

UREASE ACTIVITY IN MAIZE (*ZEA MAIZE L. CV. 704*) AS AFFECTED BY NICKEL AND NITROGEN SOURCES*

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Abstract – Nickel (Ni) is one of the essential micronutrients for higher plants and its known function is being the metal component of urease. The effects of various Ni levels on urease activity in maize (*Zea maize L.*) plants grown in two nutrient media containing urea or ammonium nitrate as two separate nitrogen sources were investigated. The experiments were performed as completely randomized blocks with three replications. Treatments included two growth media, the nitrogen of which was either urea or ammonium nitrate added at the rate of 84 mg L⁻¹ and four Ni levels (0, 0.01, 0.05 and 0.1 mg L⁻¹) supplied as NiSO₄. Plants were grown in the nutrient solutions for six weeks. On the second, fourth and sixth week of the growth period, both the leaves and root samples were taken to determine their urease activities. At the end of the sixth week, the dry weights of both the shoots and roots were also measured. Urease activity in leaves of corn supplied with urea increased significantly with the increase in Ni supply till the end of the 6th week sampling date, however in those supplied with ammonium nitrate, urease activity increased up to the 3rd Ni level and 4th week of sampling date, but was reduced at the 4th Ni level in the 6th week. Urease activity in the roots of corn plants supplied with urea was the highest at the 2nd Ni level at the end of the 2nd week. Increase in Ni levels and date of sampling resulted in a decrease in urease activity. However, in ammonium nitrate-fed plants urease activity in the 2nd week of the sampling date increased up to the 4th Ni level and for other sampling dates the activity increased up to 2nd Ni level. Further increase in Ni supply and date of sampling resulted in a decrease in urease activity. Enzyme activity was higher in the roots than in the shoots and was also higher in plants supplied with urea, compared to those fed on ammonium nitrate. In maize plants supplied with urea, the dry weights of the shoots and those of the roots were also higher.

Keywords – Urease activity, nickel, maize (*Zea maize L.*), urea, ammonium nitrate

1. INTRODUCTION

Ni, the most recently discovered essential element [1], is unique among plant nutrients in that its metabolic function was determined well before it was determined that its deficiency could disrupt plant growth. Subsequent to the discovery of its essentiality in the laboratory, Ni deficiency has now been observed under field condition in several perennial species [2]. The interest of plant scientists in the role of Ni was initiated following the discovery in 1975 [3] that it was the critical constituent of the plant enzyme, urease. Dixon and coworkers [3] discovered that Ni is a component of the urease, the only known Ni-containing enzyme in higher plants [4]. It has a molecular weight of 590 KDa and consists of six subunits, each containing two Ni atoms [5].

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Urease is a Ni dependent enzyme that catalyzes the hydrolysis of urea to form ammonia and carbon dioxide [6, 3]. It has been isolated from a wide variety of organisms, including plants, fungi and bacteria [7, 8].

Plants are able to use several forms of nitrogen. Urea is the most common form of combined N-fertilizer used in agricultural practices worldwide [9]. In Iran, of the 2052 thousand tons of N-fertilizer used in 2004, 1727 thousand tons (84%) was in urea form [10]. Urea cannot be used in plant metabolism directly [11]. The primary physiological role of urease is to allow the organism to use externally supplied and internally generated urea as a nitrogen source [7, 12]. Urease converts urea to products at a rate of at least 10^{14} times faster than the urea spontaneous decomposition rate [13]. High levels of urea are toxic to plant metabolism, causing leaf burn [14]. One way to overcome urea toxicity in higher plants is using Ni to increase urease activity [15, 4]. Without Ni supply, large amounts of urea accumulate in the leaves and symptoms of leaf tip necrosis are severe [16]. Plants suffering from Ni deficiency show high urea toxicity [17]. Urease catalyses urea hydrolysis, thus making urea-N available to be assimilated into organic compounds [18].

The first evidence of a response of a field crop to the application of a Ni fertilizer was demonstrated in 1945 for potato, wheat and bean crops [19]. Mishra and Kar [20] and Welch [21] reviewed the evidence of Ni roles in biological systems and cited many examples of yield increases in field-grown crops in response to Ni application to the crop and to the soil. Clear evidence that Ni application benefited the growth of nitrogen-fixing plant species was demonstrated by Bertrand and Dewolf [22], who reported that soil-Ni application to field-grown soybean resulted in a significant increase in nodule weight and seed yield.

