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Effect of the Sodium Nitroprusside Inducer on the Concentration of Nitric Oxide and Photosynthetic Pigments Under Stress Conditions

Abstract

Drought and salinity are major global challenges affecting crop productivity, including in Azerbaijan, where grain production is a vital sector. Sodium nitroprusside (SNP), a nitric oxide donor, has shown promise in enhancing plant stress tolerance, but its effects depend on concentration and stress type. This study investigates the impact of SNP on nitric oxide (NO) levels and photosynthetic pigments in the local durum wheat variety *Triticum durum* Desf. Revan under salinity and drought stress.

Wheat seeds were germinated under controlled laboratory conditions and exposed to 0.1 mM and 0.2 mM SNP treatments. Salinity and drought stress were simulated using sodium chloride (NaCl) and polyethylene glycol (PEG-6000), respectively.

Results showed that 0.1 mM SNP significantly enhanced chlorophyll content and NO levels under salinity stress compared to 0.2 mM, indicating a concentration-dependent response.

However, under drought stress, neither concentration of SNP led to significant improvements in NO accumulation or pigment content.

These findings suggest that low-concentration SNP treatment may enhance salinity tolerance in wheat by modulating NO levels and maintaining photosynthetic activity, while its effect under drought conditions appears limited.

Keywords: *wheat, sodium nitroprusside, abiotic stress, salinity, photosynthetic pigments*

Introduction

Wheat cultivation is a historically and economically significant part of industry in Azerbaijan. The wheat plant (*Triticum aestivum*) is one of the most important food crops worldwide. Among cereal grains, wheat has the highest protein content. More than 20% of the daily calorie intake of the world's population is supplied through wheat and wheat-based products (Aliyev et al., 2020).

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As a result of efforts to develop more productive wheat varieties with higher nutritional value, the genetic diversity of cultivated forms has decreased, making them more susceptible to pests, environmental stress, and various diseases (Baloch et al., 2017). Therefore, the use of chemicals to enhance resistance to biotic and abiotic stress factors, as well as their study, is necessary.

Salinity negatively affects seed germination, seedling growth, plant height, yield, and grain weight (Turki et al., 2012).

Exposure of wheat plants to osmotic stress can occur at all stages of development and may lead to cellular damage. The intensity and duration of osmotic stress factors determine the extent of cellular damage during wheat growth (Sarto et al., 2017), which in turn affects growth and development processes, often resulting in reduced yield (Chavoushi et al., 2020).

Research

Osmotic stress caused by drought and ion toxicity can accelerate premature leaf aging, leading to a reduction in chlorophyll content and, consequently, a decline in photosynthetic activity. At the cellular level, high salinity disrupts selective ion uptake due to low water availability, thereby affecting the accessibility of essential nutrients (Trukhachev et al., 2022). The key factor in tolerance to high salt stress is the differential distribution of K^+ and Na^+ ions. One of the most common responses to abiotic stresses is the increase in free cytosolic calcium (Ca^{2+}) levels. This suggests that ion channels could act as mechanosensors (Knight et.al, 2001).

The study of osmotic stress signaling has primarily been conducted on *Arabidopsis*, which served as a foundation for studying stress signals in wheat. During osmotic stress, signal transduction is regulated by both ABA-dependent and ABA-independent pathways. The initial signals of abiotic stresses are transmitted through an increase in reactive oxygen species (ROS) levels. These include singlet oxygen ($^1O_2$), superoxide anion radicals ($O_2^{-}\cdot$), hydroxyl radicals ($HO\cdot$), hydrogen peroxide (H_2O_2) (Reczek et al., 2015).

Studies have shown that the application of sodium nitroprusside (SNP) (200 μM) plays a protective role in preventing oxidative damage caused by salt stress in wheat (*Triticum aestivum* L.) plants (Zhang et al., 2023).

