THE BIOINDICATIVE ROLE OF ENZYME POLYMORPHISM OF SEVEN ENZYME SYSTEMS AS REACTION ON HIGH CADMIUM IONS CONCENTRATION IN MAIZE (*ZEAMAYS* L.)

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Effect of different concentrations of cadmium ions (0, 15, 60, 240 and 960 $\mu\text{mol dm}^{-3}$ in full Hoagland solutions) on enzyme polymorphism of seven enzymes – acid phosphatase (ACP), alcohol dehydrogenase (ADH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM) and their fifteen polymorphic loci – in coleoptiles, leaf blades and in the central parts of primary root systems of 14 days old seedlings in two self-pollinated maize lines and their single-cross was studied. Analyses of enzyme polymorphism showed that the biological material was homogeneous, self-pollinated lines had homozygous constitution in each analysed locus and hybrid had three loci (*Acp1*:

2/4, *Idh2*: *4/6* and *Pgd1*: *2/3.8*) with heterozygous constitution. Ions of cadmium negatively influenced seedling growth and development, high cadmium concentration (960 $\mu\text{mol dm}^{-3}$) was toxic and stopped further growth and development of maize seedlings after five days of cultivation. Enzyme fingerprint analysis of these fifteen polymorphic loci from selected enzymes supported their genotypic, organ and ontogenetic stability under high doses of cadmium ions. According to our results this group of enzymes in these polymorphic loci did not express changes in polymorphism after treatment with cadmium ions and thus cannot play the bioindicative role in this respect.

Key words: maize (*Zea mays* L.), cadmium concentrations, self-pollinated lines, single cross, starch gel electrophoresis, isoenzymes, molecular markers

It is generally known that study of stress effect on plant organisms is very often connected with analysis of both quantitative and qualitative protein content. Enzymes represent a group of proteins with catalytic effects. They are studied with the aim to explain the mechanisms of stress, reactions and coping of plant organism with stress. The toxicity symptoms observed in plants in the presence of high concentrations of toxic metals may be connected with a range of interactions on cellular level (Hall 2002). Toxicity may result from the bounding of metals to sulfanyl groups of proteins, including enzymes, what results in disruption of their

structure or/and inhibition of enzyme activity (Van Assche & Clijsters 1990). Enzymes are one of the main targets of toxic metal ions and long-term exposure of soils to such metals also results in marked decrease in soil enzyme activity (Tyler et al. 1989). The inhibition of enzymes by toxic metals may be due to masking of catalytically active groups or protein denaturation (Das et al. 1997). In addition, excess of toxic metal may stimulate the formation of free radicals and reactive oxygen species (Dietz et al. 1999; Gallego et al. 1996).

Maize (*Zea mays* L.) belongs to one of the most

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important crops of the world, ranking third after wheat and rice. From this point of view the research of xenobiotics, mainly the group of so-called “heavy metals” as Cd, Pb, Hg, Ni, Zn, Cu and other ones on plant production is of high importance. Cadmium is a non-essential element in plants (Verkleij & Schat 1990) which has been recognized as one of the most potentially hazardous of all metal pollutants since it is extremely toxic to plants, animals and humans (Cieslinski et al. 1996).

The changes of enzyme activities induced by Cd concentrations in early stages of maize development were widely studied (Leblová & Zatloukalová 1988; Lagriffoul et al. 1998; Šírká et al. 2004; Chaffai et al. 2006) on the other side, their biochemical and eco-physiological role and the meaning of multiplicity of key enzymes of bioenergetic pathways are studied very poorly.

Our research was oriented to study the effect of cadmium ions on maize seedlings. The main aim of the present study was to reveal effects of different cadmium ion concentrations on enzyme polymorphism of seven chosen enzyme systems – acid phosphatase, alcohol dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoglucosomerase and phosphoglucomutase in different maize organs and evaluate possible changes of enzyme polymorphism as bioindicators of high cadmium concentrations.

MATERIAL AND METHODS

In our experiments we used two self-pollinated maize (*Zea mays* L.) lines 3098 (♀), 3150 (♂) and their single cross (Sc 3098 × 3150) of Slovak provenience (Sempol Holding Inc., Trnava). During years 2005–2007 we have studied early stages of ontogeny, influence of different cadmium ions concentrations and analysed enzyme polymorphism during early stages of ontogeny without or after exposure to different cadmium ions concentrations using these genotypes.

