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# Genotoxicity of Co<sup>2+</sup> in plants and other organisms

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A mini review on the genotoxicity of cobalt in various groups of organisms and experimental data on the genotoxicity of Co<sup>2+</sup> to plants are presented. Two groups of plants are compared: field bean (*Vicia faba*) and pea (*Pisum sativum*) in which Co<sup>2+</sup> induces chlorophyll morphoses, and opposite to them barley (*Hordeum vulgare*) in which this phenomenon is absent. A slight mutagenic effect was observed in all plant species tested. It depended on the plant genotype. The phenomenon of induction by Co<sup>2+</sup> of chlorophyll morphoses can be used for evaluation of soil contamination with Co<sup>2+</sup>. In field bean offspring of plants with chlorophyll morphosis, chlorophyll mutations are more frequent than in offspring from normal green plants. The antimutagenic effect of Co<sup>2+</sup> on EMS was noted, but it depended on the genotype of barley.

**Key words:** Co<sup>2+</sup> genotoxicity, field bean, pea, barley, chlorophyll morphosis, visible mutations

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## INTRODUCTION

Metal ions belong to the most important and dangerous environmental factors. Their biodegradation is impossible, and they can be accumulated in the organism at the concentrations many times higher than the levels of these metals in the surrounding environment. However, the degree of bioaccumulation and genotoxic hazard differ for various metals and concrete organisms. They also depend on environmental conditions, the physiological and developmental status of the organism.

### 1. Cobalt genotoxicity in human and mammalia

Although cobalt is a micronutrient, in several fields of human activities (mainly in the manufacture of alloys) its toxic concentrations can be accumulated. So, the problem of the genotoxicity of cobalt does exist.

The action of cobalt on human and animals is very diverse. Its excess can cause gastric disturbances, polycythemia and hyperglycemia, and leads to reproductive changes. Cobalt is a metal with a marked allergic potential. Allergic dermatitis is also produced by cobalt. The beer-containing cobalt for preserving its foam may cause heart disease in heavy beer drinkers [1–3]. Cobalt is a well-known carcinogen, but only weakly genotoxic. Co<sup>2+</sup> induced changes of DNA basis, DNA breaks, sister chromatid

exchanges (SCE) in human lymphocytes and aneuploidy, but not chromosomal aberrations [2, 4–6] or significant increase of DNA migration when the ‘comet’ assay was applied [7]. Co<sup>2+</sup> induces DNA strand breaks in HeLa cells, a small number of 6-thioguanine-resistant mutants and a more pronounced frequency of SCE in V79 Chinese hamster cells [8].

However, the genotoxic hazard of cobalt can be more pronounced in its interaction with other mutagenic factors. So, Co<sup>2+</sup> induces changes in DNA of chromatin extracted from cultured human cells in the presence of H<sub>2</sub>O<sub>2</sub> [9]. Cobalt inhibits repair of the DNA damaged by direct genotoxic agents such as UV, alkylating substances, X-rays [6, 8, 10] even at low, uncytotoxic concentrations [11]. It is mechanism of a pronounced comutagenic effect of cobalt on mammalian cells [4].

### 2. Action on microorganisms

The comutagenic action of cobalt has been also confirmed on the microorganisms. In the bacterial test systems Co<sup>2+</sup> itself is inactive, but exerts comutagenic effects in combination with various chemical and physical agents [4, 8]. However, the antimutagenic action of Co<sup>2+</sup> is also shown in *Escherichia coli*. The effect depends on experimental conditions. Co<sup>2+</sup> may act either as an inhibitor or as an inducer of the

SOS functions [12]. The spectrum of mutations induced by  $\text{Co}^{2+}$  was examined by using plasmid pUB3 which was propagated after transfection into *E. coli*. Deletion and frame shifts predominated. The base substitutions were induced about two times less [13].

The antimutagenic action of cobalt is observed in *Saccharomyces cerevisiae* [14]. On the other hand, in yeast  $\text{Co}^{2+}$  induces respiratory deficiency [2] and mutations in mitochondria genes, but is weakly mutagenic or nonmutagenic to chromosomal genes [4]. It induces also a stable epigenetic switch, enhances telomeric silencing and represses the telomeric marker gene *URA3* [15].

