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Effect of Sodium nitroprusside (SNP) on growth and physiological parameters in wheat (*Triticum aestivum* L.) seedlings

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Abstract
The present study was carried out on wheat (*Triticum aestivum* L.) seedling to determine the exogenous effect sodium nitroprusside (SNP) on their growth and antioxidative defence system. Nitric oxide (NO) is a very active molecule involved in many biological pathways where it has proved to be protective against oxidative stress conditions. For this investigation, PBW 343 seeds of wheat genotype were treated with varying concentrations of SNP (0, 25, 50, 100, 200, 300, 500 µM) and growth data was taken at 7 days of treatments. Exogenous application of 100 µM SNP had growth promoting effects in wheat seedlings while; higher concentration of SNP inhibited seedling growth. In comparison to control seedlings the application of 100 µM of SNP had promoting growth in wheat seedlings and increased germination percentage, shoots and root length and also increased fresh and dry weight. The results of experiment showed exogenous application of 100 µM SNP increases photosynthetic pigments content in wheat (Chl a, Chl b, total chlorophyll and carotenoid). Increasing concentration of SNP (500 µM) shows negative effect and reduced photosynthesis pigment and protein content in germinated seedlings. Supplemented with 100 µM SNP enhances activity of H_2O_2 scavenging enzymes. A reduction of hydrogen peroxide, malondialdehyde and proline contents was observed at 100 µM SNP concentration. SNP at lower concentration enhances protein content in wheat variety PBW 343. It is concluded from the present investigation that nitric oxide acts as a stimulator at low concentrations whereas at higher concentration it acts as a stress inducer.

Keywords: *Triticum aestivum* L., Sodium nitroprusside, Hydrogen peroxide, Photosynthetic pigments

Introduction
Nitric oxide (common name) or nitrogen monoxide (systematic name) is a chemical compound with chemical formula NO. Maleck *et al.*, 2005 [1] described NO a highly toxic compound as it is a component of pollutant gasses released by industrial waste, vehicle exhaust and cigarette smoke etc. Before the discovery of NO as a signal molecule, free radicals were considered as toxic by-products of oxidative metabolism but today's research is focused on the role of this free radical in various cellular functions. Nitric oxide is highly reactive (having a lifetime of a few seconds), yet diffuses freely across membranes. NO can exist in interchangeable structures namely the nitrosonium cation (NO^+), nitroxyl anion (NO^-) and the nitroxyl radical. These attributes make nitric oxide ideal for a transient paracrine (between adjacent cells) and autocrine (within a single cell) signalling molecule [2]. NO does not bind to a single receptor due to its chemistry and diffusible behaviour. NO and its derivatives performs post translation modification signalling which is the main cellular activity of all NO targeted protein. Nitric oxide (NO) is a bioactive molecule involved in signaling process within plants [3] and plays a central role in a variety of physiological processes including response to biotic and abiotic stresses [4]. It is a gaseous multifunctional messenger molecule [5] and involved in plant response to pathogen attack, programmed cell death, herbicides, salt, drought, temperature stress, and heavy metal toxicity [4]. NO supplied exogenously has been demonstrated to provide resistance against toxicity by heavy metals like copper [6] and aluminium [7]. NO provides protection against heavy metal toxicity due to its ability to act as antioxidant and scavenge ROS [4, 5]. NO supplementation has been found to reduce Pb uptake in *Arabidopsis thaliana*, thereby reducing toxicity symptoms [8].

However, there is a paucity of information regarding the role of NO in alleviating Pb-induced toxicity.

Materials and Methods

Healthy seeds of wheat (*Triticum aestivum* L.) variety PBW 343 were purchased from KVK Nawada (Bihar). Seeds were surface sterilized with 0.1 % sodium hypochlorite for 10 min and washed under running tap water followed by rinsing 5-6 times with distilled water and germinated in petri plate at different concentration.

Sodium nitroprusside (SNP) treatment

After hydroponics culture, SNP treatment was initiated. The seedlings were treated with different concentrations SNP (0, 25, 50, 100, 200, 300, 500µM). Seedlings without SNP treatment served as a control. For each treatment triplicates were maintained. After 7 days of treatment, root and shoot samples were harvested separately and measured various physiochemical and biochemical changes.

