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A STUDY ON INTERACTIVE EFFECT OF HEAVY METALS NIKEL - MOLYBDENUM (NI-MO) ON ROOTS AND SHOOTS OF WHEAT CROP

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ABSTRACT

Interactive effects of Ni and Mo on T durum var. HI8737 resulted in a higher level of tissue weight being maintained in Mo1 and Mo2 treatments compared to Ni alone and almost intermediate levels with Ni-Mo1 and Ni-Mo2 in root and shoot. Further, for MDA and H2O2 content, Ni-Mo1 and Ni-Mo2 resulted in greater reductions compared to Ni alone, suggesting the role of Mo in mitigating the oxidative stress induced by Ni. In the binary treatments (Ni-Mo1 and Ni-Mo2), the SOD activities in the roots were substantially lower compared to the Ni treatment alone, whereas a marginal increase was observed in the shoot, indicating that Mo do not exert a prominent effect against Ni-induced O2- radical generation. Effects of Ni on CAT and APX activities were prominent in shoots of both binary treatments compared to Ni alone, indicating their participation in mitigating Ni-induced H2O2 stress in shoot tissue. It is worthwhile to discuss the research on Ni's interaction with various ions/molecules. Thus, Ni precipitation in soil occurs when Mn and Fe are present. The pre-treatment of Triticum aestivum L. cv. Samma seeds with GA3 and Ca2+ mitigates the negative effects of Ni toxicity by enhancing the antioxidant system seedling observed alleviation of Ni toxicity in the presence of Se and is related to limitation ofNi uptake. Hence, Mo may have a role in reducing Ni toxicity via forming complex.

KEY WORDS: Interactive Effect, Heavy Metals, Nikel, Molybdenum, Wheat Crop.

1. INTRODUCTION

Wheat is the predominant crop in temperate regions and is used as both human sustenance and animal feed. Wheat's success depends in part on its adaptability and high yield potential, as well as on the gluten protein fraction, which gives its dough the viscoelastic properties that enable it to be processed into bread, pasta, noodles, and other food products. It contributes

nutritional factors, beneficial phytochemicals, and dietary fiber to the human diet. This review focuses on current and future issues, such as sustaining wheat production and quality with reduced agrochemical inputs and developing lines with improved quality for specific end-uses, such as biofuels and human nutrition.

Wheat contributes more than fifty percent of the nation's caloric. Approximately 95% of total wheat production comes from bread wheat, while 4% comes from durum wheat and 1% comes from dicoccum wheat. USDA projected that world wheat production for 2013-14 would reach a record 708.89 Mt, up 53.69 Mt or 8% from 655.2 Mt in 2012-13, while global wheat consumption would reach a record 706.47 Mt. More than 200 wheat varieties have been released for cultivation in India's six mega wheat growing environments over the past four decades. The majority of these varieties are released for cultivation under conditions of irrigation, high fertility, and opportune sowing. Wheat is consumed largely as homemade chapattis or rotis (unleavened flat bread) and custom milled atta (whole meal flour) in India, whereas it is consumed predominantly as bread globally.

1.1 THE "WHOLE" TRUTH CONCERNING WHEAT

Wheat's health benefits are wholly dependent on how it is consumed. To maximize the health benefits of wheat, it is essential to choose products produced with whole wheat flour rather than those that have been refined and stripped of their natural goodness. These benefits are diminished when wheat is transformed into white flour that has been extracted and bleached to a level of sixty percent. The standard procedure for preparing the majority of wheat products removes 40% of the original wheat grain, leaving 60%. Unfortunately, the bran and germ of the wheat grain, the most nutrient-dense portion of the grain, are among the 40% that are removed. In the process of producing flour with 60% extraction, more than half of the vitamin B1, B2, B3, and E, folic acid, calcium, phosphorus, zinc, copper, and iron, as well as the dietary fiber, are lost1. Although some refining is required for palatability, safety, and even bioavailability of nutrients, there has been interest in the potential health benefits of high-fibre food products for several years.