Sodium nitroprusside and their effects on the concentration of nitric oxide and photosynthetic pigments (e.g. chlorophyll) in plants under stress conditions are of great importance. High levels of NO can promote chlorophyll synthesis in plants, which can help increase photosynthesis efficiency. At the same time, NO helps maintain the stability of the photosynthetic system by preventing oxidative stress. By using nitric oxide inducers (such as sodium nitroprusside - SNP), NO production in plants can be increased under various stress conditions. As NO levels rise, the chlorophyll content in plants increases, and photosynthesis efficiency may improve. Nitric oxide can help plants adapt better by regulating their biological response to stress, as well as strengthening their antioxidant system, reducing oxidative damage, and minimizing the loss of photosynthetic pigments (Fancy et al., 2017).

Understanding how photosynthetic pigments, which play a key role in photosynthesis and form the main structure of the photosystem change under normal and stress conditions is of great interest. Therefore, studying the variations in chlorophyll a and chlorophyll b levels in plants under stress conditions is crucial (Trukhachev et al., 2022).

The aim of the research is to demonstrate the effect of sodium nitroprusside as an inducer on the concentration of nitric oxide and photosynthetic pigments in durum wheat under drought and salinity conditions.

Materials and methods

The experiments were carried out on *Triticum durum* Desf. Revan- wheat, genotype which was obtained from the Azerbaijan Research Institute of Crop Husbandry, Ministry of Agriculture. The

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seeds of wheat were pre-treated with 0.2% potassium permanganate for 5 minutes, then, they were planted in 7 cm plastic cups containing neutral substrate (perlite, coco peat). Seeds were sown at a depth of 2-3 cm. The seedlings were grown in a phytotron (Taisite, GZX-300 E) at temperatures of 22–24°C, humidity of 65–75%, and light intensity of 4800 lux. The light cycle was 16/8 hours day/night. As a nutrient medium Steiner's solution was used with the addition of different concentrations of NaCl, PEG-6000 and SNP during the entire growth period, starting from the first days of seedling formation (Fig. 1). Two-week-old fresh leaves of wheat were used for analyses.

Substances	First experiment	Second experiment
SNP	0.2 mM	0.1 mM
PEG-6000	3%	10%
NaCl	100 mM	100 mM
Neutral substrate	Perlite	Cocobit

Table 1. Structure of experiments

The Steiner solution was prepared by adding 100 mL of macroelement-containing Component A, 100 mL of macroelement-containing Component B, 10 mL of microelement-containing Component C, and 10 mL of microelement-containing Component D to 5 liters of distilled water, then total solution was reached to 10 liters. The pH of the solution was then measured and maintained within the range of 5.8–6.3. To achieve this, dilute HNO_3 (nitric acid) or KOH (potassium hydroxide) solutions were used for pH adjustment (Steiner et al., 1961).

To investigate the effects of SNP under drought and salinity stress, six main combinations were formulated using Steiner solution. The media were prepared under controlled conditions to ensure consistency and reproducibility. These combinations served as the experimental treatments for evaluating plant responses to stress conditions.

After preparing the solutions, wheat seeds were treated with them for two weeks, with irrigation occurring 2–3 times per week. The application was conducted under controlled conditions to ensure consistent exposure to the treatment. Morphological analyses of both root and shoot, as well as some biochemical analyses of the shoot of durum wheat, were started on the 14th day of wheat seedling growth.

Determination of chlorophyll a, b, and carotenoid content: The quantification of photosynthetic pigments was performed using a modified method based on Lichtenthaler (Lichtenthaler, 1987). Approximately 0.1 g of fresh green leaf tissue was ground with 5 mL of 96% ethanol to extract chlorophylls and carotenoids. To accelerate the disruption process, quartz sand (SiO_2) was used, while chalk powder ($CaCO_3$) was added to precipitate phenolic compounds. The homogenate was filtered through filter paper into volumetric flasks with a final volume of 25 ml. Any remaining pigments on the filter paper and flask walls were washed with additional ethanol to ensure complete extraction, bringing the final extract volume to 25 ml.