The discovery that Ni is a component of the plant urease in 1975 [3] prompted a renewed interest in the role of Ni in plant life. In 1976, Polacco [23] and also in 1978 Gordon and co-workers [24] reported the enhancing effects of Ni on plant growth and development when grown hydroponically and supplied with urea as the nitrogen source. Shimada and Ando [25] reported that when tomato and soybean plants were grown in hydroponic cultures with insufficient Ni and supplied with urea as the nitrogen source, urea accumulated in their tissues and developed leaf tip necrosis. The role of Ni in urea metabolism in plant was reported by Walker and his co-workers [26] in 1985. Until then, there was no proven record for Ni as being an essential element for higher plants.

Witte and co-workers [27] studied the effects of urease activity on nitrogen distribution and nitrogen loss after spraying potato plants with urea. Good correlation was found between urease activity and ^{15}N -metabolism. In this study, nitrogen metabolism on the basis of NH_3 accumulation was evaluated.

Tan et. al [28] investigated the effects of different Ni levels on growth and N-assimilation in tomato plants supplied with urea and nitrate as two different nitrogen sources. They found a positive correlation between plants Ni content and its concentration in the nutrient media, and also demonstrated that urea toxicity symptoms are alleviated by the presence of Ni. In this study, plant growth, urea hydrolysis and chlorophyll content in plants supplied with urea, increased up to 0.1 mg Ni L^{-1} of the nutrient solution. Ni had little effect on growth and N-assimilation in plants supplied with nitrate salts.

Studies on the effects of Ni supply on growth, urease activity, urea accumulation and soluble amino acids in wheat, soybean, rape, cucurbit, sunflower and rye have shown that without Ni, urease activity in all these six plants is very low, resulting in considerable urea accumulation and also in lower plant dry weight and nitrogen content [29]. Besides the role of urease in plants receiving urea as a nitrogen source, this enzyme is also important for hydrolyzing urea derived from ureides and arginine produced in cytokinins metabolism [30, 26 and 8]. Urea produced in these processes will cause leaf burn unless detoxified by urease [31, 14, 32]. Urease activity is also very important when urea is applied as a foliar

spray. Many reports have pointed to the positive effects of urea spray on mulberry yield in relation to urease activity [33, 34].

The reaction catalyzed by urease is essential to make N-urea accessible to plants [35]. Urease activity has been detected in many plants [36, 18, 37] and is reported to be inducible by urea in several plant species [38-40].

Several direct and indirect detection methods for the reaction products catalyzed by urease have been successfully applied for the quantification of urease activity [37].

In the present study urease activity is determined in the leaves and roots of maize plants in different times of growth fed with ammonium nitrate or urea as nitrogen sources and different Ni levels.

2. MATERIALS AND METHODS

The experiments were carried out with maize (*Zea mays* L. cv. 704) in a complete factorial, randomized with three replications for each treatment. Treatments included nutrient solution cultures containing either urea or ammonium nitrate separately as two different nitrogen sources with four Ni levels (0, 0.01, 0.05 and 0.1 mg L⁻¹). Maize seeds were germinated between moist filter papers in covered Petri dishes placed inside phytotron set at 23°C. After 5 days, germinated seeds were transferred to half strength Hogland solution in the greenhouse with average 32/20°C. day/night temperatures. At this stage, the nitrogen source in the nutrient solution was ammonium nitrate. Seedlings with 5-6 leaves were transferred to 24 polyethylene containers filled with nutrient solution given in Table 1.

Table 1. Composition of nutrient solution

Nutrient	Concentration (mM)	Nutrient	Concentration (mM)
N(NH ₄ NO ₃ or Urea)	3	Fe EDDHA	0.02
K ₂ SO ₄	2	MnSO ₄	0.003
CaCl ₂	1.5	ZnSO ₄	0.002
MgSO ₄	1	CuSO ₄	0.001
NaH ₂ PO ₄	0.66	H ₃ BO ₃	0.024
		NH ₄ MO ₄	0.0001

The pH of the culture solution was adjusted at 6.0 ± 0.2 using 1M NaOH or 1M HCl. The solutions were aerated and renewed weekly. Ni was added as NiSO₄ .6H₂O.

Plants were allowed to grow for 6 weeks. At the end of the 2nd, 4th, and 6th weeks the leaves and root samples were taken for urease assay. Young leaf blades which had attained 75% of their final size and terminal 1 cm of young roots were used for the assay. Samples were immediately frozen in liquid nitrogen and were then kept at -80°C for future use. At the end of the 6th week the rest of the plants were harvested and the fresh weights of their shoots and roots were determined separately. After drying in an oven at 70°C for 72 hours, they were weighed and ground to a fine powder. Ni was extracted from dry ash samples by 2 M HCl. The total Ni contents in shoots and roots were determined by ICP (Inductively Coupled Plasma, Optima 2100 DV, USA).