### 3. Action on the higher plants

Cobalt is a trace metal for plants. However, its deficiency symptoms in plants are unknown. In contrast, several investigations on the physiological action of cobalt excess have been published, and its multiple effect was noticed on different functional systems and processes: on mitochondria respiration, cytokinesis and karyokinesis, growth, crop yield, ageing, inhibition of alkaloid and plant hormones (ethylene), RNA and DNA biosynthesis, reaction to stress conditions [see as review 16]. Especially numerous are investigations concerning the action of cobalt on the photosynthesis, photosystems I and II, chlorophylls [16–19].  $\text{Co}^{2+}$  and other metal ions ( $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ) caused appearance of a red-brown discoloration, first in the veins of unifoliate leaves and later in the petioles and stems of *Phaseolus vulgaris* [20], leaf drop, and the initial loss of leaf orientation was also observed on that plant [21]. In solution culture of *P. vulgaris*  $\text{Co}^{2+}$  induced severe chlorosis [22].

Accumulation and action of  $\text{Co}^{2+}$  in plants depend on the concentration of other metals ( $\text{Zn}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Ni}^{2+}$ ), temperature, pH of soil [16], as well as on plant species. So, the concentration of  $\text{Co}^{2+}$  in 9 medicinal plants differed significantly. In contrasting plants *Alpina galanga* and *Purearia tuberosa*  $\text{Co}^{2+}$  concentration differed about 61 times (0.16 and 7.08 ppm in dry material, respectively) [1]. Plants with unusually high concentrations of heavy metals exist and can be used for removal of such contaminants from soils, *i.e.* for phytoremediation. The other sphere of the usage of such plants is phytomining for growing crop plants to harvest the rare metals. For  $\text{Co}^{2+}$  such hyperaccumulator is *Berkheya codii* [23].

Only a few works deal with the mutagenic action of  $\text{Co}^{2+}$  ions in plants. Despite negative results on mammalian cells, in plants  $\text{Co}^{2+}$  induces chromosomal aberrations [4, 24]. These data may be explained by the action of  $\text{Co}^{2+}$  on oxidative stress and activity of enzymes introduced in the oxidative stress response of plants [25, 26].

A stimulus for  $\text{Co}^{2+}$  genotoxicity investigations in the present work was the phenomenon of chlorophyll morphosis induction in *Vicia faba* [27]. From the 17 metal ions tested, only  $\text{Co}^{2+}$  induced that phenomenon, and only after seed soaking in solutions of cobalt salts. This phenomenon is observed only in several *Fabacea* species: *Pisum sativum*, *Lens culinaris*, *Vicia sativa*. However, morphosis is absent in other *Fabaceae* species such as *Lupinus luteus*, *Glycine hispida*, *Ornithopus sativus*, *Onobrychis viciifolia*. The results were also negative for *Nicotiana tabacum*, *N. rustica*, *Lycopersicon esculentum*, *Sinapis alba*, *Brassica napus* ssp. *napus*, *Hordeum vulgare* [28, 29].

In the present work, the frequency of chlorophyll and other visible mutations was analyzed after treatment of *Vicia faba*, *Pisum sativum* and *Hordeum vulgare* with  $\text{Co}(\text{NO}_3)_2$  solutions with a special attention to the offspring of the chlorophyll morphosis plants and to the combined action of  $\text{Co}(\text{NO}_3)_2$  with the alkylating mutagen, ethylmethanesulphonate.

## MATERIALS AND METHODS

Seed material of field beans cv. 'Aušra' and pea of two cultivars, 'Grafila' and 'LŽI2114', was obtained from the Lithuanian Institute of Agriculture (Dotnuva). The both pea cultivars are attributed to the leafless, with reduced leaf plates and exceeded runners, and are resistant to lodging.

Barley cv. 'Auksiniai 3' was originally obtained also from Dotnuva, but that cultivar has been grown in Botanical Garden of Vilnius University for about 10 years. It is the initial wild type for induced mutants *tw*<sub>4</sub> (*tweaky spike*) and *be*<sub>1</sub> (*branched ear*) [30].

