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The modulatory effect of Nitric oxide molecules on the heavy metal stress in *Triticum aestivum*

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INTRODUCTION & AIM

Nitric oxide (NO) serves as a dual-functioning agent in plants, acting both as an antioxidant and a pro-oxidant, significantly influencing plant growth and development (Besson-Bard et al., 2008). NO's adaptability within cells allows it to function as an autocrine and paracrine signaling molecule. Plant metal stress generated from the toxicity of heavy metals (HMs; with higher density and atomic weight) in the environment. These inorganic compounds such as zinc (Zn), chromium (Cr), cadmium (Cd), nickel (Ni), silver (Ag), lead (Pb), and arsenic (As) are often toxic trace elements. The surplus of HMs poses significant hazards in agriculture and the environment, infiltrating the food chain and posing severe health risks to humans (Gall et al., 2015). This toxicity leads to rapid growth inhibition in plant parts, interactions with nuclear proteins and DNA, causing biomolecule deterioration, and disrupting the antioxidant machinery at the genetic level (Chaudhary et al., 2013). NO promotes seed germination and reduces seed dormancy under diverse stresses, playing a pivotal role in mitigating HM-induced reactive oxygen species (ROS). Moreover, NO triggers resistance and tolerance responses against various HMs such as Cd, Cu, Ni, Zn, and As (He et al., 2014). Therefore, this study aims to explore NO's role in alleviating metal stress in *Triticum aestivum* and uncover the physiological and biochemical changes resulting from NO treatment in metal toxicity.

METHODOLOGY

Heavy metals (Cr as K2Cr2O7 and Ni as NiSO4) at concentrations of 50µM and 100µM and NO as sodium nitroprusside (SNP; 50µM) were given hydroponically (alone and in combination). Stress parameters in the roots of test plants were assessed after 72h of treatment.

Estimation of oxidative stress

- Total Ascorbates: **Law *et al.* (1983)**
- Total Glutathione S-Transferase (GST): **Habig *et al.*, (1974)**
- Peroxidases: **Batish *et al.* (2006)**
- Polyphenol Oxidases (PPO): **Lelyveld and Pretorious (1973)**
- Hydrogen Peroxide (H_2O_2): **Velikova *et al.* (2000)**
- Superoxide Anion (SO^{2-}): **Misra and Fridovich (1972)**
- Lipid peroxidation: **Heath and Packer (1968)**
- Lipoxygenase: **Axelrod *et al.* (1981)**
- Monodehydroascorbate Reductase (MDHAR): **Hossain *et al.* (1984)**

Antioxidant enzymatic activity

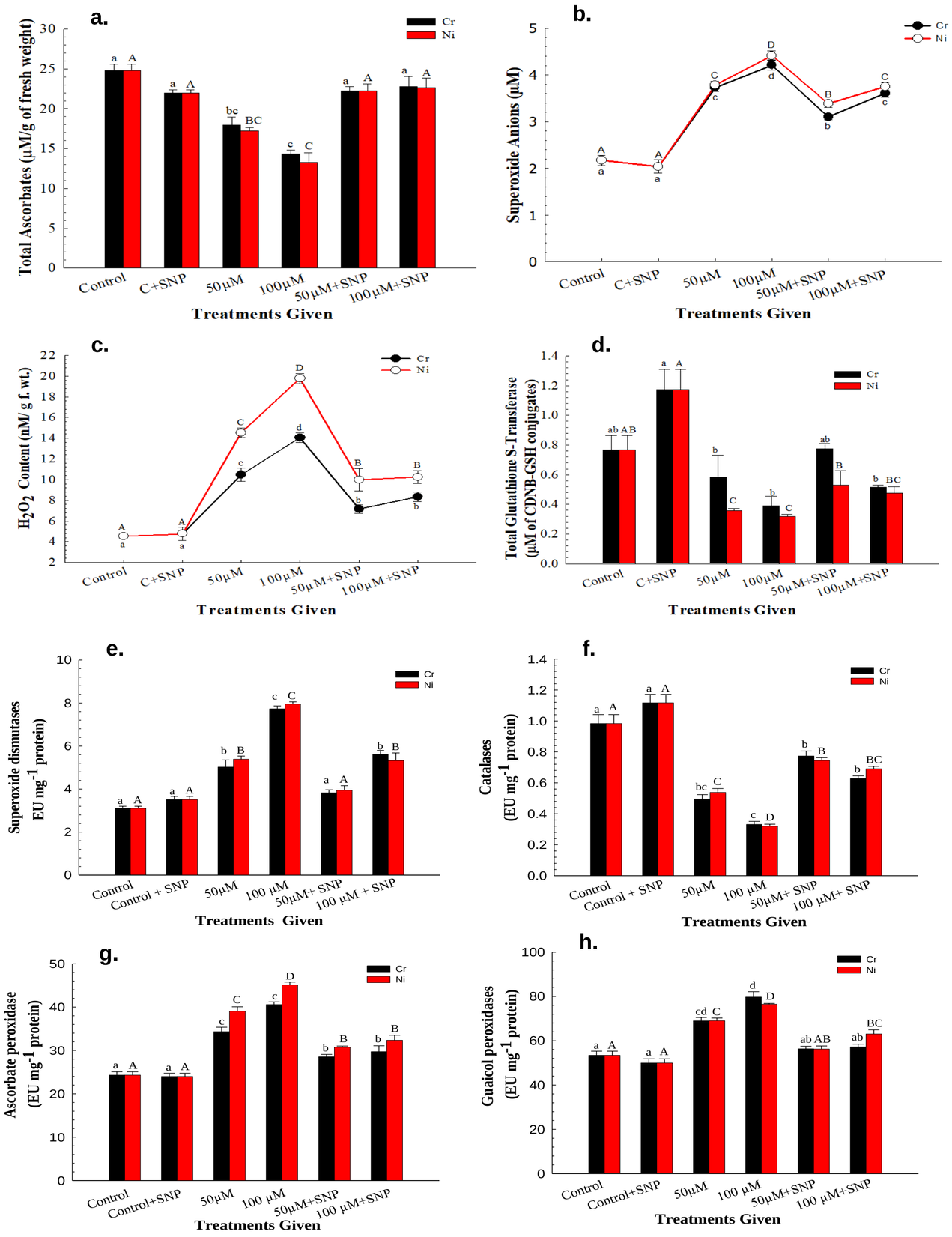
- Superoxide dismutase (SOD): **Beauchamp and Fridovich (1971)**
- Catalase (CAT): **Cakmak and Marschner (1992)**
- Ascorbate Peroxidases (APX): **Nakano and Asada (1981)**
- Guaiacol Peroxidases (GPX): **Egley *et al*. (1983)**

