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Toxicity symptoms of nickel in common bean[1]

Sintomas de toxidez de níquel em feijoeiro

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Abstract - Despite the importance of nickel (Ni) in the N metabolism of legumes, common bean (*Phaseolus vulgaris* L.) is considered very sensitive to Ni doses. The objective in this study was to characterize the toxicity symptoms of Ni in common bean cv. Pérola. The experiment was conducted in greenhouse in the campus of the Universidade Estadual do Norte Fluminense - Darcy Ribeiro, Campos dos Goytacazes - RJ. Solutions containing 0; 20; 40; 60 and 100 mg L^{-1} of Ni, in the form of nickel chloride hexahydrate ($NiCl_2.6H_2O$), were applied to shoots of common bean plants at the rate of 2.5 mL of solution per plant of common bean, 25 days after sowing. Plants of common bean treated with 0; 20; 40 and 60 mg L^{-1} of Ni showed no toxicity symptoms. Plants treated with 100 mg L^{-1} of Ni showed chlorotic leaves with gray spots that coalesced and became necrotic in a more advanced stage.

Keywords - *Phaseolus vulgaris* L. Chlorosis. Necrosis.

Resumo - Apesar de o Ni ser importante no metabolismo do N em leguminosas, o feijoeiro comum (*Phaseolus vulgaris* L.) é considerado bastante sensível a doses de Ni. Objetivou-se caracterizar os sintomas de fitotoxidez de Ni em feijoeiro comum cv. Pérola. O experimento foi conduzido em casa de vegetação na Universidade Estadual do Norte Fluminense - Darcy Ribeiro, Campos dos Goytacazes - RJ. Foram aplicados 0; 20; 40; 60 e 100 mg L^{-1} de Ni (2,5 mL de solução por planta) na forma de cloreto de níquel hexahidratado - $NiCl_2.6H_2O$, na parte aérea de plantas de feijoeiro comum aos 25 dias após a semeadura. Plantas de feijão tratadas com 0; 20; 40 e 60 mg L^{-1} de Ni não apresentaram sintomas de ftoxidez. Já as plantas que receberam a dose de 100 mg L^{-1} de Ni apresentaram folhas cloróticas e com manchas acinzentadas que coalesceram e se tornaram necróticas em um estádio mais avançado.

Palavras-chave - *Phaseolus vulgaris* L. Clorose. Necrose.

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Introduction

Up until a few years ago, nickel (Ni) was not thought to be essential for plants, with research focusing on its toxic effect (GALDO et al., 2004; KOPITTKE et al., 2007). Studies sought to understand how Ni hyperaccumulator plants are capable of absorbing, accumulating and tolerating high concentrations of this element in their tissues aiming at the use of techniques such as phytoextraction to recover contaminated land (BROADSHURTS, 2009; GIORDANO et al., 2005; INGLE et al., 2005). However, the finding that Ni is a component of the enzyme urease, which is present in numerous plant species, has drawn scientific interest from researches relating to its role in higher plants. Since then, plant developmental responses to Ni fertilization have been obtained under field conditions (WOOD et al., 2004a, b e c) and in nutrient solutions (NEVES et al., 2007; TAN; IKEDA, 2000).

Reports on developmental responses to Ni application have become common, as well as the variability among plant species and even cultivars of the same species in requirements and tissue tolerance to Ni. Small Ni amounts (0.01 to 5 $\mu g\ g^{-1}$ of dry matter) are enough to meet the needs of some higher plant species. However, high Ni concentrations can cause toxicity to plants (SEREGIN; KOZHEVNIKOVA, 2006) and even death, as verified by Berton et al. (2006), by applying 210 mg kg^{-1} of nickel sulphate ($NiSO_4.6H_2O$) in the soil.

Nickel toxic effects produce various injury symptoms, including development impairment, physiological disorders in the photosynthetic process, transport of photoassimilates, mineral nutrition, water potential of plants, chlorosis, necrosis and wilting (KRUPA et al., 1993; PANDEY; SHARMA, 2002).

Nickel stress interferes with the activity of superoxide dismutase, with possible accumulation of hydrogen peroxide (H_2O_2) in leaf tissue. It has been suggested that H_2O_2 inhibits the growth of plants treated with heavy metal (CHEN et al. 2000), acting as a substrate for peroxidases involved in the hardening of cell walls, which leads to restrictions to cell elongation (DÍAZ et al., 2001).