**Morphosis induction and treatment of plants in M<sub>1</sub>.** Seeds of field bean and pea were soaked for 15 h in 0,  $0.25 \cdot 10^{-2}$ ,  $0.5 \cdot 10^{-2}$ ,  $0.75 \cdot 10^{-2}$  and  $1.0 \cdot 10^{-2}$  M solutions of  $\text{Co}(\text{NO}_3)_2$  (Sigma). All unswollen seeds were removed. The plants were planted in the experimental field of Botanical Garden of Vilnius University. Morphosis types were determined one month after seed soaking in  $\text{Co}(\text{NO}_3)_2$  solutions. Chlorophyll morphoses of various phenotypes were observed [28, 29]. For progeny analysis, only plants of four groups were taken: normal, chlorotic, mosaic, and yellow. Seeds from these plants were harvested separately.

Grains of barley were soaked in  $\text{Co}(\text{NO}_3)_2$  and ethylmethanesulphonate (Sigma) solutions for 12 h.  $\text{Co}^{2+}$  and EMS action on cv. 'Auksiniai 3', mutants *tw*<sub>4</sub> and *be*<sub>1</sub> was tested.

**Mutations in M<sub>2</sub>.** In field beans mutations were analysed with respect to the phenotype of morphosis in M<sub>1</sub>. In M<sub>2</sub> of peas only alterations of plant pigmentation were fixed. The effect of 0;  $0.25 \cdot 10^{-2}$ ;  $0.5 \cdot$

$\cdot 10^{-2}$  and  $1.0 \cdot 10^{-2}$  M concentrations of  $\text{Co}(\text{NO}_3)_2$  was investigated.

Only alterations of pigmentation were examined also in barley treated with  $0.25 \cdot 10^{-2}$ ;  $0.5 \cdot 10^{-2}$  M  $\text{Co}(\text{NO}_3)_2$  alone or in combinations with  $1.0 \cdot 10^{-2}$  M or  $2.0 \cdot 10^{-2}$  M EMS (ethylmethanesulphonate). For positive control, both concentrations of EMS were also tested. Different genotypes in pea (cv. 'Grafilā' and 'LŽI2114') and barley (wild type – cv. 'Aukšiniai 3' and induced mutants  $tw_4$  and  $be_1$ ) were compared.

In  $M_2$  barley was grown by the families method separately from each plant in  $M_1$ . The frequency of pigmentation alterations was calculated per number of families and per number of plants.

## RESULTS AND DISCUSSION

Several experiments on peas and field beans were made on chlorophyll morphosis induction, and in all of them the dose effect of  $\text{Co}(\text{NO}_3)_2$  was very clear. The same results were also obtained with witch (*Vicia sativa*) and lentil (*Lens culinaris*).

The dose effect displayed itself in two ways:

– number of the normally green plants reduced and frequency of the chlorophyll morphoses increased proportionally to increasing the concentration of  $\text{Co}(\text{NO}_3)_2$ ;

– degree of injured plants, such as yellow or even white also increased after treatment with higher doses of  $\text{Co}(\text{NO}_3)_2$ .

The same conclusion was made in the previous works with field beans and peas [25–27]. So, it is a well-grounded proposition to use the chlorophyll morphosis test for a quantitative dosation of the biological effectivity of  $\text{Co}^{2+}$  in soils highly contaminated with that metal. Such situations really exist [2, 23].

In previous works [25, 26] it was proved that the chlorophyll alterations are uninherited and are really phenocopies of chlorophyll mutations. Dividing to groups according of the plant phenotype in  $M_1$  for  $M_2$  analysis was made only with *Vicia faba*. The uninherited nature of chlorophyll alterations was also confirmed in the present work with field beans and peas.