The absorbance of the extract was measured at 440.5 nm, 644 nm, and 662 nm using a Genesys 20 spectrophotometer (Thermo Scientific, Madison, WI, ABS).

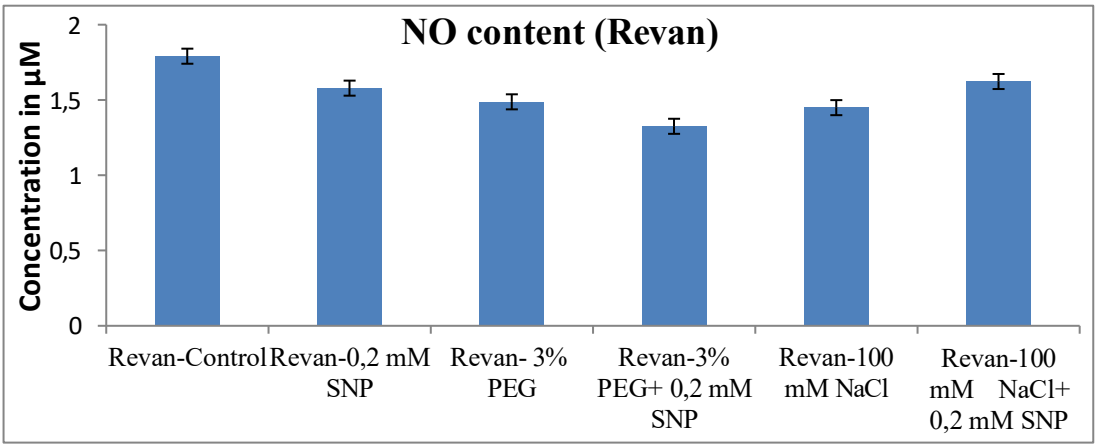
Determination of NO content: The determination of nitric oxide (NO) content in the roots and leaves of wheat plants (both control and experimental variants) was performed using a modified method based on Zhou et al (Zhou et al., 2010). Nitric oxide emissions from rice-wheat rotation fields in eastern China: effect of fertilization, soil water content, and crop residue. *Plant and soil*, *336*, 87-98. This method relies on the conversion of endogenous NO to nitrite and the subsequent quantification of nitrite using the Griess reaction (Probes, 2003). In this reaction, sulfanilic acid reacts with nitrite in the presence of phosphoric acid, forming a diazonium salt. The resulting

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diazonium salt then reacts with N-(1-naphthyl) ethylenediamine to produce an azo dye, which is quantified at a wavelength of 548 nm (Karpets et al., 2015). For the construction of a calibration curve, sodium nitrite solutions of varying concentrations were used.

Statistical analysis: Subsequently, the shoot length and root length were measured, followed by statistical analysis (unit: cm).

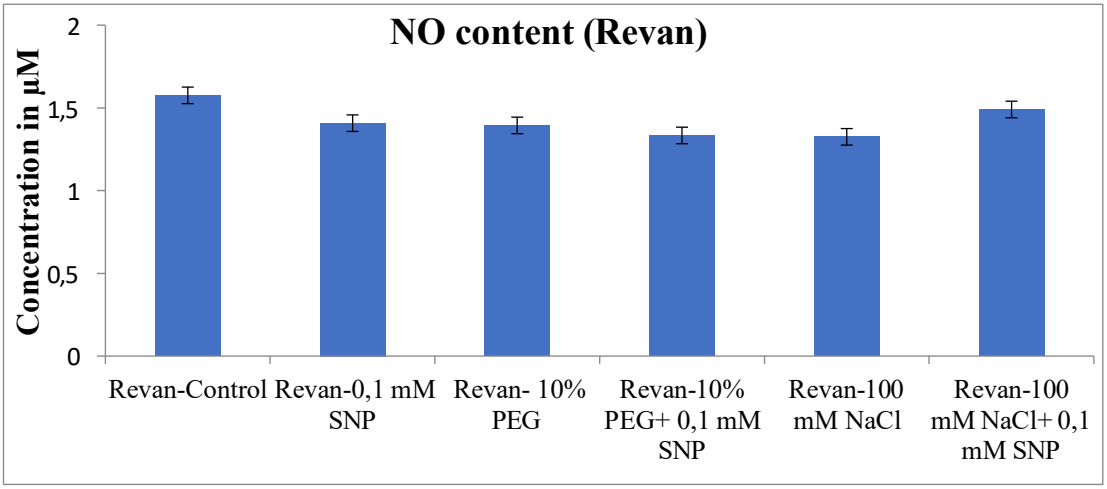
Table 4 shows how nitric oxide (NO) levels change in Revan under different treatments. The control group had the highest NO concentration (about 1.8 µM). When 0.2 mM sodium nitroprusside was added, NO levels slightly decreased. Plants treated with 3% polyethylene glycol (PEG), which causes water stress, had lower NO levels than the control. The combination of 3% PEG and 0.2 mM SNP resulted in the lowest NO concentration, suggesting that water stress affects



