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Influence of heavy metals on the accumulation of trimethylglycine, putrescine and spermine in food plants*

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Summary. Increased contents of cadmium (Cd), lead (Pb), zinc (Zn) and other heavy metals in barley plants enhanced the accumulation of trimethylglycine (betaine), putrescine and spermine. Higher contents of heavy metals in barley were caused by soil enrichment with heavy metals and by soil salinity. The highest accumulation of spermine and betaine (increase 3-fold or 5-fold in comparison to untreated soil substrates) was obtained at the highest concentration of heavy metals in plants. Consequently the betaine-N / protein-N-ratio and the spermine-N / protein-N-quotient increased 3-fold in plants with high heavy metal contents. The biomass formation was not changed significantly by the different experimental treatments.

Keywords: Amino acids – Heavy metals – Salinity – Barley – Trimethylglycine – Putrescine – Spermine

Introduction

Cadmium (Cd) promotes the formation and exudation of proteinogenic and non-protein amino acids in maize roots (Bergmann et al., 1996) and the biosynthesis of polyamines in plants, as well (Smith, 1985; Bergmann, 1996). A higher production of non-protein amino acids (nicotinamine, mugineic acid) was also obtained when essential microelements as iron, copper or zinc are in a minimum in relation to Cd (unpublished, Marscher, 1995; Stephan et al., 1996). Salinity affects bioavailability of Cd and Zn in soils and modifies the translocation of these elements in *Leucaena leucocephala* (Helal et al., 1999).

Because soil degradation and soil contamination change the bioavailability of essential and non-essential elements in soils, the effect of plant-available Cd and other heavy metals on the metal concentration and the

*Part of an oral communication presented at the 7th International Congress on Amino Acids, Bonn FRG, August 3–7, 1999 contents of trimethylglycine (betaine), putrescine and spermine were examined in barley under the influence of different soil salinity or water shortage.

Material and methods

Plant cultivation

Seeds of barley (*Hordeum vulgare L.* cv. Alexis) or spinach (*Spinacia oleracea L.* cv. Matador) were sown in Mitscherlich pots filled with 6 kg sandy substrate according to Leinhos and Bergmann (1995). The contents of macronutrients and micronutrients allowed a normal plant growth. The test-specific enrichment of soil substrates with heavy metals or NaCl is shown in Table 1.

Determination of heavy metals

The heavy metal content in the plants was determined after dry-ashing (two times, 480°C, addition of 10% NH_4NO_3 solution) by means of flame-AAS (see Machelett et al., 1998).

Determination of trimethylglycine and trigonelline

The plant material was extracted with methanol/chloroform/water [70/20/10;(v/v/v)]. The purification of the extracts was carried out by ion exchange chromatography. The qualitative and quantitative determination of trimethylglycine and trigonelline was done by HPLC following a method described by Eckert et al., 1992 (isocratic elution; column: SCX, Partisil).

Determination of putrescine and spermine

The plant material was extracted with perchloric acid (5%). After further purification the extracts were derivatized with benzoyl chloride. Quantification of the derivatized extracts was done by HPLC (isocratic elution, C18-column, silica gel: Flores and Galston, 1982).

Results and discussion

Increased contents of cadmium (Cd), lead (Pb), zinc (Zn) and other heavy metals in barley plants enhanced the accumulation of trimethylglycine (betaine), putrescine and spermine (Table 1). Higher concentrations of heavy metals in the above-ground biomass were caused by an enrichment of sandy substrates with heavy metals in water-soluble forms (Table 1, No. 2) and by addition of NaCl to the sandy substrate (Table 1, No. 4, 5, 6). The higher concentration of heavy metals or betaine and spermine was not caused by growth inhibition because the soil enrichment with heavy metals or NaCl did not affect the plant growth (unpublished). Consequently the amounts of betaine and spermine rose in the individual plants (see Table 2 on the right).

In order to distinguish metal-induced changes in betaine accumulation from the NaCl-effect the separate influence is shown in Table 1 (No. 2 and 3

Table 1. Heavy metal-stimulated accumulation of trimethylglycine (betaine), trigonelline and polyamines in barley plants (*Hordeum vulgare L.*)

No.	Soil enrichment with:		Contents of heavy metals in plants (mg kg^{-1} dry matter, stalks + ears)						Contents of N-metabolites[3,4] (mg kg^{-1} dry matter)			
	Heavy metals	NaCl	Cd	Pb	Zn	Cu	Ni	Mn	Betaine	Trigonelline	Putrescine	Spermine
1	− (No)[1]	− (No)	0.1	1.8	33	3.3	0.6	144	140[(5)]	4.6	13.7	98
2	+ (Yes)[2]	−	1.4^{+}	2.5	121^{+}	$4.7^{(+)}$	0.8	162	$197^{(+)}$	$6.5^{(+)}$	$16.0^{(+)}$	105
3	−	+ (0.1%)	0.2	1.8	43	3.5	0.6	148	$201^{(+)}$	$2.9^{(+)}$	$16.2^{(+)}$	103
4	+	+ (0.1%)	2.6^{+}	$2.6^{(+)}$	168^{+}	$4.8^{(+)}$	0.8	188	421^{+}	3.8	$16.4^{(+)}$	123
5	+	+ (0.3%)	4.3^{++}	$3.0^{(+)}$	178^{+}	6.1^{+}	1.6^{+}	255	541^{++}		$15.5^{(+)}$	121
6	+	+ (0.6%)	5.8^{++}	$6.3^{(++)}$	140^{+}	7.7^{+}	1.8^{+}	265	686^{++}		22.2^{+}	341^{+}

Significance: $^{+}\alpha \leq 0.05$; $^{++}\alpha \leq 0.01$; $^{(+)}\alpha \leq 0.1$.