Along with wheat bran, wheat germ merits its status as a health food. The germ is the vitamin and mineral-rich embryo of the wheat kernel, packed with essential B-vitamins, a high oil content, and consequently a high amount of vitamin E, a potent antioxidant that is essential for immune system functions, cancer prevention, and blood glucose control in healthy and

diabetic individuals6. Increasing evidence suggests that fibre may reduce the risk of certain chronic diseases in humans, including diabetes, cardiovascular disease, and certain types of cancer.

2. RESEARCH METHODOLOGY

Directorate of Wheat Research (DWR), Indian Agriculture Research Institute (IARI), Indore, India, provided the wheat seeds. The seeds were surface sterilized with 0.1% HgCl2 for 1-2 minutes, rinsed with milli Q water, and germinated for three days at 251o C in a petri dish lined with damp filter paper. The seedlings were transferred to a static hydroponic culture in a 50 ml beaker for four days in a growth chamber "Scientech" model SE110 (90 mW/cm2 light intensity, 55% relative humidity, and a 12-hour day/night cycle). To determine the effect of pH and temperature on germinated seedlings, the seedlings were cultivated in a Hoagland nutrient solution diluted to one-fourth strength and subjected to various combinations of pH (4,5,6) and temperature (10o, 20o, 30o C). The effect of varying pH and temperature on root and shoot tissue was measured in terms of fresh tissue weight and length, total chlorophyll content, total protein, proline content, and In-vivo NR activity. In addition, in order to examine the effect of heavy metals such as Al, Ni, and Mo on selected wheat varieties grown at pH 5 and 20oC, the germinated seedlings were sprayed with variable concentrations of AlCl3 (0, 25, 50, 100, and 200 M) for four days in a growth chamber.

2.1 ANALYTICAL PROCEDURE

The treated seedlings were used to evaluate growth in terms of parameters such as the fresh weight of the shoot and root and the length of the shoot and root. For the purpose of analyzing the effect of binary treatment on wheat varieties for 30 days, fresh tissue weight and length / fresh plant weight and length were also recorded.

Malondialdehyde (MDA) Content

Extraction

Wheat shoot and root tissues were extracted in 1.0 ml of TCA (0.1%w/v) in an ice-cold buffer, and the homogenate was centrifuged at 13,000 g for 15 minutes at 4°C using the "Sorvall RC 5B Plus."

Estimation

Double the volume of 0.5% thiobarbituric acid in 20% TCA was added to one milliliter of supernatant and incubated in a water bath for 30 minutes, after which the reaction was terminated in a cold bath. The supernatant was centrifuged for 5 minutes at 12,000 rpm and 4 degrees Celsius. Utilizing specific absorbance at 532 nm and non-specific absorbance at 600 nm, the concentration was determined. The level of lipid peroxidation was determined using the extinction coefficient (= 155 mM1 cm1) expressed as M MDA per gram of fresh weight.

H2O2 Content

Extraction

Wheat shoot and root tissues were extracted in 1.0 ml of TCA (0.1%w/v) in an ice-cold buffer, and the homogenate was centrifuged at 13,000 g for 15 minutes at 4 degrees Celsius using a "Sorvall RC 5B Plus" centrifuge.

Estimation

To 1 ml of supernatant, 1 milliliter of 10 mM sodium phosphate buffer (pH 7.5) and 2 ml of 1 M potassium iodide were added. At 390 nm, the absorbance of the sample was measured. Calculated from the extinction coefficient (=0.28 M1 cm1), the H2O2 concentration was expressed as M H2O2 g1 FW.

Antioxidative enzyme activity

Root and shoot material was homogenized with 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA in a mortar and pestle at 4 C in a cold chamber. Using the Remi "C-24" chilling centrifuge, the homogenate was centrifuged at 12,000 g for 20 minutes at 4°C. The supernatant was utilized for various enzyme tests and protein analysis.