NO production. Under salt stress (100 mM NaCl), NO levels were lower than in the control but higher than in PEG-treated plants. Adding 0.2 mM SNP to salt-treated plants increased NO levels, showing that extra NO might help plants cope with salt stress.

Table 2. Levels of NO content in the shoot under the effect of 0.2 mM SNP

Table 2 illustrates nitric oxide (NO) concentration in Revan under different treatments. The control group exhibited the highest NO level (~1.6 µM). Treatment with 0.1 mM sodium nitroprusside (SNP) led to a slight decrease in NO levels compared to the control. Plants subjected to 10% polyethylene glycol (PEG), which induces drought stress, showed a further reduction in NO concentration. The combination of 10% PEG and 0.1 mM SNP resulted in a similar NO level, indicating that PEG stress significantly affects NO accumulation. Under salt stress (100 mM NaCl), NO levels were slightly lower than the control but remained higher than in PEG-treated plants. Notably, when 0.1 mM SNP was added to salt-treated plants, NO levels increased, suggesting that SNP application can enhance NO accumulation under salt stress. These findings indicate that NO levels are affected by both drought and salt stress, with drought stress leading to a greater reduction. The addition of SNP slightly affects NO concentration, potentially influencing plant stress responses.



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Table 3. Levels of NO content in the shoot under the effect of 0.1 mM SNP

A comparison of 0.1 mM and 0.2 mM SNP treatments indicates that a higher SNP concentration (0.2 mM) is more effective in modulating NO levels in *Triticum durum* Desf. Revan. Under stress conditions, SNP application generally enhances NO accumulation more significantly in salinity stress than in drought stress. The data suggest that NO metabolism in *T. durum* Desf. Revan responds more favorably to SNP under salinity stress, potentially due to differences in stress signaling pathways. Overall, these findings highlight the concentration-dependent role of SNP in regulating NO levels and suggest its greater effectiveness in relieving salinity stress compared to drought stress.

