MŰEGYETEM 1782

Ph.D. THESIS

Kornélia Ilona Leskó

Changes of biologically active components in wheat seedlings under cadmium stress

Simonné Dr. habil Sarkadi Livia
associate professor
supervisor

Budapest University of Technology and Economics
Department of Biochemistry and Food Technology
Budapest

2005

Introduction and aims

Wheat (*Triticum aestivum* L.) is an important cereal in human feeding, because bread, flour and pasta made of wheat are basic foodstuffs. Wheat gives the greater part of cereal cultivation in the world, which indicates its outstanding importance, too. Wheat is the main cereal in Hungary. According to statistical data from 2001 a brutto production value of 138.9 billion HUF wheat was harvested from 1.2 million hectares agricultural land, from which a value of 42.9 billion HUF wheat was exported by Hungary.

Environmental stressors, like drought, changes in temperature, salinity and heavy metals, have a detrimental effect on the quality and quantity of the yield. Agricultural sector faces that problem year by year. Beside environmental factors endangering crop safety, antropogenic stressors come into prominence more and more. Toxic heavy metals widely occur in the environment resulting from various human activities, such as industry, mining, agriculture and traffic. Highway construction, industrial waste and sludge deposition might cause an increased hazard in Hungary. The greater part of toxic heavy metals has a geochemical origin in the natural, non-polluted soils. Cd content of the Hungarian soils is usually under 0.2 mg/kg, but it can reach or exceed 0.6 mg/kg on smaller areas. The acceptable value is 1 -3 mg/kg Cd, depending on soil hardness.

Acidic rains due to air polluting gases and resulting from them the more and more acidic soils encourage the heavy metal extraction, so toxic metals can easily enter the food chain through the plants and seriously damage the health of living organisms and also that of human. Cadmium is one the most toxic heavy metals, which is accumulated in the cereal grains (bran). It is essential to make clear the relation between plants and cadmium to avoid and reduced its detrimental effect.

Low molecular weight organic compounds, such as free amino acids or polyamines accumulate during cadmium stress. Proline plays an important role in osmotic homeostasis, so it well indicates the different stress effects in plants. Cadmium also induces the enzymatic and non-enzymatic antioxidant defensive system of the plants, which can be detected by the changes of stress enzyme activities and phenolic compounds eliminating reactive oxygen species.

The aim of the present study was to compare the response of wheat (*Triticum aestivum* L.) genotypes differing in drought tolerance under cadmium stress on the basis of their major biologically active compounds. Answering the question whether the investigated genotypes can be distinguished on the basis of their response to slight and severe Cd-stress. The major growth parameters (shoot and root length, weight, dry matter content) and biochemical parameters (free amino acids, polyamines, stress enzymes, total phenol and cadmium) of the hydroponically grown wheat seedlings gave the basis of comparison. *Triticum aestivum* L. cv. Chinese Spring and Cappelle Desprez cultivars differing in drought tolerance were chosen for the model experiment. The tolerance of both cultivars has been previously proved in *in vivo* and *in vitro* experiments in HAS Agricultural Research Institute.

Materials and methods

Plant samples

Triticum aestivum L. wheat cultivars: Chinese Spring moderately drought-tolerant and Cappelle Desprez drought-sensitive.

The seedlings were grown in half-strength modified Hoagland-solution for 4 weeks prior to the stress period after germination (25°C, 4 days) in a spring type growth chamber (PGV-36 chamber, Conviron, Winnipeg, Canada), at night/day temperatures of 10/14°C with 76-68 % relative humidity and 16 h illumination at max. 270 μmol m^{-2} s^{-1}. Subsequently, the night/day temperatures were changed to 13/17°C for the 5^{th}-6^{th} week.

Heavy metal stress was imposed at the beginning of the fifth week by adding cadmium(II) acetate into the nutrient solution at concentrations of 10^{-7} M and 10^{-3} M for 7 days. After the stress treatment, the plants were transferred to control nutrient solution containing no cadmium for a 7-day recovery period. Control seedlings were grown under the same conditions without cadmium treatment.