Superoxide dismutase

Outlined a procedure for measuring superoxide dismutase activity.

Assay

In a small glass test container, 0.1 ml of enzyme and 60 l of 240 M riboflavin were combined with 1 ml of reaction mixture*. These test tubes were illuminated for 8 minutes at 25 degrees Celsius in a growth chamber. A duplicate solution that was not illuminated served as the void. Simultaneously, a control tube was ran in which the enzyme was replaced with 0.1 ml of buffer. The formation of formazone was measured at a wavelength of 560 nm, and the SOD activity was expressed as units per mg of protein.

* The reaction mixture consisted of 27 ml of potassium phosphate buffer (50 mM, pH 7.8), 15 ml of 0.2 M L-methionine, 1.0 ml of 1.76 mM nitroblue tetrazolium (NBT), and 0.75 ml of Triton X-100.

3. RESULTS AND DISCUSSION

Nickel effect

The supply of 25-200 M NiCl3 substantially inhibited the growth of seedlings as measured by the fresh weight of the root and shoot. Root (R2 = 0.878) and branch (R2 = 0.806) correlations were also extraordinarily strong. To evaluate oxidative stress caused by Ni supply, MDA, H2O2, and antioxidative enzyme levels were measured. The MDA content increased considerably at all Ni concentrations, and there was a strong correlation between root (R2 = 0.746) and shoot (R2 = 0.787). The H2O2 content increased as Ni concentration increased in both the root (R2=0.912) and shoot (R2=0.953) tissues, demonstrating a perfect correlation. The root had higher levels of MDA and H2O2 than the shoot. The MDA content increased significantly at all Ni concentrations in both the root and shoot. whereas the H2O2 content of the shoot increased from 50 to 200 M to 50 to 200 M.

The supply of 25-100 M NiCl3 markedly increased SOD activity in the root but decreased it in the shoot, with a strong correlation (R2 = 0.775) only in the latter. At 200 M Ni concentration, both the root and shoot showed a significant decrease. Ni supply significantly reduced CAT activity while maintaining a robust correlation in both the root (R2=0.778) and shoot (R2=0.789) tissues. Ni supply at 25-100 M significantly increased APX activity in roots but had no effect on shoot, whereas Ni supply at 200 M decreased activity in both.

Exogenous application of Ni increased Gu-POX activity significantly at 100 M Ni only in the root, but decreased it significantly at all concentrations in the shoot, with a strong correlation (R2 = 0.778).

A compound correlation was performed between the fresh weight of the tissue and stress parameters. Except for APX, a perfect correlation was observed between the fresh weight of the shoot and CAT (+1), Gu-Pox (+0.965), SOD (+0.914), MDA (-0.958), and H2O2 (Table 1a). Root fresh weight correlated perfectly with CAT (+0.956), MDA (-0.952), and H2O2 (-0.992), but not with SOD, APX, or Gu-POX (Table 14b). Table 1a reveals a perfect correlation between CAT and all other antioxidative parameters except APX in shoot. There was a correlation between CAT and MDA and H2O2 in root tissue, as well as APX and SOD and MDA and H2O2 (Table 1b).

Table 1. Compound correlation amongst fresh tissue weight, CAT, APX, Gu-POX, SOD, MDA, H2O2 of Ni treated *T. durum* var. HI8737

a) Shoot	**SOD**	**CAT**	**APX**	**Gu-Pox**	**MDA**	**H2O2**
Weight	0.914293	0.995	-0.1296	0.964629	-0.95795	-0.96084
SOD		0.927813	0.211496	0.986199	-0.95459	-0.88471
CAT			-0.0671	0.971075	-0.97895	-0.94395
APX				0.056365	-0.12897	0.052999
Gu-POX					-0.96922	-0.91855
MDA						0.908131

b) Root	SOD	CAT	APX	Gu-POX	MDA	H2O2
Weight	0.24209	0.95626	0.4056	0.13529	-0.95247	-0.99132
SOD		0.44203	0.97593	0.43451	-0.06551	-0.36042
CAT			0.5628	0.38672	-0.87384	-0.97313
APX				0.35694	-0.21424	-0.51845
Gu-POX					0.013723	-0.18979
MDA						0.912399