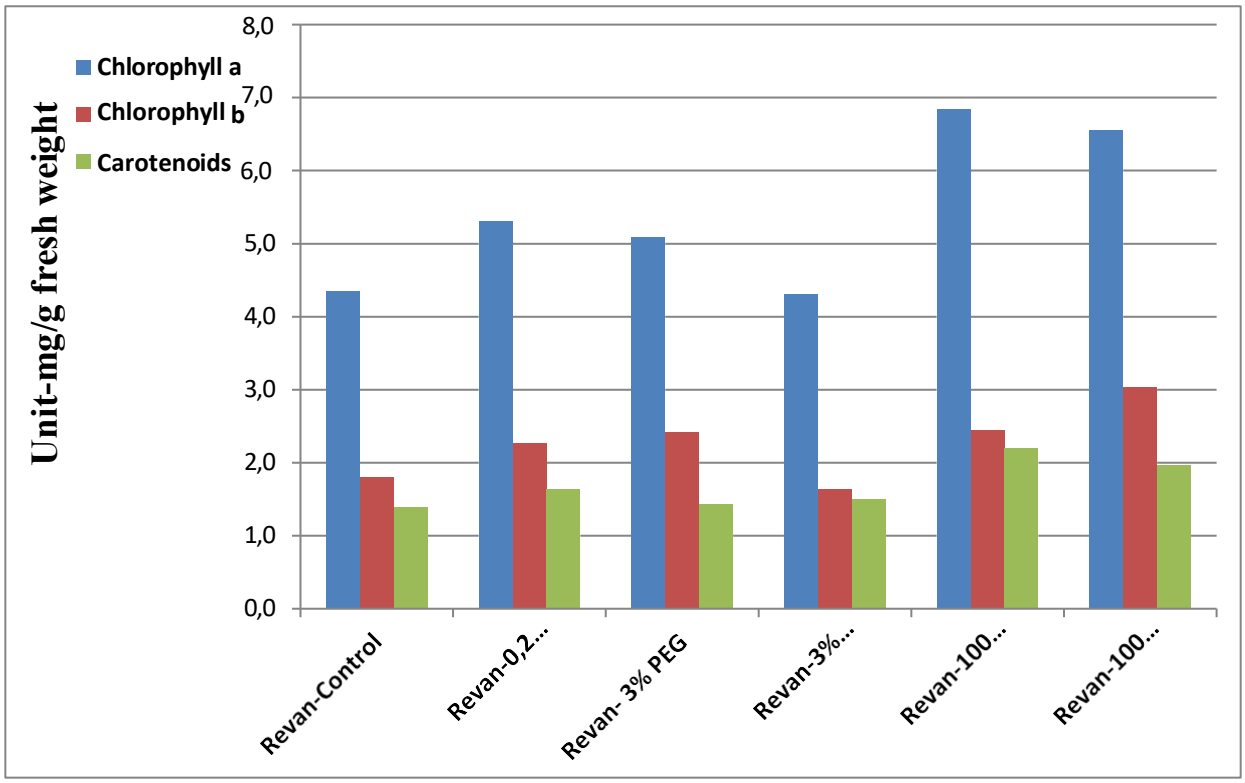


Table 4. Effect of 0.2 mM SNP to the concentrations of chlorophyll a,b and carotenoids. ofn photosynthetic system of T.durum Desf. Revan under drought and salinity stress

The control group shows moderate levels of chlorophyll a, chlorophyll b, and carotenoids. Revan-0.2 mM SNP exhibits a slight increase in chlorophyll a and chlorophyll b, while carotenoids remain relatively unchanged. In the Revan-3% PEG treatment, chlorophyll a and carotenoid levels are slightly in comparison to those in the SNP treated plants, but chlorophyll b decrease slightly, suggesting PEG-induced stress. Revan-3% PEG + 0.2 mM SNP leads to a noticeable reduction in chlorophyll a, b and carotenoids, indicating that SNP might not fully relieve PEG-induced stress. Revan-100 mM NaCl results in the highest chlorophyll a levels, suggesting that salinity stress may enhance pigment production in Revan. Despite the increase in chlorophyll a, chlorophyll b and carotenoids remain relatively stable under 100 mM NaCl, indicating a selective response to salt stress. Revan-100 mM NaCl + 0.2 mM SNP also shows high chlorophyll a levels, though slightly lower than the NaCl-only treatment. Chlorophyll b increases in the Revan-100 mM NaCl + 0.2 mM SNP treatment compared to NaCl alone, suggesting that SNP decreases some salt stress effects. Carotenoid levels in Revan-100 mM NaCl + 0.2 mM SNP are slightly higher than those in NaCl alone, indicating an improved antioxidant response. Comparing 3% PEG and 100 mM NaCl

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treatments, it is evident that salinity stress enhances chlorophyll a, whereas PEG-induced drought stress reduces it.