Maize seeds were surface sterilized with 10% H₂O₂ for 20 min, rinsed ten times with tap water (Chaffai et al. 2006), germinated and grown in heating incubator on wet filter paper in Petri dishes under dark at 25°C and 98% RH. Experimental variants were represented by seedlings cultivated in 12.5 ml of full Hoagland solution with and without of addition of cadmium in

form of Cd (NO₃)₂ · 4H₂O. Concentrations of cadmium in experimental solutions were 0, 15, 60, 240, and 960 μmol dm⁻³. The samples were represented by one coleoptilar section (11 mm long) after five days of maize grain germination, fully developed leaf lamina and central part of primary root system after fourteen days of cultivation from each variant.

The standard technique (for coleoptilar section) of horizontal starch gel electrophoresis described by Stuber with co-workers (Stuber et al. 1988) and recently published in our works (Múdry & Juráček 2001; Múdry & Kraic, 2007; Uváčková et al. 2008) was used for analysis of polymorphism in acid phosphatase (ACP, E.C. 3.1.3.2), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), malate dehydrogenase (MDH, E.C. 1.1.1.37), 6-phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44), phosphoglucosomerase (PGI, E.C. 5.3.1.9) and phosphoglucomutase (PGM, E.C. 2.7.5.1). Extracts were isolated from coleoptile section, leaf lamina and primary root system by crashing in mortar on ice with addition of several grains of pure sand. Extraction solution contained 5 ml of water, 0.84 g of sucrose and 0.42 g of sodium ascorbate. Starch gels were composed of 77.31 g of hydrolysed potato starch for electrophoresis (Starch Art Corporation, Smithville, USA), 15 g of sucrose and 600 ml of gel buffer. The compositions of gel and electrode buffers are shown in Table 1. Extracts were inserted into gels using paper wicks (Whatman 2, 11 × 1.5 mm) soaked in isolates exactly 3 cm from the cathodic end. An electrophoretic separation was running at 4°C.

After finishing of separation the gels were cut horizontally into several thin slices (app. 1.2 mm thick) and placed into boxes for staining of zones of enzymatic activity in staining buffer systems. Compositions of buffers for preparation of staining solutions are shown in Table 2.

Composition of staining media for individual enzymes:

ACP: 50 ml 0.1 mol dm⁻³ sodium acetate-acetic acid buffer (pH 5.0), 50 mg Fast Garnet GBC Salt, 50 mg MgCl₂, 50 mg α-naphtyl acid phosphate (sodium salt).

ADH: 50 ml 0.05 mol dm⁻³ Tris-HCl buffer (pH 8.0), 1 ml 95% ethanol, 20 mg β-nicotinamide adenine dinucleotide, 20 mg MTT, tetrazolium thiazolyl blue,

5 mg phenazine methosulphate.

IDH: 50 ml 0.05 mol dm⁻³ Tris-HCl buffer (pH 8.0), 50 mg MgCl₂, 150 mg DL-isocitric acid, trisodium salt, 5 mg β-nicotinamide adenine dinucleotide phosphate, sodium salt, 5 mg tetrazolium thiazolyl blue, 1 mg phenazine methosulphate.

MDH: 50 ml 0.1 mol dm⁻³ Tris-HCl buffer (pH 9.1), 100 mg DL-malic acid, neutralized, 20 mg β-nicotinamide adenine dinucleotide reduced, 10 mg nitro blue tetrazolium, 1.25 mg phenazine methosulphate.

PGM: 50 ml 0.1 mol dm⁻³ Tris-HCl buffer (pH 8.5), 100 mg MgCl₂, 250 mg α-D-glucose-1-phosphate, disodium salt, 10 mg β-nicotinamide adenine dinucleotide phosphate, sodium salt, 7.5 mg tetrazolium thiazolyl blue, 1 mg phenazine methosulphate, 37.5 units of NADP-dependent glucose-6-phosphate dehydrogenase.

PGI/PGD: 50 ml 0.05 mol dm⁻³ Tris-HCl buffer (pH 8.0), 50 mg D-fructose-6-phosphate, 20 mg 6-phosphogluconic acid, trisodium salt, 50 mg MgCl₂, 5 mg β-nicotinamide adenine dinucleotide phosphate, sodium salt, 5 mg tetrazolium thiazolyl blue, 1.5 mg phenazine methosulphate, 10 units of NADP-dependent glucose-6-phosphate dehydrogenase.

