SOME PHYSIOLOGICAL ASPECTS OF LEAD PHYTOTOXICITY

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1. Introduction

Lead phytotoxicity was examined using bean plants (*Phaseolus vulgaris* L. cv. 'Limburgse vroege') grown on hydroponics over a range of 14 concentrations of lead. Plant development was observed and the capacity of some enzymes known to be induced under oxidative stress was measured in primary leaves and roots to determine whether Pb induces oxidative stress. Soluble protein content was measured as another criterion for evaluating harmful effects of Pb. Additionally Pb content in primary leaves was determined.

2. Materials and Methods

Seeds of bean plants were germinated in wet rockwool at 22 °C during 96 h, after vernalisation (72 h). Seedlings with a root length of app. 1,5 cm were grown in 3-mm thick polystyrene squares (13 seedlings per 289 cm^2 polystyrene) by fixing the roots through 5-mm holes. The polystyrene was floated on 3 l of Hoagland solution in 3.5 l polyethylene beakers. The plants were grown for another 14 days under controlled environmental conditions (temperature: 22 °C, relative humidity: 65 %, photoperiod: 12 h light/12 h darkness, photosynthetically active radiation: 150 µmol m^{-2} s^{-1}). Lead was added as $Pb(NO_3)_2$, complexed with EDTA (1/1) in 15 different treatments: 0 (control), 2, 5, 10, 20, 30, 40, 50, 65, 80, 100, 125, 150, 200, 400 µM. In the control no EDTA was used since no effects on enzyme capacties have been detected due to EDTA (results not shown).

Morphological parameters were measured at harvest (14 days after sowing): weight of aerial parts and of primary leaves, primary leaf area (LiCor type LI-3000 areameter), stem length and root weight.

Plant material was stored at –70°C; enzyme capacities were determined spectrophotometrically (Shimadzu UV-1602) in primary leaves and roots: Ascorbate peroxidase (APOD), Dehydroxy ascorbate reductase (DHAR), Glutathion reductase (GLUR), Guajacol peroxidase (GPOD), Superoxide dismutase (SOD) and Syringaldazin peroxidase (SPOD). Preparation of the samples is described by Van Assche et al. (1988); results are expressed in mU per g fresh weight.

Soluble protein content was measured in primary leaves and roots using the Biorad method (Bradford, 1976). Enzyme capacities and protein content could not be measured on plants treated with 400 µM Pb due to the limited amount of biomass obtained. Primary leaves were used for Pb analysis with AAS (Perkin Elmer 1100B), after extraction with $HNO_3/HClO_4$ (Milestone MLS-1200 MEGA).

3. Results and Discussion

Morphological analysis showed that Pb strongly reduced the root growth, already visible at 80 µM Pb. Root weight was reduced by 90 % at 400 µM (0.22 g vs 2.15 g in control). Above ground parameters stem length and leaf surface started to decline from 150 µM. At 400 µM, both organs were reduced respectively by 42 and 57 % in comparison to control plants. Decline of aerial parts and primary leaves was only noticeable at 400 µM, and for both parameters decrease was around 50 % as compared to control plants.

In primary leaves, induction of enzyme capacity was measured for 2 enzymes. At 200 µM Pb, the capacity of DHAR and GPOD increased respectively with a factor 154 and 333 % in

comparison to the control. Induction was also noticeable at 125 µM for GPOD (fig. 1), and at 150 µM for DHAR. In roots, 5 enzymes were induced: GLUR (110 %), APOD (522 %), SOD (299 %), GPOD (300 %)(fig. 1) and SPOD (260 %). Increase was detectable at 100 µM for SPOD, and at 125 µM for GLUR, APOD, SOD and GPOD.
In roots, soluble protein content (fig. 2) was increased with 70 %, first detectable at 125 µM. In primary leaves, this content remained unchanged.
A linear relation was observed between lead concentration in the nutrient solution and lead content in primary leaves: at 400 µM Pb up to 2 370 mg Pb kg^{-1} dry weight was accumulated.

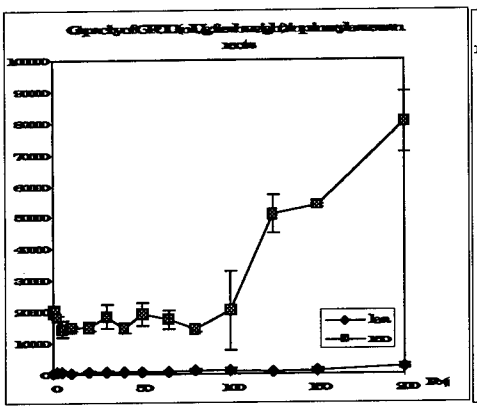

Fig. 1: Capacity of GPOD (mU/g fresh weight) as a function of Pb added in primary leaves and roots of 18 days old *Phaseolus vulgaris* seedlings grown on hydroponics.

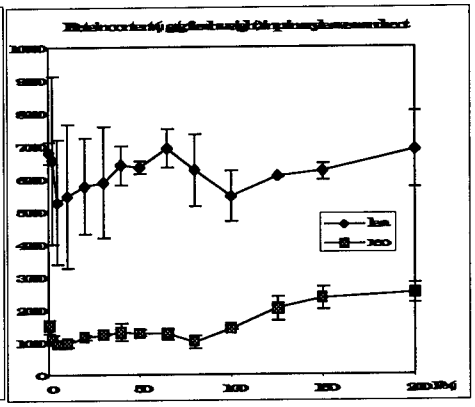

Fig. 2: Soluble protein content (µg/g fresh weight) as a function of Pb added in primary leaves and roots of 18 days old *Phaseolus vulgaris* seedlings grown on hydroponics.

4. Conclusions

Morphological analysis shows that roots respond faster and more intensively to elevated Pb concentrations than above ground plant parts: root weight is declined by 90 % while reduction of aerial plant parts is around 50 % when 400 µM Pb is added. The increased enzyme capacity in roots and partly in primary leaves indicates that oxidative stress is produced. The question however is open whether the capacity of the enzymes not induced here would increase at concentrations above 200 µM Pb. The high Pb content found in primary leaves suggests that Pb is present in the plant as Pb-EDTA since in this form lead can easely be transported to the upper plant parts (Blaylock at al., 1997; Huang et al., 1997). The linear relation found between Pb content in nutrient solution and in primary leaves suggests that this uptake is passive.

5. References

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