Determination of growth parameters

Seeds of wheat cultivars namely PBW 343 were germinated in Petri-dishes in laboratory. Each Petri-dish was layered inside with filter paper. The Petri dishes thus prepared were autoclaved. The twenty seeds were raised in each Petri-dish. Different concentrations of SNP (0, 25, 50, 100, 200, 300 and 500 µM) were added in each set of Petri-dishes, in triplicate. Germination percentage was calculated by counting the number of seeds germinated in each Petri-dish. The shoot length of 5 plants from each treatment of three replicates was measured in centimetre from the base of the plant to the growing tip of the main shoot with the help of a meter scale and expressed in centimetre (cm). The shoot length of 5 plants (5 from each replication) was measured and averaged to obtain the length of per plant for each treatment. Root length is measured after 7 day of treatments at of controlled and treated plants from the root-stem transition to the base of the root apex.

Fresh weight and Dry weight of shoot and root

Select five seedlings randomly from each pot, washed and blotted on Whatman paper. Seedling was separated into root and shoots and immediately determined fresh weight of 10 day old plant. Dry weight was determined after oven drying the shoot and root samples at 70°C until constant weight was obtained.

Determination of photosynthetic pigments contents

The chlorophyll and carotenoid (mg/g fresh weight) were determined by Wellburn (1994) [9] with minor modification. The fresh sample (0.5 gm) of control and treated plants were cut into small pieces and incubated in dimethyl sulphoxide (DMF) 5 ml covered with aluminium foil and kept in dark at 4°C for 24hrs. When the liquid become green the absorbance was measured at 664, 647 and 480 nm on UV visual Perkin-Elmer double beam spectrophotometer against DMF, as a blank.

Chl a = 11.65OD × 664 - 2.69OD × 647

Chl b = 20.81OD × 647 - 4.53OD × 664

Carotenoids = (1000 × OD480 - 0.89 × chl a – 52.02 chl b)/245

Hydrogen peroxide analysis

Estimation of hydrogen peroxide was estimated according to the method of Velikova *et al.*, (2000) [10] with minor modification. Fresh wheat leaves and root weighing 0.5 g were separately homogenized with 5 ml (0.1%) trichloroacetic acid (TCA). Then the homogenate was centrifuged at 12,000 g for 15 minute at 4°C. 4 ml assay mixture was prepared by taking 1 ml supernatant, 1ml phosphate buffer (pH 7) and 2 ml of 1M KI. The absorbance was measured at 390 nm. H_2O_2 content was determined by using the extinction coefficient (ε) of 0.28 µmole^{-1} cm^{-1} and the content was expressed as nmole g^{-1} f.wt.

Lipid peroxidation analysis

Lipid Peroxidation was estimated according to Heath & Packer (1968) [11] with minor modification. Wheat leaves and root (0.5 gm) ware homogenized in 5 ml (0.1%) trichloroacetic acid (TCA) and centrifuged at 12,000 rpm for 10 min. The assay mixture containing 1ml supernatant and 4 ml 0.5% TBA in 20% TCA, incubated at 95°C for 30 min. The reaction was transfer quickly in ice bath and centrifugation at 10,000 rpm for 10 min. The absorbance of supernatant was read at 532 nm and 600 nm by using spectrophotometer (UV-VIS). The concentration of thiobarbituric acid reactive substance (TBARS) was calculated as malondialdehyde (MDA) equivalents using the molar extinction coefficient of 155 nmole^{-1} cm^{-1}.

Estimation of proline

Proline analysis was estimated according to Bates *et al.*, (1973) [12] with some modification. 0.5 g leaves homogenized in 5ml of 3% sulphosalicyclic acid extraction buffer and filtered by Whatman No.1 filter paper. 1ml filtrate was mixed with all reagents and absorbance was read spectrophotometrically at 520 nm using toluene as a blank.

Extraction of protein

Soluble protein fractions were estimated by using Coomassie Brilliant Blue G-250 by Bradford (1976) [13] method. To protein assay 0.5 ml supernatant, 0.5 ml phosphate buffer and 3 ml of Bradford dye, were added. The samples were incubated at 37°C for 5 minutes in dark. The absorbance was recorded at 595 nm. Protein concentration was calculated with the help of standard curve using BSA.