In a study assessing the Ni effect on the content of reactive oxygen species and oxidative enzyme activity in leaves of wheat, Gajewska and Skolodowska (2007) reported that seedlings treated with 100 µM of Ni accumulated O_2^- and H_2O_2 in the leaf tissue. The increased content of reactive oxygen species was followed by inhibition in the activity of the enzymes superoxide dismutase and catalase, which are responsible for detoxifying hydrogen peroxide.

Despite the Ni importance for the N metabolism in legumes, common bean plants are considered very sensitive to Ni rates compared with other crops. Common bean plants have reduced yield significantly when the levels of Ni in the leaves were above 40 mg kg^{-1}, as well as negative effect on levels of total chlorophyll caused by a lower Mg absorption by plants under high Ni doses (PICCINI; MALAVOLTA, 1992). In soil the dose 2.3 mg kg^{-1} of Ni as $NiSO_4.6H_2O$ was sufficient to increase the metal content of the grains above 5 mg kg^{-1} fresh matter and made them unfit for human consumption (BERTON et al., 2006).

The objective in this work was to characterize toxicity symptoms of nickel in common bean cv. Pérola.

Materials and methods

The experiment was conducted in a greenhouse at the Division of Plant Mineral Nutrition of the Universidade Estadual do Norte Fluminense - Darcy Ribeiro, Campos dos Goytacazes - RJ. Common bean cv. Pérola was used as test plant.

Samples of Dystrophic Red Yellow Tb Argisol were collected at 0-20 cm depths (TAB. 1).

Table 1 - Chemical characteristics of the Dystrophic Red Yellow Tb Argisol, Campos dos Goytacazes - RJ, 2008

pH*	CE	P	K	Ca	Mg	Al	H+Al	Na	B	Mo	Mn
	µS	----- mg kg^{-1}-----		---------- $mmol_c\ kg^{-1}$ ----------				---------- mg kg^{-1} ----------			
5.62	119.71	19	180	98.6	13.4	0.5	35.06	0.41	0.51	0.021	38.1
Zn	Cu	Fe	Ni	S	C	MO	T	t	SB	V	mt
---------- mg kg^{-1} ----------				------ g kg^{-1} ------			-------- $mmol_c\ kg^{-1}$ --------			-------- % --------	
3.8	0.59	86.9	0.23	4.60	13.51	23.59	152.08	117.52	117.02	77	0.42

* pH at H_2O 1:2.5

The experiment was arranged in a randomized block design (RBD) with four replications. The treatments consisted of nickel concentrations at 0; 20; 40; 60 and 100 mg L^{-1} (2.5 mL of solution per plant) in the form of nickel chloride hexahydrate - $NiCl_2.6H_2O$.

Plants development, mineral composition of stems and leaves, as well as, urea hydrolyses in those tissues were evaluated. However, in this work it will just be shown the characterization of the toxicity symptoms of nickel, observed in plants treated with 100 mg L^{-1} of Ni.

Each experimental unit consisted of a vase with 5-kg soil capacity and four plants. Each Ni solution was added with the spreader-sticker adjuvant Adesil® (0.2 mL L^{-1}) at the time of foliar spray. The Ni solution was applied twenty-five days after sowing, when plants were at the V_4 stage, which is the vegetative stage when the third trifoliate leaf was fully expanded and the plant started branching.

Results and discussion

Plants of common bean treated with 0; 20; 40 and 60 mg L^{-1} of Ni showed no toxicity symptoms. However, bean plants that received 100 mg L^{-1} of Ni showed visual symptoms of toxicity characterized by leaf chlorosis (FIG. 1). The toxicity symptoms were observed 24 hours after Ni application. The effects appeared in young and mature tissues with predominance and higher intensity in more mature tissues.

