

Index	Correlation	Associative order
Chlorophyll *a* content	0.981	1
Belowground root length	0.971	2
Aboveground dry weight	0.970	3
Net photosynthetic rate (P_n)	0.970	4
Total chlorophyll content	0.969	5
Photochemical quenching coefficient (qP)	0.965	6
Belowground dry weight	0.954	7
Actual quantum yield of PSII photochemistry (ΦPSII)	0.953	8
Peroxidase (POD) activity	0.953	9
Chlorophyll *b* content	0.953	10
Aboveground fresh weight	0.953	11
Aboveground plant height	0.952	12
Catalase (CAT) activity	0.950	13
Belowground fresh weight	0.949	14
PSII maximum light energy conversion efficiency (F_v/F_m)	0.947	15
Superoxide dismutase (SOD)	0.945	16
Intercellular carbon dioxide concentration (C_i)	0.942	17
Transpiration rate (T_r)	0.931	18
PSII potential activity (F_v/F_0)	0.928	19
Stomatal conductance (Gs)	0.927	20
Water use efficiency (WUE)	0.905	21
Steady-state fluorescence (F_s)	0.887	22
Non-photochemical quenching coefficient (qN)	0.877	23
Carotenoid content	0.675	24

disorders (He et al. 2014). NO, which is a small ubiquitous signaling molecule, plays a vital role in the response to abiotic stress in many plants (Gadelha et al. 2017, Fancy et al. 2017, Li et al. 2018, Wei et al. 2020). In this study, compared with the control, the growth and photosynthesis of ryegrass was inhibited as shown by the decreased aboveground and underground dry weights, and the photosynthetic characteristics. The decrease in ryegrass' growth caused by B[a]P pollution could be attributed to the decrease in the leaf photosynthetic pigment content and the photosynthetic gas exchange parameters. Moreover, B[a]P stress caused a decrease in F_v/F_0, F_v/F_m and ΦPSII suggesting a decline in PSII function, and indicating that photosynthetic units and membrane-bound electron transfer processes were disrupted under this stress.

After SNP application, the chlorophyll *b,* total chlorophyll, and carotenoid contents increased compared with those under B[a]P stress, implying that the release of NO from SNP increased the photosynthetic pigments of ryegrass and maintained a high photosynthetic rate. The protective effect of SNP on photosynthesis had a positive effect on plant growth as shown by the increased plant height, underground dry weight, and fresh weight. This result is consistent with the work of Ahanger et al. (2019) who reported that the exogenous application of NO evidently contributed to the improved growth and photosynthetic parameters of salt-stressed *Vigna angularis.* Moreover, the exogenous application of NO reportedly enhances the carotenoid synthesis, effectively channels additional energy, and increases the quantum production of PSII in stressed plants, resulting in enhanced photosynthesis and growth (Ahmad et al. 2021). In this study, 200 µmol L^{-1} SNP treatment increased F_v/F_0, F_v/F_m, and F_s, implying that SNP alleviated the disruption of PSII caused by 30 µmol L^{-1} B[a]P stress.

The exogenous application of SNP was able to increase the SOD, POD and CAT activities. Ahmad et al. (2018) reported that the observed decline in lipid peroxidation and membrane leakage in NO-treated tomato plants can be attributed to the upregulation of the antioxidant system, which rapidly eliminates ROS, including H_2O_2. Our results show that SNP treatments increased activities of CAT and POD thus alleviating oxidative stress and preventing damage of the photosynthetic apparatus.

Grey correlation analysis is one of the most commonly used multivariable statistical methods (Xiao et al. 2021). The gray correlation analysis enabled the combined evaluation value of 24 measured indicators, thus avoiding the limitation of using individual trait indicators to describe the response of ryegrass. According to the comparison of gray correlation, the chlorophyll *a* content, the underground root length, and the above ground dry weight have the highest correlation with resistance to B[a]P stress in ryegrass, which can be used as the analysis index for reflecting the response effect of ryegrass to stress. The results show that the comprehensive evaluation of ryegrass indicators using gray system theory is practical and feasible. The PCA showed that the application of 200 µmol L^{-1} SNP had an obvious alleviating effect on ryegrass' growth and physiological properties under 30 µmol L^{-1} B[a]P stress. NO could exert protective effects by increasing the plant tolerance to stress conditions, but high concentrations of NO may be toxic to plants due to their high reactivity (Reda et al. 2018).

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