Statistical analysis

All experiments were performed in triplicate and expressed as mean difference. Analysis of variance (ANOVA) was done by using graph pad prism, version 5.01(graph pad software, La Jolla, Ca, USA). Differences at $p \leq 0.05$ were considered significant and mean ± standard error (SE).

Results

Effect of SNP on germination

SNP had a strong stimulating effect on germination of wheat seeds at lower concentration. Out of the various concentrations used 100 µM SNP showed maximum increase in germination percentage. Figure 1 shows the increase germination percentage by 15.38% but at higher concentration the promoting effect was inhibited. At 500 µM SNP treatment decreased germination rate 42.30 % as compare to control.

Effect of SNP on shoot and root length

The shoot and root length was measured after 7 days of treatment and it was observed that length of shoot and root was gradually increased up to 100 µM SNP but decreased thereafter. SNP treatment of 100 µM showed maximum increased in shoot and root length and increased length by 25.89 % in shoot and 41.11 % in root as compared to control. As depicted in Figure 1 the maximum reduction in root and shoot length at 500 µM SNP treatment by 49.13% in shoot and 53.33% in root comparison to control.

Effect of SNP on shoots and roots fresh weight

We observed that at 100 µM treatment maximum increased fresh weight and dry weight of germinated seedlings (Fig.1) and decreased thereafter. SNP treatment of 100 µM increased shoot and root fresh weight by 11.0% and 20.37 % respectively as compared to control. The maximum reduction in fresh weight of root and shoot observed significantly at 500 µM SNP by 52.75 % in shoot and 49.17 % in root comparison to control.

Effect of SNP on shoots and roots dry weight

SNP treatment of 100 µM increased dry weight of shoot and root by 20.90% and 36.58 respectively as compression to control where as 500 µM SNP decreased in shoot and root dry weight 52.96% and 52.43% respectively (Fig.1). The maximum reduction in dry weight of root and shoot observed significantly at 500 µM SNP concentrations.

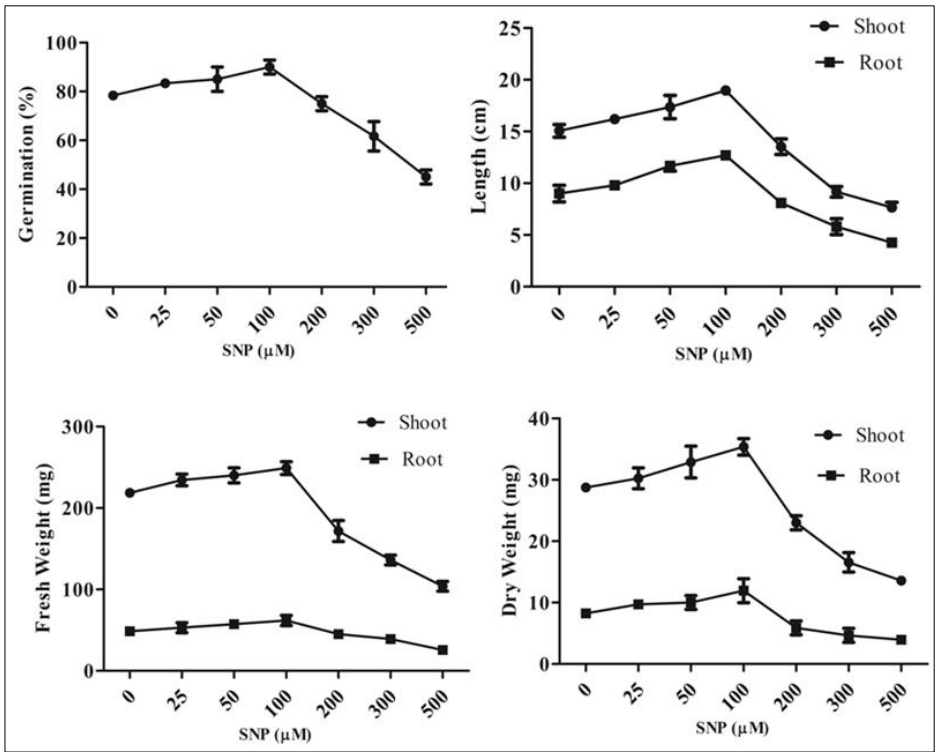

Fig 1: Effect of SNP on germination percentage, shoot and root length, fresh and dry weight of germinated seedling. Data represent the mean (±SE) was calculated from three replications for each treatment. All values are significantly different at p≤0.05 applying post hoc Tukey's test.

