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The influence of lead ions on nitrogen metabolism of lupin embryos cultivated *in vitro**

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Legumes show rather high tolerance to different types of environment [1]. One of the reasons for this tolerance may be high nitrogen supply due to symbiosis with Rhizobium.

To test the possible role of nitrogen supply in overcoming the negative impact of lead ions on lupin embryos the effect of lead on the activities of the oxidative (MDH[1]) and fermentative respiratory enzymes (ADH) was studied. MDH is a key enzyme of the main anaplerotic pathway leading to assimilation of nitrogen into organic compounds (2 - 4). ADH activities might be of importance because Pb inhibits the mitochondrial respiration (not shown). Aspartate aminotransferase (AspAT), the enzyme of nitrogen metabolism has been included for comparative purposes.

The seeds of *Lupinus luteus* L. cv Ventus were sterilized with 75% ethanol for 1 min, followed by 8% H_2O_2 for 10 min, and washing 5 times in sterile water. The portions of 10 seeds were swelled for 24 h on Petri dishes and the embryonic axes isolated under sterile conditions were transferred either to Hoagland complete medium [Table 1; (1, 2, 3, 4)] or the same medium devoid of nitrogen [Table 1; (5, 6, 7, 8)]. After three days of cultivation, the lupin embryos were re-transferred to the same media either containing nitrogen and/or lead or devoid of these components (3 and 7). $PbCl_2$ was used at the concentration of 38 mM, i.e. at the concentration which reduces growth by 50% of lupin seedlings inhibition (Rucińska, R., Przymusiński, R. & Gwóźdź, E., in preparation).

After 24 h treatment the embryos were withdrawn for the analysis. The activity of the following enzymes was measured using the Specord spectrophotometer at 25°C; alcohol dehydrogenase (ADH, EC 1.1.1.1) according to [5], cytoplasmic malate dehydrogenase (MDH, EC 1.1.1.37) according to [6] and aspartate aminotransferase (EC 2.6.1.1.) according to [7]. Electrophoresis in 8% polyacrylamide non-denatured gel was performed after [8], staining for detection of ADH and MDH isoenzymes was carried out according [9] and that of AspAT after [10].

Lead at 38 μM concentration decreased the activities of MDH and AspAT both in the nitrogen starved and non-starved embryos; this effect was less evident in the case of the former enzyme: in the non starved cultures MDH and AspAT were inhibited by about 10 and 30%, respectively (Table 1 expt. 3 *vs* 4) while in the nitrogen-starved embryos the inhibition reached effect was greater and amounted up to 20 and 65%, respectively (7 *vs* 8).

Thus this quantitative difference in the response of the two enzymes to Pb is associated with the difference in the reaction to nitrogen availability. Moreover the addition of nitrogen to both types of cultures reduced evidently Pb toxicity affecting MDH and AspAT activities (2 *vs* 4 and 6 *vs* 8). Nitrogen added to the non-

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[1]Abbreviations: ADH, alcohol dehydrogenase; AspAT, aspartate aminotransferase; MDH, malate dehydrogenase

Table 1

The effect of lead on the activity of malate dehydrogenase (MDH), alcohol dehydrogenase (ADH) and aspartate aminotransferase (AspAT) in the nitrogen-starved and non-starved lupin embryos.

The embryos were kept for 3 days in the nutrient media containing nitrogen (non-starved cultures, variants 1 to 4) and on the nutrient media devoid of nitrogen (starved cultures, variants 5 to 8). After three days, the embryos were incubated for 24 h on the media devoid of lead: 1, 3, 5, 7, and supplied with lead: 2, 4, 6, 8. The means with confidence limit ($P < 0.05$) of the results are given

Cultures	Treatment	Enzymatic activity (U/shoot)		
		MDH	ADH	AspAT
I. Nitrogen non-starved cultures	1. Nitrogen	8.01 ± 0.25	0.125 ± 0.006	0.818 ± 0.030
	2. Nitrogen + lead	7.48 ± 0.22	0.358 ± 0.018	0.622 ± 0.026
	3. None	7.62 ± 0.19	0.147 ± 0.005	0.760 ± 0.028
	4. Lead	6.95 ± 0.26	0.380 ± 0.022	0.520 ± 0.026
II. Nitrogen starved cultures	5. Nitrogen	9.40 ± 0.31	0.211 ± 0.011	0.693 ± 0.033
	6. Nitrogen + lead	8.59 ± 0.34	0.326 ± 0.020	0.434 ± 0.020
	7. None	6.10 ± 0.12	0.240 ± 0.011	0.517 ± 0.021
	8. Lead	4.98 ± 0.12	0.292 ± 0.021	0.190 ± 0.014

starved embryos reduced Pb effect on MDH activity by 30% and that of AspAT by 15% (2 *vs* 4) while when added to the starved embryos the increase in both activities amounted up to 40 and 220%, respectively (6 *vs* 8). The protective effect of nitrogen may be partially due to either induction of the synthesis of both enzymes and/or activation by metabolites: the MDH activity and AspAT activities were raised by nitrogen in the nitrogen starved embryos by 30 and 60%, respectively.

However, the data obtained imply a more complex interaction of N with Pb since the effect of nitrogen in the intoxicated embryos was higher than the effect in the control non Pb treated embryos.

The response of ADH to Pb was dramatically different as Pb stimulated this activity by about 60% (3 *vs* 4) in the nitrogen non-starved cultures, but only negligible in the starved cultures (7 *vs* 8). Nitrogen added to the non-starved Pb intoxicated embryos had practically no effect (2 *vs* 4) and but a slight stimulating effect (40%) in the nitrogen starved cultures.

Moreover, nitrogen did not induce/activate ADH activity in the control embryos (1 *vs* 3 and 5 *vs* 7). These latter results are consistent with a lack of nitrogen effect on ADH activity irrespective of Pb toxicity.

The mechanism of Pb stimulation of ADH activity should be elucidated. In connection with this finding it is noteworthy that Pb had no effect on the spectrum of MDH and AspAT isoenzymes while a new isoform of ADH appeared on the Pb treated embryos (not shown).

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