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Heavy metal detoxification by organic acids in barley seedlings

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ABSTRACT Changes in the amount of metal complexing organic acids were investigated in barley leaves during 0,1, 0,5, 1mM Ni treatment parallel with the measurement of Ni accumulation. The content of five organic acids (oxaloacetate, citrate, malate, succinate and fumarate) was measured in the leaves of barley seedlings hoping to identify them as parts of nickel detoxification mechanisms. Besides a 3 fold increase of citrate and malate, the amount of oxalate and fumarate increased by 5-6 fold. It was found that oxalate and fumarate are also take part in nickel complexation mechanisms. **Acta Biol Szeged 49(1-2):99-101 (2005)**

KEY WORDS

nickel
organic acids
HPLC

Once an essential element is absorbed by root cells, mechanisms must be present to maintain the solubility and further availability for use in mineral nutrition. Similar processes affect polluting elements as well. Microelements, such as nickel can get into plants easily, since their uptake channels are developed through the evolution. Nickel, is an essential heavy metal in the forming of urease enzyme (Klucas et al. 1983). When in excess amount, nickel tolerance mostly achieved through organic acids. Organic acids, such as citrate, fumarate, malate, succinate and oxaloacetate play an important role in several major metabolic pathways in plants such as in fatty acid biosynthesis and oxidation, glyoxalate acid cycle, carbohydrate biosynthesis and especially have significance in the citrate cycle of the respiratory processes (Popova and Pinheiro de Cavalho 1998). Citrate and malate are supposed to be responsible for chelation and transportation of nickel into the vacuoles (Brooks et al. 1981), however citrate seems to be most likely the nickel chelator (Tatár et al. 2000). On the other hand we did not find information whether other organic acids of citratre cycle also take part in the detoxification mechanisms or not. That is why we investigated the amount of five organic acids (oxaloacetate, citrate, malate, succinate and fumarate) in barley seedlings hoping to identify more that play role in nickel detoxification mechanisms.

Materials and Methods

Growth conditions

Barley (*Hordeum vulgare* L. „Triangel") seeds germinated for three days then grown on modified Hoagland solution (Hegedűs et al. 2001) for seven days. 0,1; 0,5 and 1mM $NiSO_4$ was added to the solution of ten days old seedlings. The growth conditions were: 20°C; 90 µE $m_{-2}\cdot s_{-1}$ light intensity; 12-12 h light/dark period. Seedlings were sampled for organic acid, and Ni content measurement 1, 4 and 7 days after treatment. The experiment was repeated three times.

Organic acid content measurement

Shoots (0.8 g) of barley seedlings were ground in liquid nitrogen and quartz sand then were centrifuged then filtered through 45 µm nylon membrane of which 20 µl and the measurement of organic acids with RP HPLC (Waters).

Ni content measurement

0.2 g of powdered dry material was dissolved in 2 cm^3 of HNO_3:H_2O_2 mixture (1:1 v/v). The nickel content of the filtered solution was determined by an ICAP 61E Plasma Spectrometer (Horváth et al. 1996).

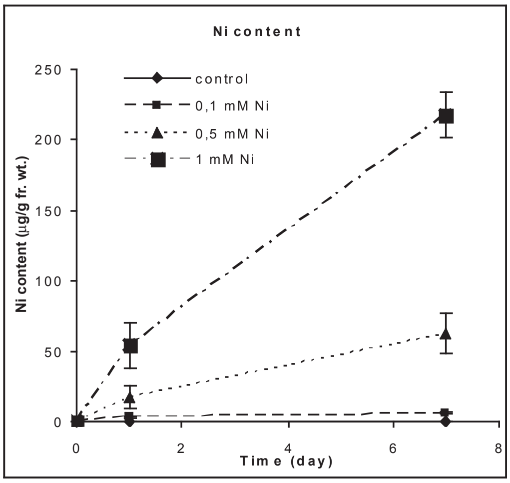

Figure 1. Ni content of leaves of barley seedlings.

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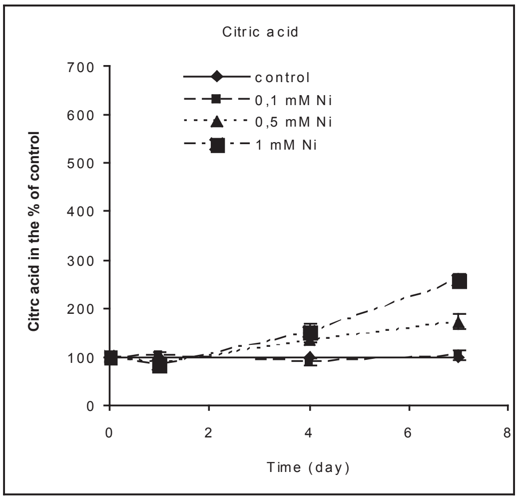