RESULTS AND DISCUSSION

Cadmium is an element strongly phytotoxic with negative impact on maize plant growth and development mainly under higher than natural concentrations. Šíroká et al. (2004) confirmed high sensitivity of maize seedlings to Cd as even the lowest 1 μmol concentration (30% reduction of root growth). From

T a b l e 1

Electrode and gel buffers formulae (Stuber et al. 1988)

System	Electrode buffer	Gel buffer
B pH 5.7	0.065 mol dm ⁻³ L-histidine 0.02 mol dm ⁻³ citric acid H ₂ O pH adjusted with citric acid	0.009 mol dm ⁻³ L-histidine 0.003 mol dm ⁻³ citric acid H ₂ O (1:6 dilution of electrode buffer)
C pH 8.3	0.19 mol dm ⁻³ boric acid 0.04 mol dm ⁻³ lithium hydroxide pH adjusted with LiOH	9 parts Tris-citric acid buffer (0.05 mol dm ⁻³ Trizma base, 0.007 mol dm ⁻³ citric acid H ₂ O, pH 8.3) + 1 part Electrode C buffer
D pH 6.5	0.065 mol dm ⁻³ L-histidine 0.007 mol dm ⁻³ citric acid H ₂ O pH adjusted with citric acid	0.16 mol dm ⁻³ L-histidine 0.002 mol dm ⁻³ citric acid H ₂ O (1:3 dilution of electrode buffer)

T a b l e 2

Staining buffers (STUBER et al. 1988)

Enzyme	Buffer pH	Components
ADH, IDH, PGI, 6-PGD	8.0	0.05 mol dm ⁻³ Trizma base, titrate to pH 8.0 with HCl
ACP	5.0	0.1 mol dm ⁻³ Sodium acetate 3H ₂ O, titrate to pH 5.0 with 1 N HCl
PGM	8.5	0.1 mol dm ⁻³ Trizma base, titrate to pH 8.5 with HCl
MDH	9.1	0.1 mol dm ⁻³ Trizma base, titrate to pH 9.1 with HCl

our visual evaluation of maize seedlings the toxicity of cadmium ions treatments negatively influenced organ growth and development. At the highest concentration after five days of cultivation growth and development of seedlings were stopped and root browning as well as and leaf red-brownish discolouration were observed (Fig. 1) similarly as published by Arduini et al. (1994), Malone et al. (1977) and for *Sorghum bicolor* (L.) Moench. – Kuriakose & Prasad (2007). Our observation confirms fact that maize belongs to plant species very sensitive to higher concentrations of Cd than naturally occurring in Cd polluted soils ($1 \mu\text{mol dm}^{-3}$) and concentrations nearly 1 mmol dm^{-3} Cd causes acute stress (Sanita di Toppi & Gabrielli 1999).

Description and genetic interpretation of isozymograms is based on knowledge accumulated over nearly five decades of study of maize enzyme multiplicity in the world (extent of allozyme variation in studied loci, structure of the enzyme – monomeric, dimeric or tetrameric, existence of intraloci and interloci interactions, alleles detected and their frequencies in entire set of maize genotypes analysed and published, possible comigration of bands of one locus with bands of another locus, presence of null alleles, lightly and darkly staining bands, etc.). Alleles at analysed loci are classified according to their migration distances. For MDH loci, larger numbers correspond to greater anodal migration. For other enzymes larger numbers correspond to slower migration. The symbol for recessive null allele is *n*. *Mmm* is a modifier locus, the alleles of which affect the migration of certain MDH bands (Goodman et al. 1980; Newton & Schwartz 1980). *Glu1:6-7* means that enzymes encoded by the alleles 6 and 7 co-mi-

grated (Bourgoin-Greneche et al. 1998).

The group of seven enzymes which were chosen for our experimental work represents those which are very frequently used by many laboratories in the world for checking genotypic variability, identity and homogeneity of most of plant species (maize – Múdry & Kraic 2007; Stuber et al. 1988; chickpea – Múdry et al. 1998; amaranth – Chan & Sun 1997; Múdry et al. 2011; Yudin et al. 2010; oats – Gonzáles-Andrés et al. 2005; sunflower – Carrera et al. 2002, Rosmarinus-Zaouali & Boussaid 2008 etc.). For these purposes mostly standardized methods for enzyme polymorphism analysis are used to avoid undesirable factors affecting the results (Múdry et al. 2011).