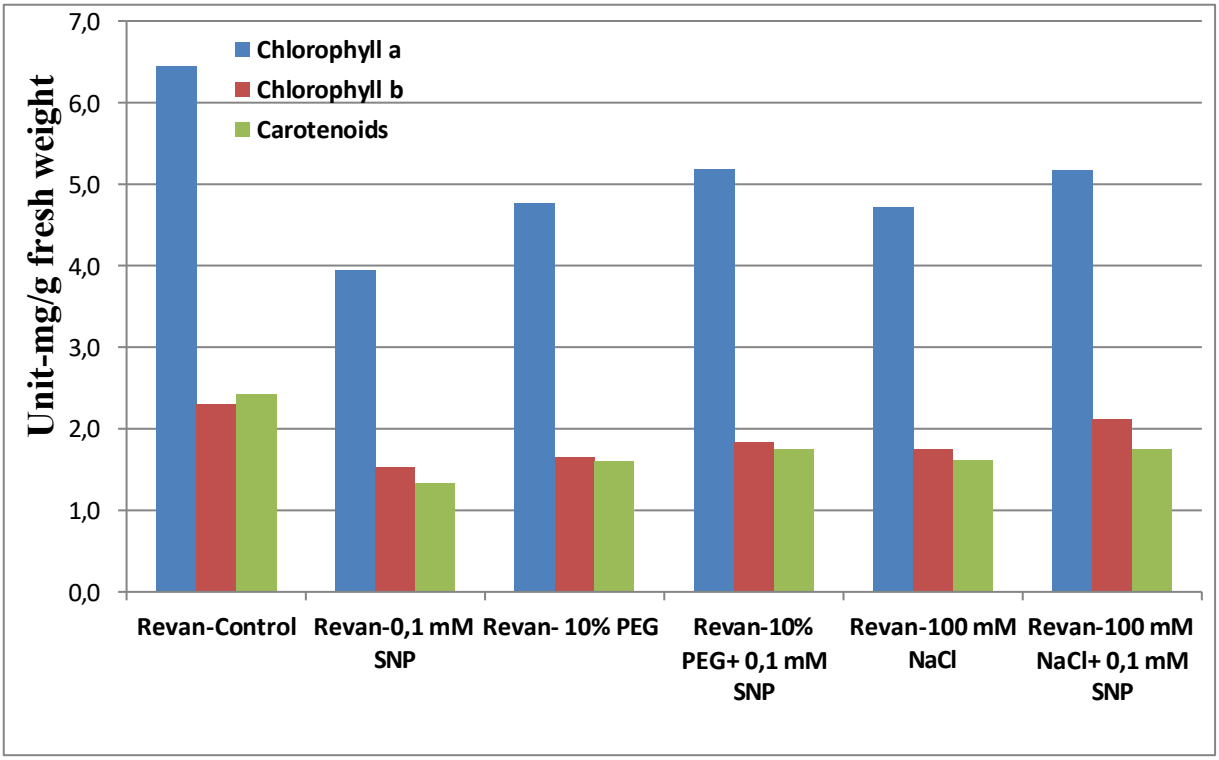


Table 5. Effect of 0.1 mM SNP to the concentrations of chlorophyll a,b and carotenoids. ofn photosynthetic system of T.durum Desf. Revan under drought and salinity stress

The control group has the highest chlorophyll a, chlorophyll b, and carotenoids levels. Revan-0.1 mM SNP shows a decline in chlorophyll a compared to the control, suggesting that SNP at this concentration might have a slight stress-inducing effect. In the Revan-10% PEG treatment, chlorophyll a decreases further compared to the SNP-only treatment, while chlorophyll b and carotenoids remain relatively unchanged, indicating PEG-induced drought stress. Revan-10% PEG + 0.1 mM SNP results in a higher chlorophyll a content than PEG alone. Chlorophyll b and carotenoids also show slight increases in the PEG + SNP treatment compared to PEG alone, supporting the idea of a protective SNP effect. Revan-100 mM NaCl has similar levels of chlorophyll a, b and carotenoid, similar to PEG stress. The Revan-100 mM NaCl + 0.1 mM SNP treatment results in higher chlorophyll a levels than NaCl alone, indicating that SNP has a positive role in maintaining pigment stability under salinity stress. Chlorophyll b and carotenoid content are also slightly elevated in the NaCl + SNP condition, suggesting improved antioxidant potential (Qu, Tian, Zhou, Li, Zhou, Wang, Dong, 2023).