Samplings were carried out after the 7-day stress and after the 7-day recovery (prior to the stress period also from control plants). Shoots and roots were separated and homogenised from 15 plant in each case. The homogeneous plant tissue was analysed.

Determination of growth parameters

Length, fresh weight, dry weight, dry matter content were measured by standard methods.

Free amino acid analysis

Free amino acids were extracted with 7% perchloric acid from fresh plant tissue. After filtration, free amino acids were determined by ion-exchange liquid chromatography with ninhydrin post-column derivatisation. The analysis was carried out using a BIOTRONIK LC 3000 (Germany) automatic amino acid analyser.

Polyamine analysis

OPLC (over-pressured layer chromatography) with the Personal OPLC BS 50 (OPLC-NIT, Hungary) automatic chromatograph was used for separation of the dansyl amines. The plates were developed with hexane-*n*-butanol-triethylamine, 90 + 10 + 9.1 (*v/v*), as mobile phase A, and hexane-*n*-butanol, 8 + 2 (*v/v*), as mobile phase B with overrun development and a stepwise gradient. Off-line quantitative evaluation of the dansyl amines was accomplished by fluorodensitometry by means of a CAMAG (Muttenz, Switzerland) TLC Scanner3 using CATS 4 (V 4.05) software.

Determination of stress enzyme activites

Preparation of cell free plant tissue extract

Method of Pandolfini et al. (1992) was used for the preparation of cell free plant tissue extract. All enzyme activities (guaiacol peroxidase, ascorbate peroxidase and gluthatione reductase) were determined after centrifugation (10000× g, 30 min, 4 °C). Activities were kinetically measured using Varian DMS 100S UV/VIS spectrophotometer.

Guaiacol peroxidase (EC 1.11.1.7)

The formation of tetraguaiacol product from guaiacol substrate was determined at 470 nm (ε = 26.6 $mmol^{-1}cm^{-1}$) by the modified method of Chance and Maehly (1955).

Ascorbate peroxidase (EC 1.11.1.11)

Enzymatic oxidation of the ascorbic acid substrate was determined at 290 nm wavelength (ε= 2.8 $mmol^{-1}$ cm^{-1}) (Nakano and Asada, 1981).

Gluthatione reductase (EC 1.8.1.7)

Gluthatione reductase reduces GSSG using NADPH and the thiol-groups of the formed GSH bind to the 2-nitro-5-thiobenzoate anion, which was measured at 412 nm (ε= 14.15 $mmol^{-1}$ cm^{-1}) (Smith et al., 1988).

Determination of the total phenol content

Total phenol content referred to gallic acid was measured by the method of Singleton and Rossi (1965) using Varian DMS 100S UV/VIS spectrophotometer at 760 nm.

Determination of the cadmium content

After digestion with nitric acid and hydrogen peroxide, cadmium accumulation was investigated by inductively coupled plasma-atomic absorption spectroscopy using ICP-AAS IRIS Thermo Jarrel Ash ICAP 61 (Thermo Jarell Ash Corp., Franklin, MA, USA) equipment.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), Duncan's test and correlation analysis using STATISTICA 6.1 (StatSoft Inc., Tulsa, OK, USA) and SPSS for Windows 11.0.1. (SPSS Inc., Chicago, Illinois, USA) softwares.

New scientific results

1. This study was the first time to investigate the responses to cadmium stress of wheat genotypes differing in drought tolerance [*Triticum aestivum* L. cv. Chinese Spring (moderately tolerant) and Cappelle Desprez (sensitive)] chosen for the model experiment. The present findings prove, that the drought-tolerant cultivar tolerated cadmium exposure better than the sensitive one, and the biologically active components play a considerable role beyond this cross-tolerance.