Warthington method was used for urease bioassay [41], with some modifications. Warthington has adopted an assay method where the hydrolysis of urea is measured by coupling ammonia production (equation 1) to a glutamate dehydrogenase reaction (equation 2), thereby the inhibitory effects of the produced ammonia on urea cannot occur and it remains active until total consumption of substrate. Na-pi buffer was replaced by Tris-HCl as a more appropriate buffer for proteins and to avoid the inhibition of urease by Na [42]. Moreover, EDTA was added as a stabilizer and since urease has been reported to be susceptible to oxidation, DTT was added to the extraction mixture [37].

Reduction in urea concentration in equation (1) was monitored by spectrophotometer at 340 nm and expressed versus protein content of the samples and considered as the rate of urease activity.



The protein content of the samples was determined by Bradford method, using BSA (bovine serum albumin) as a standard [43].

A statistical analysis was made using analysis of variance (SPSS), and the mean was separated by Duncan's multiple range test (DMRT) at the 5% level.

3. RESULTS

The amounts of Ni accumulated in the shoots and roots of plants supplied with either urea or ammonium nitrate during 6 weeks of experiments are shown in Fig. 1. Plants supplied with urea have accumulated more Ni than those receiving ammonium nitrate. In both groups Ni accumulation was greater in the roots.

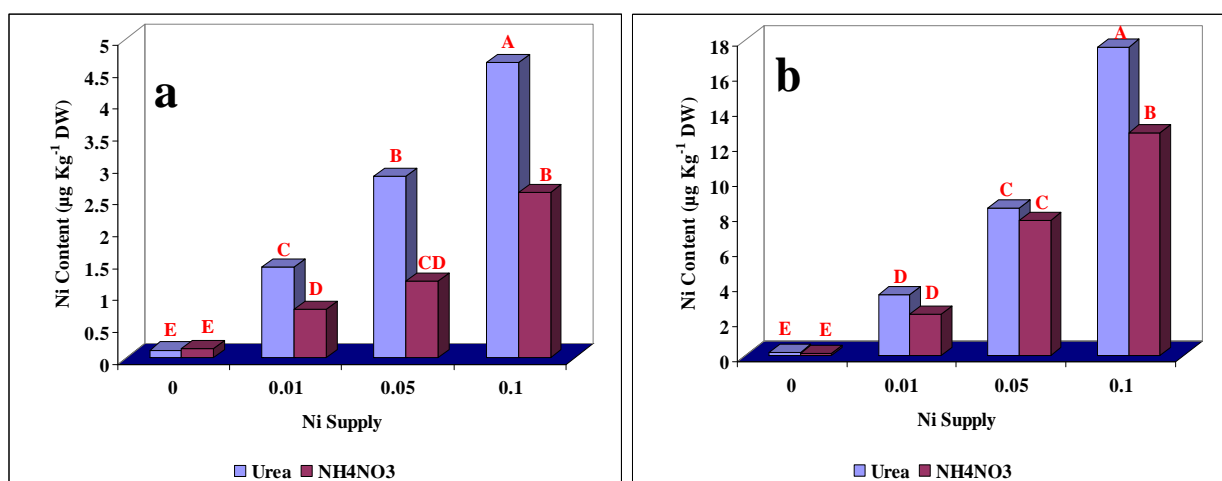


Fig. 1. Absorption of Ni by shoots(a) and roots (b) of maize plants supplied with either urea or ammonium nitrate. Different letters refer to significant differences at the level of $p \leq 0.05$ by Duncan's test

Effects of nitrogen sources and Ni-levels on maize leaves urease activity are shown in Fig. 2. When supplied with ammonium nitrate, urease activity in maize leaves increased with the increase in Ni-concentration up to third levels of Ni ($p \leq 0.05$). In the fourth week, urease activity was different ($p \leq 0.05$) from the other two sampling times (2nd and 6th week). Urease activity in the leaves of plants supplied with urea was increased ($p \leq 0.01$) with the increase in Ni-supply. The enzyme activity at the 4th and 6th week sampling time was different ($p \leq 0.05$) from that in the 2nd week. Forms of N-supply (urea or ammonium nitrate) had a significant ($p \leq 0.05$) effect on urease activity.