Molybdenum effect

The supply of 0.25 – 5 M Mo had no effect on the fresh weight of the shoot, but at 2 and 5 M Mo, the fresh weight of the roots increased considerably (R2=0.614). The content of MDA and H2O2 was higher in the shoot than in the root. In the root, MDA content decreased at all Mo concentrations with a very strong correlation (R2= 0.820), whereas in the shoot, MDA content decreased at and above 0.5 M with a perfect correlation. The H2O2 concentration decreased significantly at and above 0.5 M Mo in the root (R2= 0.551) and at all concentrations in the shoot (R2= 0.831).

Supply of Mo significantly increased SOD activity in root at and above 0.5 M, resulting in a strong correlation (R2 = 0.768). The Mo effect was less pronounced in the shoot and its activity was lower than in the root. At concentrations of 2 and 5 M Mo, CAT activity was substantially elevated in both the root and shoot. The observed correlation was stronger between the root (R2=0.864) and the shoot (R2=0.724). At 0.5-2 M Mo, root APX activity increased significantly, while shoot APX activity increased at all concentrations, albeit at a lower rate than root. The Gu-POX activity increased substantially in the root at concentrations of 2 and 5 M, with R2 =0.570, but was unaffected in the shoot.

Table 2a reveals a correlation between the fresh weight of shoots with SOD (0.536) and Gu-Pox (0.521). For antioxidative enzymes, a perfect correlation was observed between SOD and Gu-Pox (0.943) and between SOD and CAT (0.548). Table 2a reveals that only CAT exhibited a robust correlation with MDA (-0.891) and H2O2 (-0.874). Except for H2O2, all

oxidative parameters exhibited a correlation with the fresh weight of the root. SOD correlated with all parameters for antioxidative enzymes. While only Gu-Pox (0.916) and MDA (-0.706) correlate with CAT. (Table 2b) APX exhibited a strong correlation with MDA (-0.853) and H2O2 (-0.764).

TABLE-2 Compound correlation amongst fresh tissue weight, SOD, CAT, APX, Gu-POX, MDA, H_2O_2 of Mo treated *T. durum* var. HI8737

a) Shoot	SOD	CAT	APX	Gu-Pox	MDA	H2O2
Weight	0.536427	-0.18458	0.348215	0.520963	0.343969	0.116767
SOD		0.547856	0.118769	0.942752	-0.5348	-0.48421
CAT			-0.09573	0.427397	-0.89144	-0.87377
APX				-0.1328	-0.05028	-0.2451
Gu-Pox					-0.37047	-0.2313
MDA						0.88151

b) Root	SOD	CAT	APX	Gu-Pox	MDA	H2O2
Weight	0.73433	0.76895	0.54695	0.748689	-0.83806	-0.40063
SOD		0.55754	0.62958	0.529898	-0.87747	-0.86315
CAT			0.4667	0.916346	-0.70596	-0.39723
APX				0.151792	-0.85321	-0.76411
Gu-Pox					-0.11117	0.135381
MDA						0.775575

Interactive effect of Ni-Mo on HI8737

The effects of Mo (0.5 and 2 M) and Ni (100 M) on fresh weight, stress parameters such as MDA, H2O2 content, antioxidative enzymes such as SOD, CAT, and APX, and a key constituent of nitrogen metabolism, NR activity and NO content, were investigated. Upon binary treatment, Mo1 and Mo2 maintained a higher level of tissue weight than Ni, which exerted almost intermediate levels with Ni-Mo1 and Ni-Mo2 in both root and shoot. Root MDA content was significantly reduced in both Ni-Mo1 and Ni-Mo2 treatments compared to Ni alone, whereas shoot MDA content was significantly reduced only in the Ni-Mo2 treatment. MDA levels were substantially higher in the shoots of Ni-Mo1 and Ni-Mo2 treatments compared to their respective Mo controls, but only in the roots of Ni-Mo1-treated plants. With both shoot and root, Ni-Mo1 and Ni-Mo2 treatments demonstrated a significant decrease in H2O2 content compared to Ni alone. With Ni-Mo1 treatment, the H2O2 content was significantly higher in both the root and shoot when compared to Mo1, but only in the root when compared to Mo2.