Various changes in field bean were noted after  $\text{Co}(\text{NO}_3)_2$  treatment in  $M_2$  [26]. In the present work they were divided into two groups – chlorophyll mutations and others, and inheritance of alterations observed in  $M_2$  was examined in  $M_3$ – $M_5$ . After such analysis only real mutations were selected (Table 1). The mutagenic effect of  $\text{Co}^{2+}$  was strongly dependent from the concentration of  $\text{Co}(\text{NO}_3)_2$ . The frequency of chlorophyll mutations increased so slightly after treatment with  $1.0 \cdot 10^{-2}$  M  $\text{Co}(\text{NO}_3)_2$  that the effect was not statistically significant. However, the among progenies of plants that in  $M_1$  were with chlorophyll morphosis, the frequency of mutations was distributed differently. It was significantly higher among the progenies from chlorotic plants after

Table 1. Chlorophyll and other mutations in the progeny of the chlorophyll morphosis plants induced by  $\text{Co}(\text{NO}_3)_2$  in field bean *Vicia faba*

$\text{Co}(\text{NO}_3)_2$ $\times 10^{-2}$ M	Phenotype in $M_1$	Number of plants in $M_2$	Frequency of mutations in $M_2$ , % <sup>1</sup>		
			Chlorophyll	Others	Sum
0	Only normal	6900	$0.03 \pm 0.02$	0	$0.03 \pm 0.02$
0.25×	Normal	4356	0	0	0
	Chlorotic	1551	$0.26 \pm 0.13$	$0.52 \pm 0.18$	$0.78 \pm 0.22$
	Mosaic	6386	0	0	0
	Yellow	212	0	0	0
	Total	12505	$0.03 \pm 0.02$	$0.06 \pm 0.02$	$0.09 \pm 0.03$
0.5×	Normal	4608	0	0	0
	Chlorotic	2933	0	0	0
	Mosaic	7933	0	0	0
	Yellow	1245	0	0	0
	Total	16719	0	0	0
1.0×	Normal	4032	0	0	0
	Chlorotic	753	$0.27 \pm 0.19$	0	$0.27 \pm 0.19$
	Mosaic	6739	0	0	0
	Yellow	1521	$0.26 \pm 0.13$	0	$0.26 \pm 0.13$
	Total	13045	$0.05 \pm 0.02$	0	$0.05 \pm 0.02$

<sup>1</sup> Results were corrected on the basis of the  $M_3$ – $M_5$  investigation.

Table 2. Comparison of changes in plant pigmentation induced by Co(NO<sub>3</sub>)<sub>2</sub> in two pea cultivars

Co(NO <sub>3</sub> ) ×10 <sup>-2</sup> M	Number of tested plants in M <sub>2</sub>	Alterations in M <sub>2</sub> , %										Total frequency
		Full plant				Mosaic						
		Yellow	Brightened	Brightened with altered shape of leaves	Sum	With white part (s)	With yellow part (s)	With brightened part (s)	Sum			
0	5245	0	0	0	0	0	0.019	0	0.019 ± 0.019	0.019 ± 0.019	0.019 ± 0.019	0.019 ± 0.019
0.25×	6398	0.078	0.188	0	0.266 ± 0.064	0.266	0.047	0.188	0.501 ± 0.088	0.501 ± 0.088	0.767 ± 0.109	0.767 ± 0.109
0.5×	6288	0	0.080	0.048	0.128 ± 0.045	0.080	0.032	0.255	0.367 ± 0.076	0.367 ± 0.076	0.495 ± 0.089	0.495 ± 0.089
1.0×	6679	0.045	0.090	0.075	0.210 ± 0.056	0.135	0.135	0.190	0.460 ± 0.083	0.460 ± 0.083	0.670 ± 0.100	0.670 ± 0.100
Common <sup>1</sup>	19365	0.123	0.358	0.123	0.604 ± 0.056	0.481	0.214	0.633	1.328 ± 0.082	1.328 ± 0.082	1.932 ± 0.099	1.932 ± 0.099
0	7478	0	0	0	0	0	0.013	0	0.013 ± 0.013	0.013 ± 0.013	0.013 ± 0.013	0.013 ± 0.013
0.25×	7543	0.013	0.027	0.013	0.053 ± 0.026	0.040	0.026	0.053	0.119 ± 0.040	0.119 ± 0.040	0.172 ± 0.048	0.172 ± 0.048
0.5×	7108	0.028	0.028	0.141	0.197 ± 0.053	0.056	0.070	0	0.126 ± 0.042	0.126 ± 0.042	0.323 ± 0.067	0.323 ± 0.067
1.0×	6811	0.073	0.015	0.015	0.103 ± 0.039	0.086	0.030	0	0.116 ± 0.041	0.116 ± 0.041	0.219 ± 0.057	0.219 ± 0.057
Common <sup>1</sup>	21462	0.114	0.070	0.169	0.353 ± 0.002	0.182	0.126	0.053	0.361 ± 0.041	0.361 ± 0.041	0.714 ± 0.057	0.714 ± 0.057

1 – Common sum of frequencies for all concentrations of Co(NO<sub>3</sub>)<sub>2</sub> tested.

treatment in M<sub>1</sub> with 0.25 · 10<sup>-2</sup> and 1.0 · 10<sup>-2</sup>M Co(NO<sub>3</sub>)<sub>2</sub>.