Effects of nitrogen sources and Ni-levels on maize roots urease activity are shown in Fig. 3. Times of sampling and various Ni levels had no significant effects on the roots urease activity in plants supplied with ammonium nitrate. However, the roots urease activity in plants given urea was affected by Ni-supply. The effects of the second Ni level (0.05 mg Ni L⁻¹) were different ($p \leq 0.05$) from the others. Urease activity at the second week sampling time was different ($p \leq 0.01$) from the other sampling times. Nitrogen sources (urea or ammonium nitrate) in growth media had a significant effect ($p \leq 0.01$) on the plant roots urease activity.

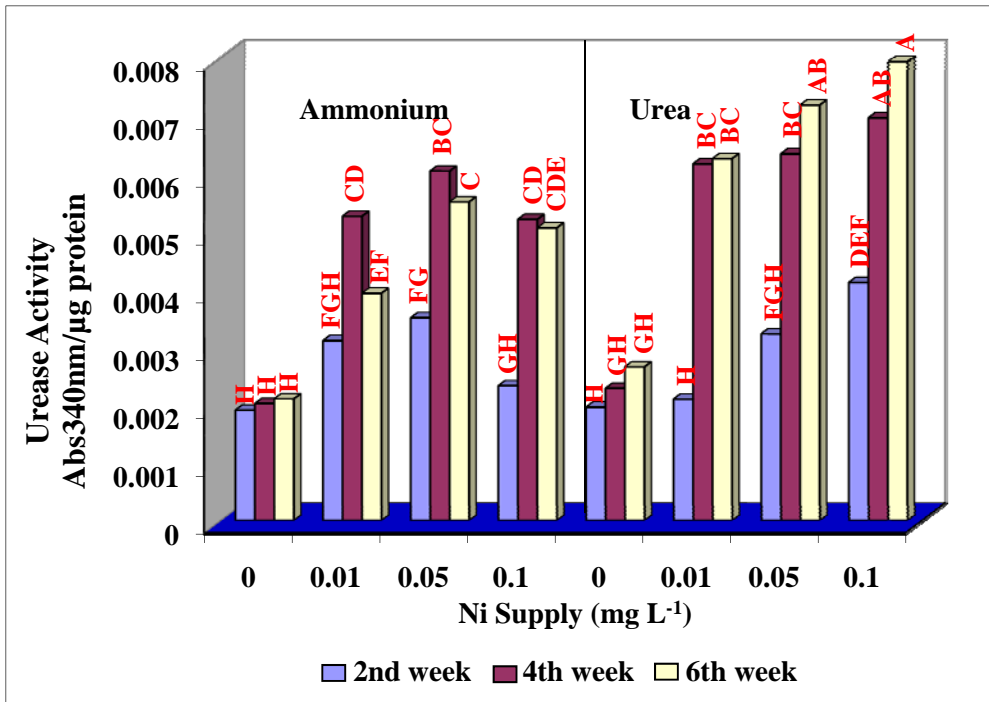


Fig. 2. Influence of nickel concentrations in the nutrient solution on urease activity of maize leaves supplied with urea or ammonium nitrate. Different letters refer to significant differences at the level of $p \leq 0.05$ by Duncan's test