Acid phosphatase – it catalyses the hydrolysis of phosphomonoesters important in a variety of biochemical reactions including the formation of sucrose in photosynthesis (Goodman & Stuber 1983). In maize genotypes enzyme polymorphism associated with *Acp1* locus has been most widely studied. Isoenzymes associated with the *Acp1* and *Acp2* loci have dimeric quaternary structure (El-Metainy & Omar 1981). Subcellular location of isoenzymes expressed by the *Acp1* locus is cytosol in roots, leaves, coleoptiles, scutella and pollen (Efron 1970). Šíroká et al. (2004) detected the inhibition of acid phosphatase at higher Cd concentrations (50 , 250 and $1000 \mu\text{mol dm}^{-3}$) in the apical parts of roots but slight increase was detected in coleoptiles especially at the highest $1000 \mu\text{mol dm}^{-3}$ concentration of Cd. Under our experimental conditions we have detected three isophorms of *Acp1* locus (2 , 4 and $2/4$) unchanged by increasing concentrations of cadmium ions.

Fig. 1. Demonstration of the effect of cadmium ions concentrations in full Hoagland solutions on growth and development of maize seedlings fourteen days cultivated in heating incubator under constant conditions

Alcohol dehydrogenase enzymes can be differentiated on the basis of their substrate specificities and type of cofactor required. NAD-dependent ADH acts on primary and secondary aliphatic alcohols (Hart 1969). These isoenzymes, quaternary structure of which is dimeric, are encoded by two genes *Adh1* and *Adh2* in maize (Schwartz 1960, 1969; Freeling & Schwartz 1973; Freeling 1974). Anaerobic conditions can induce ADH activity in seedling roots as a result of synthesis of both *Adh1* and *Adh2* homodimers and intragenic and intergenic heterodimers (Freeling & Schwartz 1973; Ferl et al. 1980). Subcellular location of these isoenzymes is cytosol. In our analysis only one and unchanged (by cadmium) isoform of ADH was detected – *Adh1*: 4. The changes of enzyme activities evoked by Cd concentrations in early stages of maize development led to the decrease of ADH activity in germinating seeds (Leblová & Tichý 1989).

Isocitrate dehydrogenase (NADP⁺) is one of the enzymes of the tricarboxylic acid pathway. It is involved in the movement of reducing power between mitochondria and the cytosol (Lehninger 1982) and catalyzes the oxidation of isocitrate to α -ketoglutarate. In maize, IDH isoenzymes have dimeric quaternary structure and they are encoded by two loci – *Idh1* and *Idh2* (Stuber & Goodman 1980). Subcellular location of IDH isoenzymes is cytosol (Ni et al. 1987) and they are active in the maize plant tissues of roots, leaves, coleoptiles, scutella and pollen (Goodman & Stuber 1983). Changes in both growth parameters and enzyme activities in roots and leaves of 14-day-old maize plants as a result of Cd uptake were studied (Lagriffoul et al. 1998). In this work they pointed out that IDH activity in the third leaf of maize seedlings decreased. Four isoforms of IDH unchanged by cadmium were detected in our samples – *Idh1*: 4, *Idh2*: 4, 6 and 4/6.

Malate dehydrogenase catalyses the conversion of malate to oxaloacetate and they are compartmentalized in microbodies, mitochondria and cytosol (Gottlieb 1981a; Ting et al. 1975). Both the cytosolic and mitochondrial forms of MDH have dimeric quaternary structure and they are active in the roots, shoots, endosperm, scutellum, hypocotyl, coleoptile, adult leaves and pollen and the microbody forms are active in maize endosperm and scutella (Longo & Scandalios 1969; Ting et al. 1975; Yang & Scandalios 1975; Goodman et al. 1980; Newton 1980; Newton & Schwartz 1980). According to our analyses all six loci are monomor-

phic for enzyme polymorphism. There were detected isoenzymes – *Mdh1*: 6, *Mdh2*: 3, *Mdh3*: 16, *Mdh4*: 12, *Mdh5*: 12 and *Mmm*: M. A marked decrease in MDH activity was observed in roots and increased activity in shoots of Cd-treated plants (Chaffai et al. 2006).

6-phosphogluconate dehydrogenase operates in the pentose phosphate pathway, where it catalyzes the breakdown of 6-phosphogluconate to ribulose-5-phosphate and carbon dioxide. Quaternary structure is dimeric (Gottlieb 1981b). In maize this enzyme is encoded by two loci *Pgd1*, *Pgd2* and products of both loci are active in tissues of roots, leaves, coleoptiles, scutella and pollen (Goodman & Stuber 1983). In two loci we have detected four isoenzymes of PGD – *Pgd1*: 2, 3.8, 2/3.8 and *Pgd2*: 5 unchanged by acting of cadmium.