Compared to Ni alone, the Ni-Mo1 and Ni-Mo2 treatments significantly reduced the SOD activity in the root but increased it in the shoot. As opposed to Mo1 and Mo2, Ni-Mo1 and Ni-Mo2 treatments resulted in significantly lower levels in both tissues. Only the Ni-Mo1 and Ni-Mo2 treatments significantly increased CAT activity in the shoot compared to Ni alone. Only the Ni-Mo2 treatment resulted in a significant reduction in root and shoot activity when compared to Mo2. The activity of APX in the shoot was substantially higher in the Ni-Mo1 and Ni-Mo2 treatments compared to Ni alone, whereas the root activity increased only in the Ni-Mo1 treatment. In comparison to Mo1 and Mo2 interventions, Ni-Mo1 and Ni-Mo2 significantly reduced the observed activity in shoot and root tissues, respectively.

NR activity of root tissue was substantially elevated in Ni-Mo1 and Ni-Mo2 treatments as compared to Ni alone, but only in Ni-Mo2 treatment of the shoot. The activity was substantially higher in both the shoot and root of the Ni-Mo1 treatment compared to the Mo1 and Mo2 treatments, but only in the shoot of the Ni-Mo2 treatment. All treatments resulted in a higher level of NO content in the root and shoot compared to Ni alone; however, the Ni-Mo1 and Ni-Mo1 treatments had a minimal effect compared to Mo1 and Mo2, respectively.

4. CONCLUSION

Ni is a growth-required micronutrient that plays a crucial function in metalloenzymes such as urease, hydrogenase, methyl-CoM reductase, glyoxalase (family I), peptide deformylase, NiSOD, etc. These enzymes are involved in several metabolic processes, including ureolysis, hydrogen metabolism, methane biogenesis, and acetogenesis. Ni availability is pH dependent, and below pH 5.5, Ni enrichment in wheat root can occur. Toxicities caused by excessive Ni negatively impact plant growth. Multiple studies have demonstrated that Ni toxicity is manifested in wheat growth parameters such as shoot length, shoot weight, root length, and root weight. In addition, Ni's toxic effects suppress root growth by inhibiting cell proliferation. In the presence of Ni, Gajewska and Skodowska's research indicated that the root is more sensitive than the stalk.

Ni supply slowed the growth of T. durum var. HI8737 wheat seedlings as measured by the fresh weight of the stem and root in this study. The retardation of growth may be the result of oxidative stress induced by Ni toxicity. Thus, both the MDA and H2O2 content of the tissue of Ni-treated seedlings were found to be substantially elevated, with a strong correlation between the two variables. The effect was more pronounced in root tissue than in stalk because higher levels of stress parameters are maintained in root tissue. In addition, a correlation analysis of the fresh weight of shoot and root tissues with MDA and H2O2 levels revealed a perfect correlation. The correlation between MDA and H2O2 was also perfect in both the shoot (+0.908) and the root (+0.912). The effects of Ni on the growth of Triticum durum var. 8737 wheat appear to be mediated by oxidative stress as a result of increased lipid peroxidation and H2O2 concentration. In wheat, an increase in lipid peroxidation has also been reported. In addition, an increase in H2O2 content has been reported in various wheat varieties.