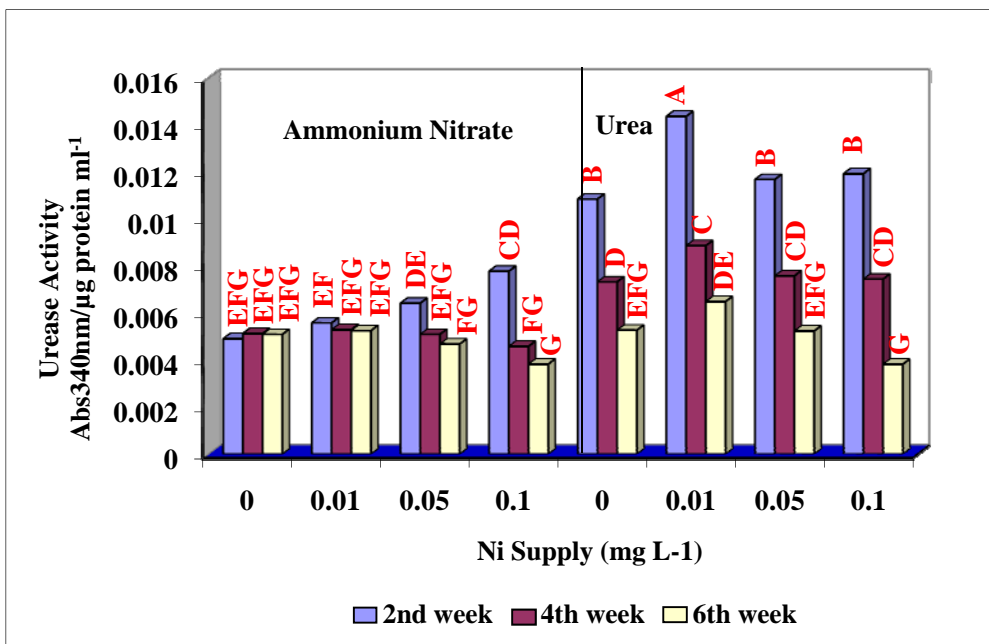


Fig. 3. Influence of nickel concentrations in the nutrient solution on urease activity in roots of maize plants supplied with urea or ammonium nitrate. Different letters refer to significant differences at the level of $p \leq 0.05$ by Duncan's test

Effects of various Ni levels on shoot dry weight in plants grown in media containing urea or ammonium nitrate are shown in Fig. 4. The effects were not significant in plants supplied with ammonium nitrate, although the plants which received the third Ni level (0.05 mg Ni L⁻¹) had the highest shoot dry weight. In contrast, in the plants that received urea as the N-source, the effects of various Ni levels on their shoot dry weight was significant ($p \leq 0.01$). The highest shoot dry weight was obtained in plants supplied with the third and fourth (0.05 and 0.1 mg Ni L⁻¹) Ni levels.

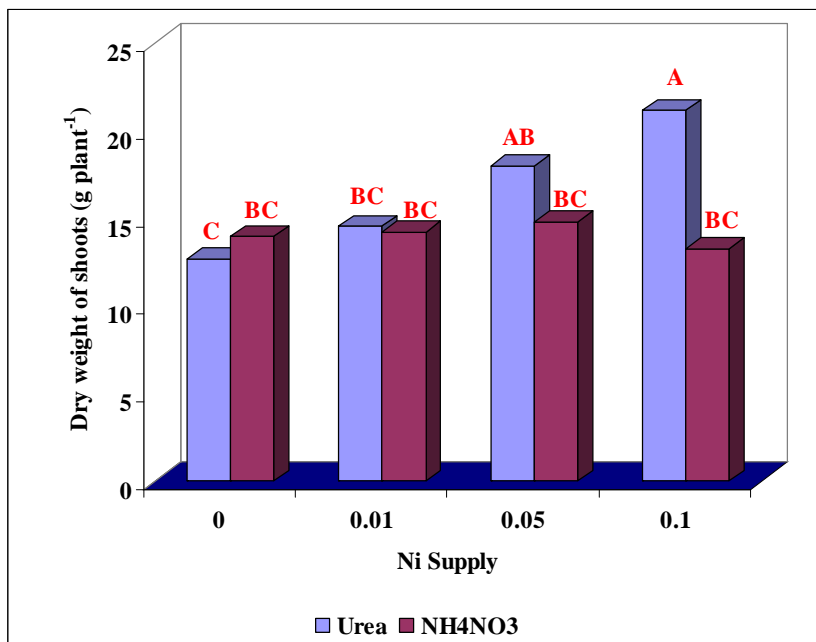


Fig. 4. Influence of nickel concentrations in the nutrient solution on dry matter production in leaves of maize plants supplied with urea or ammonium nitrate. Different letters refer to significant differences at the level of $p \leq 0.05$ by Duncan's test

The effects of various Ni levels on the maize roots dry weight supplied either with ammonium nitrate or urea are shown in Fig. 5. Roots dry weight was the highest at the second Ni level (0.01 mg Ni L⁻¹) in plants grown in media containing ammonium nitrate. However, the difference was not significant among various Ni levels. Dry root weight of plants that received urea was the highest at the second Ni level and the differences among various Ni treatments were significant ($P \leq 0.05$).

