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ZINC TOXICITY AND ITS ALLEVIATION BY NITRATE AND AMMONIA IN CORN SEEDLINGS (*ZEA MAYS* L.) WITH SPECIAL REFERENCE TO NITRATE REDUCTASE ACTIVITY

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The inhibitory effect of zinc on Nitrate Reductase Activity (NRA) was assessed by supplying zinc through the incubation medium and also through the rooting medium. At lower concentrations zinc had a promotory role on NRA, however, at higher concentrations zinc had inhibitory role and the rate of inhibition was proportional to the concentration of zinc. Nitrate, a substrate of NR only at lower concentrations can help to a certain extent to overcome the zinc toxicity. Ammonium, an end product of nitrate reduction, in general alleviates the zinc toxicity.

Keywords: Alleviation; Ammonium; Nitrate reductase; Toxicity; *Zea mays;* Zinc.

Introduction

Metal toxicity (especially by pollutants) is an increasing problem in agriculture due to urbanization and industrialisation[1]. Uptake and accumulation of heavy metals at toxic levels often lead to phytotoxicity to many plants[2,3] which limit the plant growth[4,5] cause physioligocal disorders[6] affect biomembranes and metabolism[7]. Besides there are many reports which say that heavy metals inhibit many important enzymes like nitrate reductase, corbonic anhydrace etc[8,9].

Nitrate Reductase (NR) is the first enzyme that particularly reduces the nitrate to nitrite[10]. Various factors like light[11], temperature[12], oxygen concentration[13], pH of the medium[14] and heavy metal pollutants[15], influence the nitrate assimilation to a certain extent.

As the pollution by heavy metals from various sources, affect the plant growth and yield in highly industrialised areas, it becomes an inevitable aspect to study the effect of heavy metals on NR. When the heavy metal inhibit the NRA, it is essential to find out a measure to alleviate the heavy metal effect on NRA.

Materials and Method

Certified seeds of *Zea mays* L. were obtained from Tamil Nadu Agricultural University, Coimbatore-646 003, India. Seeds were surface sterilized with 75% ethyl alcohol for three minutes followed by rinsing with glass distilled water repeatedly prior to germination[16].

Surface sterilized seeds were soaked in glass distilled water for 12 hours and transfered to petriplates for germination (10 seeds per plate). After 24 hours germinated seeds were transfered and cultured by paper towel method (10 seedlings per towel) in modified Hoagland medium containing 1.9 mM $CaSO_4$, 4.7 mM K_2SO_4, 1.0 mM $MgSO_4$, 0.25 mM FeEDTA, and 100 mM KNO_3. Minor elements were added as 1 ml stock/ liter. The stock contained in g/1 3.72 KCl, 1.54 H_3BO_3, 0.83 $MnSO_4.4H_2O$, 0.57 $ZnSO_4.7H_2O$, 0.125 $CuSO_4.5H_2O$, and 0.12 $H_2MoO_4.2H_2O$[16]. Plants were subjected to 10 hours light and 14 hours dark and the culture medium was changed alternate days.

Sampling was carried out on 7th day

after sowing. Roots and leaves were collected and cut into 1 cm pieces. The samples were further randomized in ice cold 0.1 M potassium phosphate buffer (pH 7.5) containing 30 mg/ml chloramphenicol to prevent microbial activity and 1% n-propanol as a surfactant[17]. From this randomized root and leaf bits 0.3 g sample was used for enzyme assay.

Zinc Toxicity Study: Zinc was supplied in the form of $ZnSO_4$. Zinc toxicity study was performed in two ways.

a. Through the Incubation Medium: Different concentrations of zinc was supplied in the incubation medium during the assay and the level of inhibition of NRA was estimated. In this study the concentration of zinc in the tissue will be equal to the concentration of zinc in the medium.

b. Through the Rooting Medium: Plants were grown in the nutrient medium. On 6th day plants were subjected to different concentrations of zinc which were given through rooting medium for 24 hours (a day prior to estimation). Then the sampling was carried out as described above and the activity of NR was measured.

Alleviation Study: The seedlings were kept for 24 hours only in 25 mM Zinc. Nitrate (NO_3) and ammonia (NH_4) were chosen to alleviate the zinc toxicity. During the assay the samples were collected from seedlings grown in 25 mM zinc, the nitrate and ammonia were supplied through the incubation medium in various concentrations.