Effect of SNP on photosynthetic parameters

Chlorophyll content is very useful indicator of heavy metal toxicity in plants. We studied the effect of SNP on chl a, chl b total chlorophyll and carotenoid content in wheat seedlings growing in different SNP concentration. Application of 100 µM SNP enhance photosynthetic pigments contents in wheat seedlings. Fig.2 depicted that application of 100 µM SNP increased chlorophyll a content 27.41 %, chlorophyll b 64.80%, carotenoid 27.27 % and total chlorophyll 40.39 % as compared to control. Application 500 µM SNP decreased chlorophyll a & b, carotenoid and total chlorophyll by 56.71%, 64.45%, 57.02% and 59.36% respectively as compared to control.

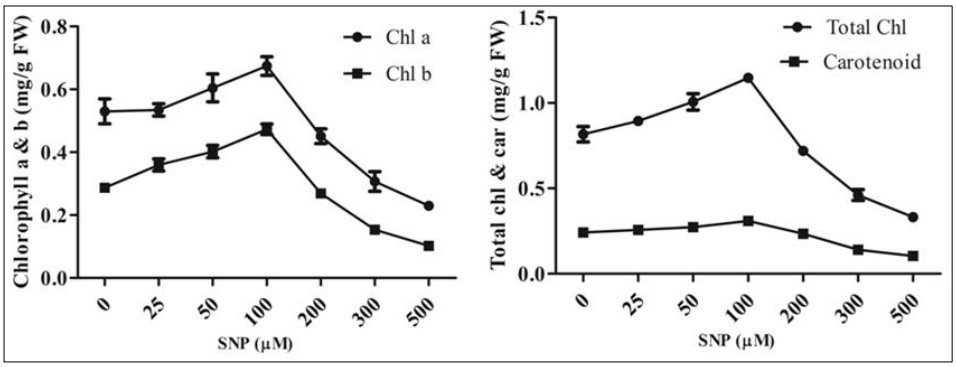

Fig 2: Effect of SNP on Chl a, Chl b, Total Chl and carotenoid content of germinated seedling. Data represent the mean (±SE) was calculated from three replications for each treatment. All values are significantly different at p≤0.05 applying post hoc Tukey's test.

Effect of SNP on Hydrogen peroxide content

Hydrogen peroxide produces under oxidative stress. Effects of SNP content has been shown in Fig. 3. H_2O_2 content decreased as compared to control upto 100µM SNP treatments but increased at higher concentrations of SNP. The amount of H_2O_2 decreased by in 26.01% shoot and 43.41% in root as compared to control. The maximum reduction of H_2O_2 content at 100 µM of SNP concentration out of the various concentrations used. The highest H_2O_2 reduction rates were observed in root treated with 100 µM SNP and the similar reduction pattern is also recorded in shoot as compared to the control. The amount of H_2O_2 increased significantly at 500 µM of SNP 49.10 % in root comparison to control.

Effect of SNP on Lipid peroxidation content

In wheat seedling the level of lipid peroxides measure in terms of MDA. MDA content decrease at 100 µM SNP treatments but increased at higher concentration of SNP. Out of the various concentrations used 100 µM SNP led to maximum reduction in MDA content. Fig.3 depicted that MDA content decreased by 45.6 % in shoot and 29.8 % in root as compared to control. The maximum reduction of MDA content at 100 µM of SNP concentration out of the various concentrations used. The highest MDA reduction rates were observed in shoot treated with 100 µM SNP and the similar reduction pattern is also recorded in root as compared to the control. The amount of MDA increased significantly at 500 µM of SNP 19.70 % in root comparison to control.