[1] Heavy metal content in uncontaminated soil substrate (in mg kg^{-1}): Cd 0.12 mg, Pb 37 mg, Zn 10 mg, Cu 8 mg, Ni 7 mg, Mn 23 mg.

[2] Soil enrichment with heavy metals (in mg kg^{-1} = ppm): Cd 1.5 mg, Zn 40 mg, Cu 20 mg, Ni 7 mg as water soluble salts.

[3] The yield of above-ground biomass was not reduced by soil treatments.

[4] In an experiment with spinach (*Spinacia oleracea L.*) a soil contamination with 1.2 ppm Cd or 40 ppm Pb resulted in a 20-fold higher content of Cd or a 4-fold higher concentration of Pb in spinach leaves followed by higher contents in betaine (increase 200%) and spermine (50%).

[(5)] Water shortage by lowering soil moisture, from 60% of water capacity to 40%, caused an increase of betaine content about 100%. Under drought and the soil enrichment with heavy metals and NaCl (according to variant No. 5) the betaine accumulation increased up to 1,100%.

Table 2. Influence of different cadmium : copper ratios on the quotients of betaine-N / protein-N and spermine-N / protein-N

Variants[1] according to Table 1	Ratio[2] of Cd : Cu [Mol/Mol]	Quotient[3] Betaine-N[mg] / Protein-N [g]	Quotient[3] Spermine-N[mg] / Protein-N [g]	Quotient[3] Spermine-N[mg] / Putrescine-N [mg]	Amounts[3] [in μg plant^{-1}]	
					Betaine	Spermine
A 1	0.017 : 1	0.72	0.107	6.1	200.6	14.1
2	$0.168:1^{+}$	$0.99^{(+)}$	0.126	6.8	$350.6^{(+)}$	16.9
3	0.034 : 1	$1.27^{(+)}$	0.110	5.8	$362.6^{(+)}$	18.7
4	$0.305:1^{++}$	2.20^{+}	0.135	7.4	822.3^{+}	$23.9^{(+)}$
5	$0.396:1^{++}$	2.80^{+}	0.187^{+}	$9.6^{(+)}$	872.1^{+}	27.7^{+}
6	$0.426:1^{++}$	3.30^{+}	0.351^{++}	13.2^{+}	663.1^{+}	33.0^{+}
B 1 (+D)[1]	0.017 : 1	$1.35^{(+)}$	0.232^{+}	$11.2^{(+)}$	$390.2^{(+)}$	31.0
2 (+D)	$0.132:1^{+}$	2.03^{+}	0.276^{+}	13.4^{+}	621.4^{+}	39.8
5 (+D)	$0.360:1^{++}$	5.78^{++}	0.078	1.1^{++}	$1,644.0^{++}$	10.4

[1] See Table 1 for soil enrichment with heavy metals and NaCl. **A**: Plant cultivation without water shortage (60% of water capacity). **B**: Plant cultivation with a moderate water shortage (40% of the water capacity). The plants of variants 1 (+D), 2 (+D) and 5 (+D) were cultivated under the same soil properties as shown in Table 1.
[2] The ratio of Cd to Cu was determined in the biomass (stalks + ears) according to Table 1.
[3] The quantities of betaine, spermine and putrescine were estimated in the above-ground biomass (stalks + ears) at the beginning of graining. For protein analysis the KJELDAHL – and LOWRY-method were used.
Significance: $^{+}\alpha \leq 0.05$; $^{++}\alpha < 0.01$; $^{(+)}\alpha < 0.1$ in relation to variant-No. A 1.

in comparison to No.1): The heavy metal enrichment produced an increase of betaine content about 40% (No. 2 in comparison to No. 1), and by addition of NaCl (1 g per kg sandy soil) a similar increase in the betaine concentration was obtained (compare in Table 1 No. 3 with No. 1). However a simultaneous enrichment of soil substrate with heavy metals and NaCl acted synergistically on the betaine accumulation in barley (Table 1, No. 4, 5, 6). – Thus, a simultaneous soil treatment with heavy metals and 1 g NaCl per kg soil substrate produced a betaine increase about 281 ppm, but a separate treatment with heavy metals or NaCl resulted only in an enhanced betaine accumulation of 57 mg and 61 mg per kg dry matter, respectively (see Table 1, as above-mentioned).

Such an interaction of heavy metals with salinity factors in soils and plants is present under field conditions and a stronger soil salinity might increase the contents of heavy metals and specific metabolites in plant products considerably (see Bergmann, 1996; Eckert et al., 1992; Helal et al., 1999; Smith, 1985).

Finally the heavy metal contamination and soil salinity modified the Cd/Cu-ratio on the one hand and the betaine-N/protein-N-ratio or spermine-N/protein-N ratio in barley on the other hand (Table 2). Consequently more nitrogen was found in soluble N-metabolites under stress (Bergmann, 1996).

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