RESULTS & DISCUSSION

Table 1. Changes in the **a) Peroxidases, b) Polyphenol Oxidase, c) Lipoxygenase, d) MDHAR, e) Lipid Peroxidation** in *Triticum aestivum* against to the heavy metals alone or in combination of SNP. Data represented as mean±SE.
*Different alphabets at different treatments represents significant difference at p≤0.05 after applying *post hoc* Tukey's test.

Treatment Given	Peroxidases (Kat s^{-1} mg^{-1} protein)	PPO (Kat s^{-1} mg^{-1} protein)	Lipoxygenase (EU mg^{-1} protein)	MDHAR (µM min^{-1} mg-1 protein)	MDA content (nM g^{-1} f.wt.)
Control	0.29±0.01a	0.48±0.02a	1.63±0.05a	6.21±0.29ab	3.74±0.06a
Control + SNP	0.27±0.01a	0.50±0.01a	1.78±0.05ab	5.68±0.23a	3.67±0.04a
50µM Cr	0.57±0.01c	0.67±0.02c	2.26±0.06c	7.94±0.28cd	5.23±0.05c
100µM Cr	0.82±0.0.01d	0.87±0.02d	2.93±0.05d	8.67±0.10d	6.09±0.15d
50µM Cr + SNP	0.45±0.00b	0.55±0.02ab	1.75±0.06a	6.45±0.06ab	4.41±0.08b
100µM Cr + SNP	0.52±0.04bc	0.64±0.02bc	2.07±0.10bc	7.10±0.14bc	4.95±0.08c
50µM Ni	0.59±0.01c	0.65±0.00c	2.63±0.07c	7.91±0.23bc	5.28±0.03c
100µM Ni	0.69±0.01d	0.85±0.03d	2.99±0.09c	8.57±0.49c	6.17±0.07d
50µM Ni + SNP	0.48±0.03b	0.57±0.03bc	1.88±0.12ab	6.66±0.12ab	4.71±0.07b
100µM Ni + SNP	0.50±0.03b	0.65±0.01c	2.14±0.14b	7.01±0.20ab	5.12±0.06c

Figure 1. Changes in the **a) Total Ascorbates, b) Superoxide anions, c) H_2O_2 content, d) Total GST, e) Superoxide dismutase, f) Catalase, g) Ascorbate peroxidase, and h) Guaiacol peroxidase,** in *Triticum aestivum* against to the heavy metals alone or in combination of SNP. Data represented as mean±SE.
*Different alphabets at different treatments represents significant difference at p≤0.05 after applying *post hoc* Tukey's test.



CONCLUSION

The NO molecule acts as a stress inducer when applied separately in some parameters. However, in combination with both Cr and Ni, it acts as a stress reliever suggesting its possible use in agricultural systems located in areas abundant in these two heavy metals.

FUTURE WORK / REFERENCES

Further, the interaction of NO molecules with heavy metals will be explored by in –gel activities of anti-oxidative enzymes through SDS-PAGE and use of different standard dyes.
Data gathered from these experiments will be analyzed statistically to reach final conclusion of the study.