Discussion

Several studies have shown that application of exogenous NO significantly influences biomass accumulation, growth, and yield irrespective of salinity stress. Exogenous NO alleviates salt-induced oxidative damage and improves plant growth and yield potential by regulating osmotic balance, mineral homeostasis, photosynthetic machinery, the metabolism of reactive oxygen species, and the antioxidant defense mechanism (Tahjib-Ul-Arif et al., 2022). NO as a free radical can directly alter proteins, enzyme activities, gene transcription, and post-translational modifications that benefit functional recovery from drought (Santisree et al., 2015). Different

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experiments under drought and salinity stress reveal positive effect on exogenous es application of NO donors, SNP (Cai et al., 2015; Bhardwaj et al., 2021)

Our results also prove that SNP can fight against drought and salinity stress. But still these findings highlight the concentration-dependent role of SNP in regulating NO levels and its greater effectiveness in alleviating salinity stress compared to drought stress. Different studies report varying results: Maslennikova et al. found a decrease in NO content in wheat under salinity stress with 200 µM SNP, while Qu et al. observed increased NO content in soybean under drought stress with rising SNP concentrations. These results are opposite of ours, suggesting genotype-specific responses to SNP and stress itself (Maslennikova, Knyazeva, Vershinina, Titenkov, Lastochkina, 2023).

Green algae and higher plants utilize chlorophyll a and b and a variety of carotenoids to capture light for photosynthesis. Other pigments utilized by photosynthetic organisms, such as chlorophyll c, fucoxanthin, and phycobilins, absorb light in all regions of the visible spectrum. Thus, chlorophylls are light collectors, whereas carotenoids, apart from participating in light harvesting, are also involved in photoprotection. Within the first group, Chl a is present in the reaction centers and the antennae of both photosystems, whereas the presence of Chl b is restricted to light harvesting systems. Among carotenoids, b-Car is almost exclusively located in the core complexes of both photosystems, where it plays an essential role as a quencher of Chl triplets and singlet oxygen. In the literature, application of SNP improves chlorophyll content. Without a stressor, the SNP treatment delayed chlorophyll degradation, thus color was maintained, and shelf-life was extended in broccoli. Relative to the untreated control, the SNP treatment suppressed the activity of the chlorophyll-degrading enzymes, such as chlorophyllase, chlorophyll degrading peroxidase (Shi et al., 2016). Under drought stress, different plants gave signs of improvement by the application of SNP. For instance, Farouk at al. found that SNP application increased chlorophyll, carotenoid, pheophytin a (primary electron acceptor in photosystem II), Chl a and Chl b in marjoram herb (Farouk et al., 2021). Or, the SNP application to the drought-stressed plants increased the Chl b about 3.5 times and had no significant impact on Chl a and carotenoids compared with the drought stressed safflower plants, which is similar to our results (Chavoushi et al., 2020).

Conclusion

Our study investigates the effects of sodium nitroprusside (SNP) on *Triticum durum* to relieve the effect of both drought and salinity stress. As a result of our research, a corresponding increase in the germination rate was observed with the application of sodium nitroprusside. We observed that a 0.1 mM SNP treatment significantly improves chlorophyll content in wheat, showing better results compared to 0.2 mM SNP. Furthermore, the application of SNP under salinity stress leads to an increase in nitric oxide content, highlighting its potential in enhancing stress tolerance. However, when applied under drought stress conditions, the same SNP concentrations did not show any significant improvement in NO levels or chlorophyll content.

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