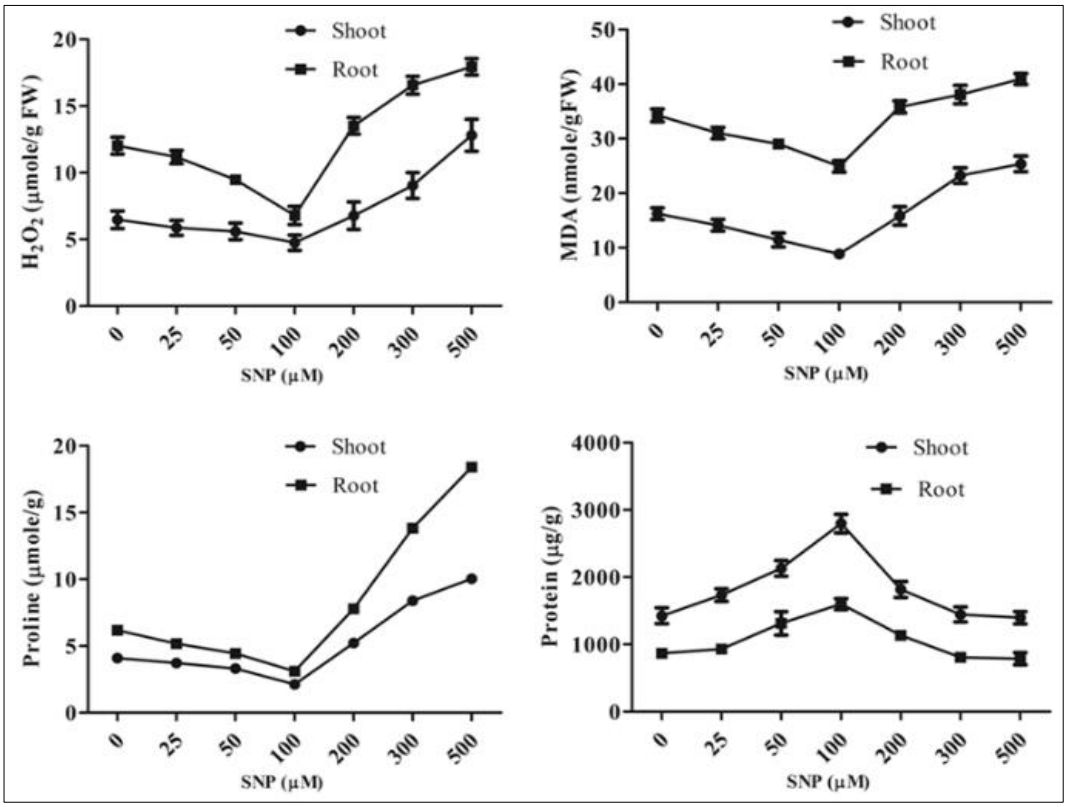

Fig 3: Effect of SNP induced change in proline, hydrogen peroxide, lipid peroxidation, soluble protein content in germinated seedlings. Data represent the mean (±SE) was calculated from three replications for each treatment. All values are significantly different at p≤0.05 applying post hoc Tukey's test.

Effect of SNP on proline content

Data of proline content presented in Fig. 3 which elucidated that the treatments had significant reduction in proline content at 100 µM SNP where as higher concentration of SNP increased proline content. Among all treatments, the minimum proline content was recorded at 100 µM SNP and maximum at 500 µM SNP as compared to control. Application of 100 µM SNP Proline content decreased by 47.5 % in shoot and 49.10 % in root as compared to control whereas at 500 µM SNP the increasement of proline content 150 % in shoot and 201% in root as compare to untreated plant. The maximum reduction of proline content was observed in root at 100 µM SNP concentrations.

Effect of SNP on protein content

Amount of protein content at growth periods are presented in Fig.3. The variations of total protein content were statistically partly significant during crop growth period. There was a gradual and significant increase in protein content of SNP treated plants as compared to control plants. SNP concentration upto 100 µM increased protein content but decreased it thereafter. At 100 µM SNP proteins content increased by 94.80 % in shoot and 84.65 % in root as compare to control. Out of the various concentration used100 µM SNP led to maximum increase in protein content. Protein content decreased by 1.96 % in shoot and 8.90 % in root when 500 µM SNP was supplied as compare to control. SNP enhanced protein content by minimizing the damaging effect of metal stress in wheat. A decrease in the protein concentration would be a typical symptom of oxidative stress and has frequently been observed in drought stressed plant.