***In Vitro* Nitrate Reductase Activity Assay:** Freshly harvested leaf and root bits (0.3 g fw) were taken in vials containing 5 ml of incubation buffer i.e., chilled O.1 M KH_2PO_4 - KOH buffer pH 7.5[18]. Anerobiosis was controlled by evacuating the vials using a vaccum pump for three minutes and the vaccum was released to facilitate infiltration of the incubation buffer in to the plant samples. Infiltered plant samples were incubated at 30° C in dark. After the specific periods 0.2 ml of aliquotes of incubation medium were removed from each vial, 0.3 ml of 0.1% Sulfanilamide in 1.5 N HCl was added (for better reaction the mixture was left for 5 to 10 minutes) followed by 0.1 ml of 0.2% N-1 (napthy1) - ethylene diamine dihydrochloride. The mixture was brought in to a final volume of 4 ml using buffer and optical density determined at 540 nm. Activity was calculated from a standard curve on nitrite and the results were expressed as μ mol NO_2^- formed g^{-1} FW[16].

Results and Discussion

Heavy metal toxicity is one of the alarming problems of this decade which possess a potential threat both to the human life and crop productivity. Consequently finding a suitable treatment to over come this problem becomes essential for crops. When the supply of one mineral affects or alters the absorption, distribution and function of another nutrient, these two minerals are considered as interacting elements and the principle behind is called mineral interaction. In other words, supply of one mineral can induce a deficiency of another element eventhough the latter is present in sufficient quantity. In the first phase the level of NRA inhibition by various concentrations on zinc was studied by supplying zinc through the incubation medium during the assay and through the rooting medium and their ability to alleviate the zinc inhibition of NRA was studied.

Effect of zinc on NRA was studied by supplying the heavy metal through the incubation medium. During the assay zinc was found to promote the NRA at lower concentrations up to 4 mM of zinc. The

promotive effect was more felt in leaves than in roots (Fig. 1).

Above 6 mM zinc there was a decrease in NRA due to the increase in the concentration of zinc both in leaves and in roots. At 14 mM zinc NRA was only 13.0% of control in leaves and in roots there was complete inhibition in and above 12 mM zinc. The present study reveals that zinc has a definite inhibitory role on NRA. The purpose of supplying zinc through the incubation medium is to create known concentrations of zinc inside the cells so that the relationship between the concentration of zinc and NRA could be derived.

However, for the plants zinc is available only t' rough the rooting medium. Therefore, it is also essential to understand the effect of different concentrations of zinc that are present in the medium in which the plants are grown. For this purpose on 6th day the seedlings were transfered to various concentrations of zinc (5, 10, 15, 20, 25, 30 and 35 mM) in which the seedlings were kept for 24 hours. When zinc was supplied to the seedlings through the culture medium zinc had a promotive effect on NRA up to 10 mM zinc. At 10 mM zinc the increase in NRA was 50% and 100% over control in leaves and roots respectively. Above this concentration zinc had an inhibitory effect on NRA. The inhibitory rate of 8 mM when supplied through incubation medium compared with inhibitory rate at 25 mM in the rooting medium (Fig. 2). Therefore, it could be inferred that when the available zinc concentration in the rooting medium exceeds 10 mM zinc has inhibitory role on NRA.

Though the direct involvement of zinc on NRA has not yet been conclusively proved. Seethambaram and Das[19] have showed

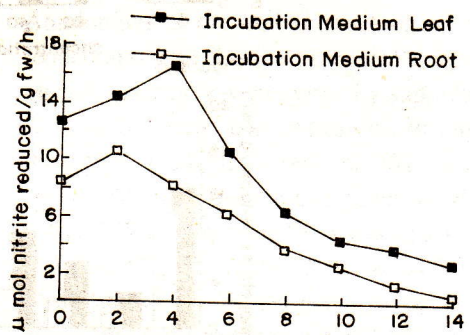

Figure I

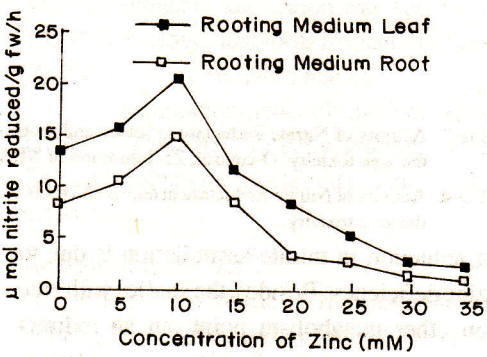

Fig.1. Activity of Nitrate Reductase in leaves and roots of *Zea mays* L. when zine was supplied in the incubation medium.

Fig.2. Activity of Nitrate Reductase in leaves and roots of *Zea mays* L. when zinc was supplied in the rooting medium.