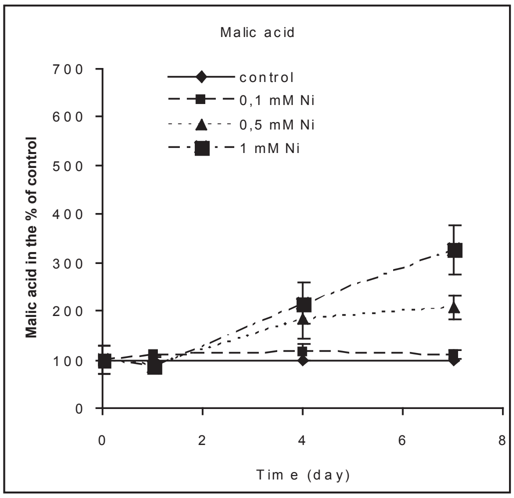

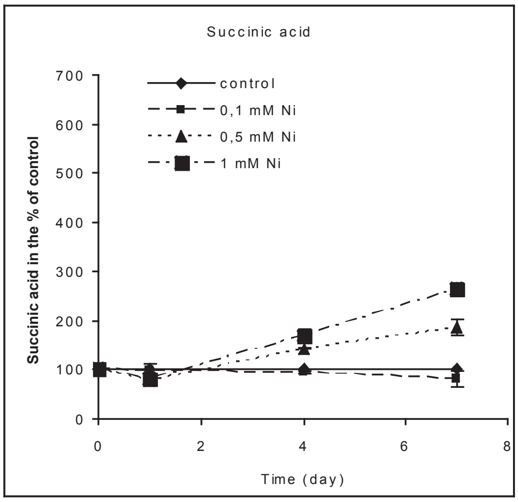

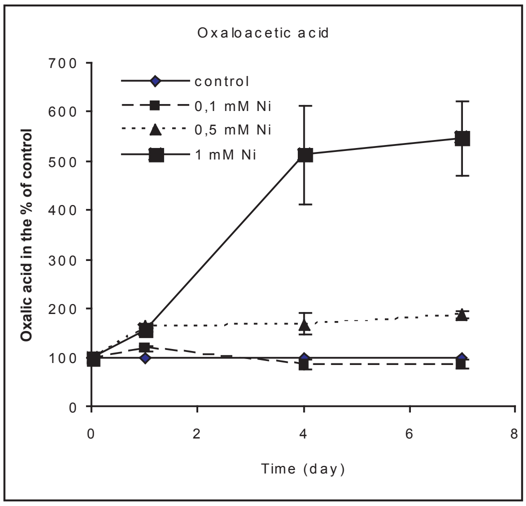

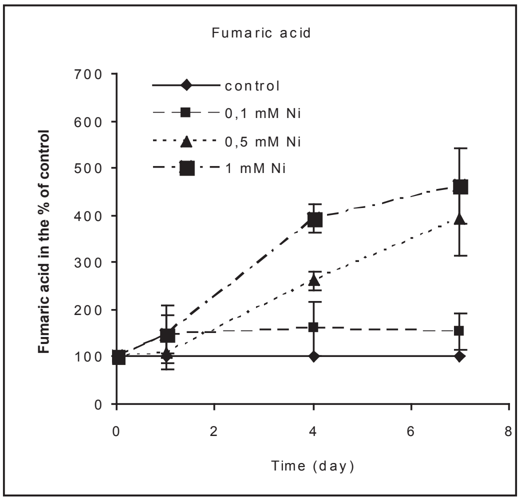

Figure 2. Changes of organic acid contents of in the percentage of control.

Results

The nickel accumulation increased significantly, although the slope of the curves was not proportional to the applied concentration. After 7 days of treatment, the nickel content of the 1 mM $NiSO_4$ treated seedlings was 250 times than that of the controls, but at 0,1 mM treatment it was only six times higher (Fig. 1).

The organic acid content of nickel treated seedlings increased during the experiment in a concentration dependent way (Fig. 2), although not in a similar rate. In the 1 mM nickel treated seedlings the oxalate and fumarate contents were approximately six times higher, but the rest of the five identified organic acid (malate, citrate and succinate) contents were only three times higher than that of the control. Furthermore, the content of oxalate- and fumarate increased during the first day of nickel treatment, whereas the others began to increase later, between the first and fourth day.

Treatments with 0,5 mM nickel also increased all organic acid contents, but only approximately to their double except for fumarate (four times higher). The lowest nickel concentration caused the most pronounced change in the level of fumarate. It has to be noted that the absolute values of fumarate and oxaloacetate were approximately one and two orders of magnitude lower than the values of malate, citrate and succinate (data not shown).

Discussion

Barley leaves are able to accumulate considerable amount of nickel in a short time, but the highest concentration of nickel in the mineral solution (1mM) causes more rapid accumulation than the lowest. (0,1mM). As consequence of nickel accumulation, the organic acid content increased, which shows an enhanced need for these compounds in order to maintain homeostatic conditions. Tatár et al. found citrate, malate and fumarate in the xylem sap of cucumber, indicating that these organic acids take part in the transport mechanism of nickel. According to our findings, the amount of oxaloacetate and succinate also increased. Consequently, they also play role in the detoxification mechanisms of nickel poisoning, not in the transportation, but in the chelation processes, since the majority of the accumulated organic acids are stored in the vacuole to where the transportation of nickel directs (Farré et al. 2001). Citrate and malate supposed to chelate nickel however there is no direct evidence for this (Brooks et al. 1998; Tatár et al. 2000).

Although the absolute value of oxaloacetate and fumarate was much lower than in case of malate, citrate or succinate, they seemed to react the most rapidly to increased nickel availability. In order to achieve the increased amount of citrate and malate, which are the most abundant organic acids in barley leaves; first, other intermediates of the Krebs cycle are synthesized in an enhanced level. Furthermore, some of the components of mitochondrial electron transport chain of Krebs cycle leak out a few electrons and produce free oxygen radicals. It is believed that heavy metals enhance these leakages. The probable sites seem to be complex I and ubiquinone-cytochrome b region (Lund et al. 1991). The rapid rise of fumarate and oxaloacetate level may be related to this change of the electron transport route.

Acknowledgements

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