In the present report, Ni treatment of T. durum var. HI8737 increased SOD activity up to 100 M, but decreased at 200 M in the root and decreased at all concentrations with a strong correlation in the shoot. This indicates that root SOD is more efficient at removing O2 . CAT activity was decreased in both the root (R2=0.789) and the shoot (R2=0.778). Moreover, compound correlation analyses revealed a perfect negative correlation (R= •0.943;-0.973) between CAT and H2O2 content in both the stem and the root, indicating a diminished

capacity of CAT to withstand increasing H2O2 stress. Other enzymes, such as APX and Gu-POX, contribute to the reduction of H2O2-induced oxidative stress. Gu-POX plays a significant function in cell wall lignification in addition to antioxidative enzyme. Ni supplementation up to 100 M substantially increased APX activity in roots and demonstrated a strong correlation with only SOD in roots. While Gu-POX activity decreased substantially at all concentrations in shoots with a strong correlation (R2 = 0.778) (Figure 32d) and perfect correlation with all other parameters except APX in shoots. Ascorbate can also be related to a marginal change in APX activity with a substantial increase in Gu-POX activity at 100 M in the root, and insignificant changes in APX activity with a substantial decrease in Gu-POX activity in the shoot. Ascorbate, a reducing substrate, is essential for the maintenance of APX. When administered externally, it is known to reduce Gu-POX activity in apoplast as well as cell wall. Therefore, it is evident that endogenous ascorbate may have affected the activity of APX and Gu-POX. Due to APX's high substrate affinity, a high H2O2 content can be efficiently removed by APX in roots where a decrease in CAT may have led to SOD–APX activity rather than SOD–CAT activity. In addition to detoxification, high Gu-POX may have contributed to root cell wall strengthening. Thus demonstrating a superior coping mechanism as a result of an increase in Ni concentration up to 100 M. Therefore, it is probable that CAT cannot reduce free hydrogen peroxide, whereas APX and Gu-POX play a role in detoxification.

Molybdenum is an essential micronutrient whose toxicity is uncommon, while its deficiency is common in acidic soils. Mo deficiency or excess in soil results in the production of immature seedlings that lack vigor and germination potential. A high dose of Mo is detrimental to wheat growth. While it has been reported that low concentrations of Mo stimulate wheat growth as measured by dry weight and various root growth parameters, there was no change in wheat growth as measured by fresh weight. Additionally, Mo application to wheat decreased MDA and H2O2.

At high concentrations, i.e. 2 and 5 M, a significant increase in root weight was observed for T. durum var. HI8737, indicating that Mo may promote root growth. In addition, there was a significant decrease in MDA and H2O2 content in both the shoot (R2=0.820; 0.551) and the root (R2=0.971; 0.831) as well as a perfect intercorrelation between the shoot (R=0.882) and the root (R=0.776). Thus, suggesting that Mo has the potential to reduce oxidative stress.

It has also been reported that Mo has an effect on antioxidative enzymes under various stress conditions. Mo application may help Triticum aestivum L. var. Huamai 8 surmount low-temperature stress by increasing antioxidant enzyme activity such as SOD, CAT, and POX. In addition, studies conducted on Mo-efficient (97003) and Mo-inefficient (97014) wheat varieties under drought stress and various N sources indicate that application of Mo enhanced antioxidative enzyme activity, including SOD, CAT, APX, and POD.

Mo effects on T. durum var. HI8737 wheat seedlings have shown an increase in SOD activity in both the root and shoot, with a higher level and stronger correlation in the root. Also, the activity correlated more strongly with MDA and H2O2 content in the root than in the stalk, suggesting a role for Mo in scavenging oxidative stress-induced generation of O2- radical in the root. Also observed was an increase in CAT, which correlated significantly with MDA and H2O2 content in the shoot tissue. The correlation between APX activity and MDA and H2O2 in the root only was pronounced when Mo supply was greater in the root compared to the shoot. Increased CAT and APX activity, as well as decreased MDA and H2O2 levels, indicate that the administration of Mo has the potential to increase antioxidative parameters, thereby overcoming stress.

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