Leaf chlorosis observed may be due to the presence of Ni, which at high levels can affect pigment accumulation in the plant, reflecting changes in the chlorophyll/ carotenoid ratio and chlorophyll *a/b*, in which carotenoids are more sensitive than chlorophyll, and chlorophyll *b* is more susceptible than chlorophyll *a*. Thus, the evolution of this effect reaches the photosynthetic apparatus of the plant, interfering with the photochemical efficiency of photosystem II. Both photosystem I and II are inhibited by the Ni concentration, but the photosystem II is more strongly affected by disturbances in the Calvin cycle and the inhibition of electron transport, because of excessive amounts of ATP and NADPH accumulated by the inefficiency of reactions taking place in the dark phase (KRUPA et al., 1993).

Although the toxicity caused by heavy metals is widely studied in plants, the mechanisms of Ni toxicity still remain unclear. However, numerous evidences indicate that the Ni toxicity can be attributed also to oxidative stress at the cellular level (BACCOUCH et al., 2001; GONNELLI et al., 2001). Under stress conditions, including exposure to excessive concentrations of heavy metals, there is imbalance and removal of reactive oxygen

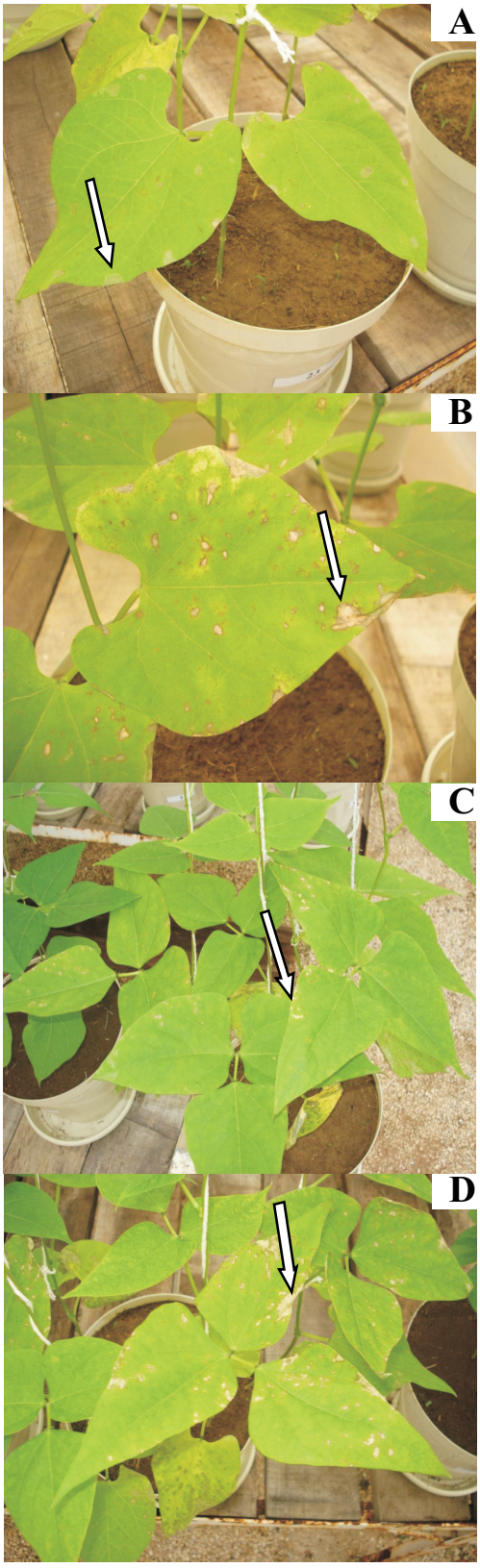

Figure 1 - Toxicity symptoms in common bean cv. Pérola grown in Red Yellow Tb Argisol after Ni foliar application of nickel. (A) Initial gray spots, (B, C and D) Chlorosis and necrosis

species in plant tissues (GRATÃO et al., 2005; GRATÃO et al., 2008). This may consequently lead to oxidative damage of macromolecules such as lipids, proteins and nucleic acids (KEHRER, 2000).

Besides chlorosis, young leaves and/or mature leaves initially showed random and irregular gray spots in the limbo, which at more advanced stages, coalesced and became necrotic. These results are similar to those reported by (PICCINI; MALAVOLTA, 1992) for common beans and may be related to hindering respiratory activity and denaturation of the cell protoplasm, which leads to tissue death in these areas.

Conclusion

Nickel toxicity in common bean plants is characterized by chlorotic leaves with gray spots that coalesce and become necrotic in a more advanced stage.

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