In M<sub>2</sub> of peas only changes in plant pigmentation were estimated, but the action of Co<sup>2+</sup> was tested on two different cultivars, 'Grafilia' and 'LŽI2114' (Table 2). The frequency of changed plants increased very significantly. In cv. 'Grafilia' it was about 40 times higher than in progenies of untreated plants. The effect of cobalt on cv. 'LŽI2114' was slighter – the maximal increase was about 25 times.

Fully changed plants were absent in the progenies of the untreated plants (Table 2).

The genotoxicity of Co(NO<sub>3</sub>)<sub>2</sub> to pea was determined only from the M<sub>2</sub> analysis. All alterations of plant pigmentation were divided into two groups: equally and fully changed or mosaics (Table 2). Only mosaics were detected among progenies of the untreated plants (number 0). So, appearance of fully altered plants must be attributed only to the action of Co<sup>2+</sup>. It allowed us to summarize the results for all three concentrations of Co(NO<sub>3</sub>)<sub>2</sub> and to compare the common genotoxicity of Co<sup>2+</sup> on two pea cultivars, 'Grafilia' and 'LŽI2114'. The common frequency of fully altered plants in cv. 'Grafilia' was 1.7 times higher than in cv. 'LŽI2114' (t = 4.5; P < 0.001). The difference between the cultivars in the common sum of mosaics was even higher – 3.7 times (t = 10.6; P < 0.001). The sum for both groups of the altered plants differed over 1.9 times (t = 13.4; P < 0.001). So, dependence of Co<sup>2+</sup> genotoxicity on the plant genotype is obvious, and cv. 'Grafilia' is significantly more mutable than cv. 'LŽI2114'. However, it should be noted that the difference between two cultivars was due to a high frequency of one type of colour alteration – fully or mosaic brightened plants (Table 2).

In barley, dependence of the genotoxicity of Co(NO<sub>3</sub>)<sub>2</sub> on plant genotype was examined on a more concrete genetical material – on two induced recessive monogenic mutants *tw*<sub>4</sub> (belonging to the family of mutants *tweaky spike*) and *be*<sub>1</sub> (*branched ear*). Both mutants are unstable, but in different way: *tw*<sub>4</sub> is genetically unstable, reversions *tw* → *Tw* to normal type arise. Mutant *be*<sub>1</sub> is phenotypically unstable. Its instability is displayed in the following manner: the lower part of the ear is branched, while the upper part of the ear is frequently two-row,

**Table 3. Mutagenic action of Co(NO<sub>3</sub>)<sub>2</sub> alone and in combination with EMS (ethylmethanesulphonate) in M<sub>2</sub> of normal type barley and of morphogenetic mutants**