Discussion

NO is a crucial gaseous signalling molecule in plant. NO plays significant role in regulating many important

physiological processes in plant [14]. In recent studies reported that NO has been established as a key messenger molecule in resistant responses in plant against heavy metal including lead, arsenic and copper [15, 16]. Qiao and Fan (2008) [17] reported that, NO can functional both beneficial and harmful effect depending on the concentration and location of NO in plant cell. The dual function of NO as a potent oxidant and effective antioxidant generally depends on the status of environments [18]. In our study SNP upto 100μM concentration enhanced germination percentage and where as higher concentration it shows inhibitory effect. In plant during germination NO acts as germination stimulator. Application of 100μM SNP increased germination percentage (Fig.1) similar result was reported by [19] and [20].

Seedling growth of wheat measured in terms of plant length was adversely affected by SNP concentration SNP at lower concentration the plant shoot and root growth increased significantly while higher concentration inhibit plant shoot and root length. A significant increase in shoot and root length was observed in 100 μM SNP treated seedlings compare to control (Fig. 1). Application of 100 μM SNP increase in shoot and root length by 25.89% and 41.11 % respectively while at 500 μM SNP treatment decreased shoot and root length by 49.13% and 53.3% respectively. Similar results were reported by [19] *Senna macranthera* and [21] *Chrysanthemum*. Root elongation is important to absorbed water and mineral nutrients. In our study root length increased more at 100 μM SNP treatment.

In our study reported that increased in fresh and dry weight of germinated seedling upto 100 μM SNP treatment where as increased SNP concentration decreased fresh and dry weight. Similar result was also reported in wheat by [22] in wheat seedling to salt stress. More reduction in fresh and dry weight of shoot and root at 500 μM SNP concentration. Tian *et al*., (2015) [22] reported that at 0.10 mM relative water content increased in wheat seedling under salt stress. Photosynthetic pigments are useful parameters for measurement of lead toxicity in plants such as total chlorophyll and carotenoid. In our study we observed the effect SNP, 100 μM increased total chlorophyll and carotenoid content as compared to control. Similar result was also found by [23] *chrysanthemum* and [24] and in wheat seedling.

In plant heavy metal caused oxidative damage due to production of reactive oxygen species. Antioxidant enzyme and certain metabolites present in plant to resist oxidative damage and play important role in adaptation and survival of plants during stress [25, 26]. Hydrogen peroxide content was decreased in shoot and root after exposure to 100 μM SNP over control. Hydrogen peroxide content was observed lower in root than shoot significantly. Such an observation was also reported by [27] in wheat seedling and [28] in pea seedlings. The present study suggests that SNP reduced metal toxicity and induces some of the key enzymes of antioxidant defence system in wheat seedlings. Our results show that increase in Pb concentration increase in the amount of lipid peroxides which indicate that Pb induces oxidative stress in wheat seedling. Similar observation was reported by [27, 29] in wheat and [28] in pea seedlings.

Plant can defend themselves from oxidative stress by producing compatible solutes like proline. Proline accumulation is an indicator of heavy metal stress in plants. The concentration of proline was constrictively decreased up to 100 μM SNP when the concentration of SNP increased and reduced toxic effect on plant. Similar to our results, increased in proline content was also reported [24] in wheat and [30] in cucumber seedlings. Protein contents in the leaves and root increased upto 100 μM SNP treatment where as higher concentration the protein content decreased. Similar result was observed by [28] in pea seedlings during Cd stress.

Conclusion

In summary, the present study concludes that exogenous NO supply partially ameliorates heavy metal toxicity and provide protection, but could not restore the plant growth on prolonged heavy metal exposure. NO helped to ameliorate the oxidative stress, as it did not reverse the increased activity of the enzymes in response to increasing dose and duration of treatment. SNP ameliorated metal toxicity significantly during the time period at which maximum concentration of NO from SNP solution was recorded. Nevertheless, further studies are required to elucidate the changes at the molecular levels and the particular isoenzymes, proteins that are involved in mediating the ameliorating action of NO.

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