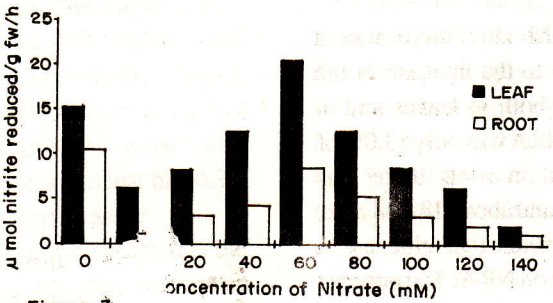

Figure 3

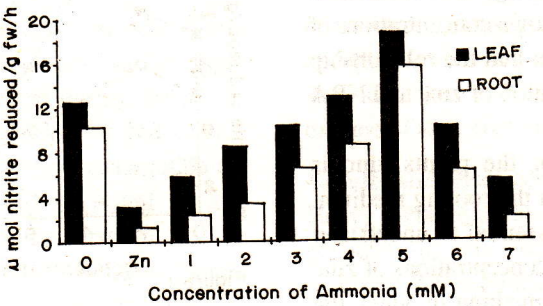

Fig.3. Activity of Nitrate Reductase in leaves and roots of *Zea mays* L. when Nitrate (KNO_3) was supplied to alleviate the zinc toxicity. O-control; Zn-Inhibition of NRA by zinc.

Fig.4. Activity of Nitrate Reductase in leaves and roots of *Zea mays* L. when Ammonium (NH_4) was supplied to alleviate the zinc toxicity.

a reduction in nitrate assimilation is due to zinc deficiency. Besides, the studies with zinc on other metabolism point out an indirect involvement of zinc in nitrate assimilation. Zn^{++} ions stimulate endogenous photophosphorylation in thylakoid membranes[20]; Zn is essential for the activity of RuBP carboxylase and PEP carboxylase[21]; Aldolase enzyme of both photosynthetic and glycolytic pathways require zinc[22]; high concentrations of zinc depressed the available P concentration inside the cell. Thus inhibiting the rate of glycolysis[23] and Zinc toxicity leads to chlorosis in young leaves. Thus interfering with photochemical reactions of photosynthesis[2].

Leblova *et al.*[24] have reported that when plants were exposed to 1 mM zinc the adverse effect was felt from 3 to 14 days. However, the present study reveals that 6 mM Zn could affect the NO_3^- reduction even after 24 hours treatment. When zinc was supplied through the rooting medium the inhibitory effect was more felt in roots than the leaves. It has been reported that when the seedlings were kept in high concentration of zinc, 30 to 70% of this metal ion was present in the roots and only 5 to 14% in the leaves[24]. This finding explains the higher inhibitory role of zinc in the roots.

Another important observation recorded in the present study is that when the zinc was supplied through the rooting medium there was no inhibiton of NRA even up to 10 mM zinc. But the same concentration when supplied through the incubation medium it could cause 74% inhibition of NRA. Compartmentation of zinc as soluble complexes of malate and oxalate[25] in the vacuoles could be a possible reason for the lesser impact of zinc when supplied through the nutrient medium. Secondly when zinc reaches the leaves through xylem is strongly bound to the plant protein. Therefore, the concentraion of free Zn^{2+} as divalent action is much lesser[26]

Effect of Nitrogen

Nitrate is the substrate for NR. To study the substrate alleviation of zinc toxicity, it was supplied in the form of $NaNO_3$. Supply of NO_3^- through the incubation medium (in addition to 100 mM $NaNO_3$ which was supplied as substrate for NR) reveals that further addition of substrate generally shows the inhibition of NRA, however, the zinc toxicity has been alleviated by NO_3^- to certain extent. However, among the alleviation observed the maximum NRA was at 40 mM NO_3^- where 8.33 and 5.83 µM NO_2^- formed per g fw per hour in leaves and roots respectively (Fig. 3) and the percentage of alleviation was maximum at 40 mM and was 54.52 and 50.0% in leaves and roots respectively. At 100 mM NO_3^- complete inhibition of NRA was observed both in leaves and roots. Thus higher concentration of NO_3^- further inhibits the NRA in addition to zinc inhibition.

Nitrate and ammonium are the major sources of inorganic nitrogen taken up by the roots of higher plants. External supply of nitrate enhances the NRA[16,27-29]. However, higher concentration of nitrate inhibit the NRA[30-32]

Thus nitrate at lower concentration enhances the NRA and thus showing some amount of alleviation and at higher concentration it further inhibit the NRA.

Effect of Ammonium

Ammonium is the end product of nitrate reduction system. The end product alleviation on zinc toxicity was measured by supplying NH_4 at different concentrations through incubation medium. Supply of NH_4 in all concentration shows the alleviation of zinc toxicity. The maximum NRA was observed at 5 mM NH_4 where the NRA was 20.0 and 15.83 µmol NO_2 formed per g fw per h in leaves and roots respectively and NRA was less in the adverse conditions of NH_4 (Fig. 4). The percentage of alleviation was 166.7 and 155.03% in leaves and roots respectively and the NRA was more felt in leaves.

Ammonium generally inhibits cation uptake[33]. Thus NH_4 directly interacts with Zn^{2+} and reduces the availability of zinc at the active sites.

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