Treatment	Number of plants analysed in M <sub>2</sub>						Frequency of mutations in M <sub>2</sub> , %					
	Families			Plants			Evaluation of families			Evaluation of plants		
	A3	tw <sub>4</sub>	be <sub>1</sub>	A3	tw <sub>4</sub>	be <sub>1</sub>	A3	tw <sub>4</sub>	be <sub>1</sub>	A3	tw <sub>4</sub>	be <sub>1</sub>
0	48	49	69	1498	700	1403	0	2.04 ± 2.04	0	0	0.14 ± 0.14	0
Co(NO <sub>3</sub> ) <sub>2</sub> 0.25 · 10 <sup>-2</sup> M	65	39	68	2200	523	2110	7.69 ± 3.33	10.26 ± 4.92	1.47 ± 1.47	1.14 ± 0.23	1.15 ± 0.47	0.43 ± 0.14
Co(NO <sub>3</sub> ) <sub>2</sub> 0.25 · 10 <sup>-2</sup> M + EMS 1.0 · 10 <sup>-2</sup> M	63	61	70	1440	753	1394	6.35 ± 3.10	8.20 ± 3.54	5.71 ± 2.79	0.28 ± 0.14	0.66 ± 0.30	0.43 ± 0.18
Co(NO <sub>3</sub> ) <sub>2</sub> 0.25 · 10 <sup>-2</sup> M + EMS 2.0 · 10 <sup>-2</sup> M	88	51	73	2338	576	2221	10.23 ± 3.25	11.76 ± 4.56	9.59 ± 3.47	0.64 ± 0.16	1.56 ± 0.52	0.36 ± 0.13
Co(NO <sub>3</sub> ) <sub>2</sub> 0.5 · 10 <sup>-2</sup> M	67	92	65	2698	1133	2471	5.97 ± 2.92	2.17 ± 1.53	4.62 ± 2.62	0.78 ± 0.17	0.18 ± 0.13	0.20 ± 0.09
Co(NO <sub>3</sub> ) <sub>2</sub> 0.5 · 10 <sup>-2</sup> M + EMS 1.0 · 10 <sup>-2</sup> M	66	74	59	2378	1091	1740	10.61 ± 3.82	10.81 ± 3.63	3.39 ± 2.38	0.93 ± 0.20	1.28 ± 0.34	0.34 ± 0.14
Co(NO <sub>3</sub> ) <sub>2</sub> 0.5 · 10 <sup>-2</sup> M + EMS 2.0 · 10 <sup>-2</sup> M	85	59	73	3467	706	1553	16.47 ± 0.63	15.25 ± 1.35	6.85 ± 0.64	0.46 ± 0.11	1.98 ± 0.52	0.52 ± 0.18
EMS 1.0 · 10 <sup>-2</sup> M	64	47	96	2737	639	1990	15.63 ± 4.58	8.51 ± 4.11	6.25 ± 2.48	0.84 ± 0.17	0.63 ± 0.31	0.80 ± 0.20
EMS 2.0 · 10 <sup>-2</sup> M	71	48	103	2393	671	3061	18.31 ± 4.62	10.42 ± 4.46	9.71 ± 2.93	1.30 ± 0.23	0.89 ± 0.36	0.82 ± 0.16

A3 – cv. 'Auksiniai 3'; tw<sub>4</sub> – mutant *tweaky spike*; be<sub>1</sub> – mutant *branched ear*.

and in different ears of the same plant that trait is expressed to various extent. Both mutants *tw<sub>4</sub>* and *be<sub>1</sub>* have an altered ear structure [31] and were compared with the wild type (*WT*) which has the normal ear structure. It is the barley cv. 'Auksiniai 3' which is also an initial form from which both mutants originate. So, the wild type and both mutants differ in one gene.

Spontaneous chlorophyll mutations were detected only among the progenies of *tw<sub>4</sub>* (Table 3). Among the progenies of untreated M<sub>1</sub> plants of *WT* or *be<sub>1</sub>* chlorophyll mutations didn't arise at all. Appearance of chlorophyll mutations among treated plants of *tw<sub>4</sub>* can be explained by genetical instability of *tw* type mutants. It may be extended also to plant pigmentation genes.

In *WT* (cv. 'Auksiniai 3') and *be<sub>1</sub>* mutant the chlorophyll mutations arose only after treatment with Co(NO<sub>3</sub>)<sub>2</sub>, and the mutagenic action of Co<sup>2+</sup> was relatively strong. Cobalt was of about the same mutagenic effectivity as the popular strong mutagen ethylmethanesulphonate (EMS), which is traditionally used as a positive control in mutagenesis investigations.

Results obtained with barley confirmed the conclusion made on the basis of the Co<sup>2+</sup> action on pea: genotoxicity of Co<sup>2+</sup> depends to a significant extent on the plant genotype even in the range of the same plant species. This dependence was rather neglected in the previous works on the genotoxicity of cobalt to the plants [4, 24]. It must be also taken into account in investigations of the physiological toxicity of cobalt.