Phosphoglucose isomerase, in scientific literature known as glucosephosphate isomerase or phosphohexose isomerase, functions in glycolysis (Freeling 1983). PGI isoenzymes are usually encoded by two loci, quaternary structure is dimeric and subcellular location was present in cytoplasm and plastids (Gottlieb 1981b). Only one locus, *Pgi1*, has been studied extensively in

Fig. 2. Diagrams of ACP, ADH, IDH, MDH, PGD, PGI and PGM isozymograms

T a b l e 3

Enzyme polymorphism of three maize (*Zea mays* L.) genotype organs cultivated under different concentrations of cadmium
Result obtained after five and fourteen days attack of ions of cadmium in germinating solutions

Genotype organ	Days of germination concentration of Cd [μmol dm ⁻³]	Number of analysed samples	Enzymes Loci and alleles														
			ACP <i>Acp1</i>	ADH <i>Adh1</i>	IDH <i>Idh1</i>	2	MDH <i>Mdh1</i>	2	3	4	5	Mmm	PGD <i>Pgd1</i>	2	PGI <i>Pgi1</i>	PGM <i>Pgm1</i>	2
3098 (♀)	5 days																
coleoptile	0	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
coleoptile	15	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
coleoptile	60	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
coleoptile	240	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
coleoptile	960	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
3150 (♂)	5 days																
coleoptile	0	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
coleoptile	15	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
coleoptile	60	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
coleoptile	240	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
coleoptile	960	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
Sc3098 × 3150	5 days																
coleoptile	0	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
coleoptile	15	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
coleoptile	60	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
coleoptile	240	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
coleoptile	960	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
3098 (♀)	14 days																
leaf lamina	0	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
leaf lamina	15	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
leaf lamina	60	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
leaf lamina	240	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
3150 (♂)	14 days																
leaf lamina	0	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
leaf lamina	15	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
leaf lamina	60	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
leaf lamina	240	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
Sc3098 × 3150	14 days																
leaf lamina	0	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
leaf lamina	15	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
leaf lamina	60	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
leaf lamina	240	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
3098 (♀)	14 days																
root system	0	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
root system	15	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
root system	60	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
root system	240	1	2	4	4	6	6	3	16	12	12	M	3.8	5	4	9	4
3150 (♂)	14 days																
root system	0	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
root system	15	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
root system	60	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
root system	240	1	4	4	4	4	6	3	16	12	12	M	2	5	4	9	4
Sc3098 × 3150	14 days																
root system	0	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
root system	15	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
root system	60	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4
root system	240	1	2/4	4	4	4/6	6	3	16	12	12	M	2/3.8	5	4	9	4

maize. Its products are active in the cytoplasm of tissues as roots, leaves, coleoptiles, scutella and pollen. Isoenzymes of the second locus have not been studied in detail. Their subcellular locations are plastids (Goodman & Stuber 1983). Only one isophorm of PGI was present in locus *Pgi1* – *Pgi1*: 4 in all our experimental samples.

Phosphoglucumutase is one of the glycolytic enzymes. Quaternary structure of its isomers is monomeric. Plants generally contain two loci. Isoenzymes of one locus are usually active in the chloroplast while those of the other locus are usually active in the cytoplasm (Gottlieb 1981b). In maize, quaternary structure of the PGM is monomeric and encoded by two loci, *Pgm1* and *Pgm2* (Stuber & Goodman 1983). No PGM activity has been detected in the plastids (etioplast) or mitochondria. PGM activities were detected in tissues of roots, coleoptiles, leaves, scutella and pollen. In our experiment both loci of PGM were monomorphic and unchanged by increasing cadmium ions concentrations – *Pgm1*: 9 and *Pgm2*: 4.

CONCLUSION

The standardised method of horizontal starch gel electrophoresis, we have used in this experimental work, was sensitive to analyse enzyme polymorphism in studied loci but global impact of cadmium ions on enzyme polymorphism in other loci (*Acp2* and *Adh2*) needs further research.

Results of the effects of cadmium ions on enzyme polymorphism are shown in Table 3 and Fig. 2. All experimental genotypes of maize were homogeneous on the basis of enzyme polymorphism, self-pollinated lines had homozygous constitution in each analysed locus and hybrid had three loci (*Acp1*: 2/4, *Idh2*: 4/6 and *Pgd1*: 2/3.8) with heterozygous constitutions. The coincidence of constitutions in all analysed loci responsible for enzyme polymorphism expression supported their genotypic, organ and ontogenetic stability at high doses of cadmium ions. According to our results this group of enzymes of these fifteen polymorphic loci (very frequently used by many laboratories in the world for checking genotypic variability, identity and homogeneity of maize seed lots) did not play the bioindicative role by means of changed polymorphism as response on high concentrations of cadmium ions.

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