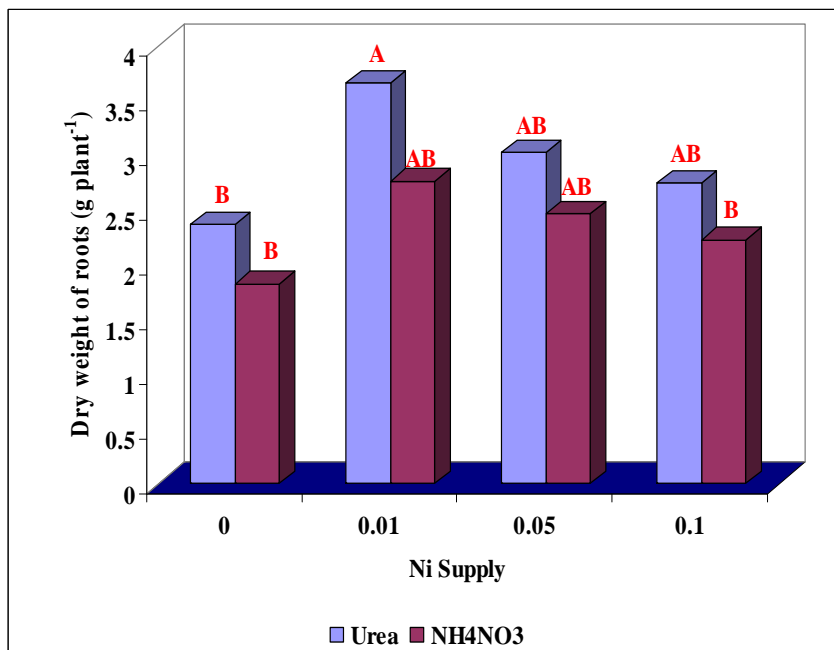


Fig. 5. Influence of nickel concentrations in the nutrient solution on dry matter production in roots of maize plants supplied with urea or ammonium nitrate. Different letters refer to significant differences at the level of $p \leq 0.05$ by Duncan's test

4. DISCUSSION

It has been shown in other plants that Ni is readily taken up by plant roots, and up to a certain concentrations, the rate of its uptake is positively correlated with the external Ni concentrations [44]. In the present study Ni content in plant tissue increased significantly along with the increase in Ni supply. In those plants which were fed on urea, the amounts of Ni in shoots and roots were higher than that of those receiving ammonium nitrate. One may expect that to consume urea as a nitrogen source, the plant prefer to absorb more Ni, thereby increasing urease content. From the results presented here, however, it is more likely that the capacity for Ni uptake, even in those plants fed on urea, was limited so that capacity reached the highest level soon after the treatments (by the second week). Regardless of the forms of nitrogen supply at any external Ni concentration, more Ni was accumulated in the roots than in the shoots (Fig. 1). It should be noted however, that in addition to the external concentration of Ni, the extent to which plants accumulate Ni depends on species, developmental stage and plant organ as well [35, 45 and 46]. Despite the limited uptake of Ni by maize, in general, urease activity of its young leaves significantly increased by increasing the Ni supply, compared to that of the control plants. The positive effects of Ni on dry weight could be due to the detoxification of urea produced in plants by various metabolic pathways [30, 8]. In the absence of Ni, no urea detoxification will take place, resulting in less growth [31, 14 and 8]. In plants supplied with ammonium nitrate the reduction in urease activity at higher Ni concentrations may have resulted from Ni toxicity in the absence of high enough urea to be used as urease substrate. Besides, at relatively high concentrations, Ni interferes and competes with essential divalent cations absorption such as Fe, Mg, Ca and Mn by plant roots [47]. Another important point in plants supplied with ammonium nitrate was the increase in urease activity at all Ni levels up to the 4th week and its decrease in the 6th week. Since Ni is taken up by plants very rapidly, too much Ni accumulation with time could have toxic effects and a decrease in urease activity [44]. A higher rate of urease activity and dry matter production can be attributed to the presence of urea in the media and thus more availability of N-urea for growth and development [27-29, 35, 38-40]. In a similar study Gerendas and Sattelmacher [29] used urea as the nitrogen source and reported a significant increase in urease activity in the leaves of six plants, wheat, soybean, rape, cucurbit, sunflower and rye. In their studies, in the absence of Ni, urease activity was reduced drastically. Similar findings have been reported by Eskew and co-workers [31] for legumes and by Korgmeier and co-workers [17] for tobacco plants. As a whole, urease activity was higher in the roots than in the shoots, both in ammonium nitrate and urea fed plants. This, particularly for urea fed plants, could be attributed to higher Ni accumulation in the roots and the presence of urea as the substrate of urease. Our study emphasizes the importance of Ni for maximum nitrogen efficiency, growth and development and also for prevention of urea toxicity when supplied externally as N-fertilizer or produced in various plant metabolic pathways.

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