Evaluation of Co<sup>2+</sup> genotoxicity in barley mutants is strongly complicated by the dependence of the mutagenic action on the concentration of Co(NO<sub>3</sub>)<sub>2</sub> (Table 3). After treatment of barley with 0.25 · 10<sup>-2</sup> M Co(NO<sub>3</sub>)<sub>2</sub> the level of chlorophyll mutations in *WT* and *tw<sub>4</sub>* was the same, while after treatment with 0.5 · 10<sup>-2</sup> M Co(NO<sub>3</sub>)<sub>2</sub> the frequency of chlorophyll mutations in *WT* was four times higher than in *tw<sub>4</sub>* if the calculated as the percentage of the altered plants. If the mutagenic effect was determined as % of altered families, the difference between *WT* and *tw<sub>4</sub>* was obvious even after treatment with 0.25 · 10<sup>-2</sup> M Co(NO<sub>3</sub>)<sub>2</sub>.

The mutagenic action of Co<sup>2+</sup> to *tw<sub>4</sub>* would be impossible to display if to test only with 0.5 · 10<sup>-2</sup> M Co(NO<sub>3</sub>)<sub>2</sub>. The level of chlorophyll mutations in *tw<sub>4</sub>* after treatment with 0.5 · 10<sup>-2</sup> M Co(NO<sub>3</sub>)<sub>2</sub> was the same as in *tw<sub>4</sub>* plants untreated with Co<sup>2+</sup>. It was the same in both methods of the calculation of mutation frequency. In the other mutant, *be<sub>1</sub>*, 0.25 · 10<sup>-2</sup> M Co(NO<sub>3</sub>)<sub>2</sub> was about twice more effective as 0.5 · 10<sup>-2</sup> M Co(NO<sub>3</sub>)<sub>2</sub> (Table 3).

The mutagenic action of EMS alone also depended on the method of determination of mutation frequency. When the frequency was determined for

plants in M<sub>2</sub>, no significant differences between the genotypes tested was observed (Table 3). If the frequency was calculated per number of the families tested, a real difference between plant genotypes existed. However, the lowest mutability to EMS was observed in mutant *be*<sub>1</sub>, while to Co<sup>2+</sup> it was in *tw*<sub>4</sub>.

Contradictory results concerning the interaction of Co<sup>2+</sup> with known mutagenic factors such as alkylating substances or ionizing radiation were obtained also in previous works. Dependence on the organism was noted. So, in mammalia Co<sup>2+</sup> in most cases exhibited a pronounced genotoxicity of these factors [6, 8, 10], while in bacteria contradictory effects were noted [4, 8, 12]. In yeast *Saccharomyces cerevisiae* cobalt acts as antimutagen [14]. In the present work, only a slight decrease in chlorophyll mutations was observed in cv. 'Auksiniai 3', but the difference was statistically significant only for the combination 0.5 · 10<sup>-2</sup> M Co(NO<sub>3</sub>)<sub>2</sub> + 2.0 · 10<sup>-2</sup> M EMS (*t* = 2.1; *P* < 0.05).

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#### KOBALTO GENOTOKSIŠKUMAS AUGALAMS IR KITIEMS ORGANIZMAMS

##### S a n t r a u k a

Trumpai apžvelgiamas kobalto genotoksiškumas įvairioms organizmų grupėms. Pateikiami eksperimentiniai duomenys apie Co<sup>2+</sup> genotoksiškumą augalams. Lyginamos dvi augalų grupės. Pašarinėse pupose (*Vicia faba*) ir žirniuose (*Pisum sativum*) Co<sup>2+</sup> sukelia chlorofilo morfozes, o miežiuose (*Hordeum vulgare*) ne. Silpnas mutageninis poveikis nustatytas visiems tirtiems augalams, tačiau jis priklausė nuo augalų genotipo. Chlorofilo morfozės gali būti taikomos įvertinant dirvos užterštumą Co<sup>2+</sup>. Nepastebėta chlorofilinių mutacijų skirtumų tarp pupų, kurios buvo kilusios iš augalų M<sub>1</sub> su chlorofilo morfozėmis arba iš normaliai žalių augalų. Miežiuose aptiktas antimutageninis Co<sup>2+</sup> poveikis etilmetansulfonatui (EMS). Šis reiškinys irgi priklauso nuo miežių genotipo.