2. On the basis of the results of growth parameters, it was established that cadmium caused stunting and chlorosis in wheat seedlings. Cadmium treatment reduced the biomass, which was confirmed by the changes of fresh and dry weight. Dry matter content of both cultivars significantly increased at the 10^{-3} M Cd-concentration.

3. On the basis of the results concerning the free amino acids, it was found that wheat genotypes differing in drought tolerance are significantly different regarding their response to cadmium stress. Beside total free amino acid (TFAA) content, the well-

known stress marker proline (Pro) also proved to be suitable for distinguishing the two wheat cultivars differing in drought tolerance.

TFAA concentration in Chinese Spring (CS) shoots (1.6×) and roots (1,5×) increased due to the 10^{-3} M Cd treatment compared to the control, and a further 1.5-times increase was found in both plant organs during recovery. Cappelle Desprez (CD), on the other hand, gave a greater response to the less severe cadmium stress (10^{-7} M), with an increase in the TFAA content of 1.3× in the shoots and 1.6× in the roots compared to the control, after which the TFAA concentration decreased to the control level during the recovery period.

The Pro accumulation was 61-fold higher in CS shoots than in CD during the recovery period at the higher cadmium concentration. In the shoots, the ratios of glutamic acid and proline showed significant differences in the two cultivars during cadmium stress. The ratio of Glu decreased to more than half, while that of the proline increased 3-times in CS. On the other hand, Glu ratio of CD increased by 12% and that of the proline decreased to the one third at the 10^{-3} M Cd-concentration compared to the control during recovery. Comparing the ratio of GABA and Glu in the roots, CS mostly accumulated GABA, while CD mainly accumulated Glu due to the Cd treatment.

4. On the basis of the results concerning the polyamines it was observed that putrescine (Put) content in the shoots and spermidine (Spd) content in the shoots and roots proved to be suitable for distinguishing between the two genotypes. Put level in the sensitive CD shoots increased with Cd concentration (2-3×), while there were no significant changes in the moderately tolerant CS. Changes of Spd-content showed a similar tendency in both plant organs. The high Spd level of the control CD samples significantly decreased after the 10^{-7} M Cd-stress (to the one third). Less decrease (to the two third) was observed compared to the slight stress due to the 10^{-3} M Cd-treatment. Contrarily, the low Spd level of CS increased 3-6-times at the severe Cd-stress.

5. The 10^{-3} M Cd-stress induced the antioxidant defensive system (POD, APX, GR, phenolic compounds) in order to eliminate oxidative damage. The moderately tolerant cultivar seemed to be much more effective in the ability to eliminate the reactive oxygen species, than the sensitive one.

Guaiacol peroxidase (POD) enzyme activity significantly increased in the shoots of both cultivars at the 10^{-3} M Cd-treatment, and in the sensitive CD to a higher extent (CS: 2.4×, CD: 3.4×). POD activity decreased in the roots of both genotypes due to the 10^{-3} M Cd-stress, and in CS to a higher extent (CS: 0.5×, CD: 0.8×). Response to cadmium stress of the roots could have been reduced after the 7-day stress, while the increased activity of the shoots well demonstrated the beginning period of stress response against oxidative damage.

Changes of ascorbate peroxidase (APX) activity in both plant organs showed a similar tendency to that of the POD in the shoots. Gluthatione reductase (GR) activity similarly changed to APX, however significant changes were not found. Total phenol content significantly decreased after 10^{-7} M Cd-treatment in Cappelle Desprez shoot (0.7×) compared to the control, while considerably increased due to the 10^{-3} M cadmium-stress in both cultivars and plant organs (in the shoots of CS: 1.7×, CD: 1.2×; in the roots of CS: 1.4×, CD: 1.9×).

6. Concerning the cadmium content of wheat seedlings, it was stated that roots had one order of magnitude higher cadmium level than the shoots. The drought sensitive cultivar accumulated more cadmium than the moderately tolerant in all cases. Cadmium content of the roots significantly increased compared to the control, with the Cd-concentration. In Cappelle Desprez shoots, Cd content increased during recovery, while increase was observed in CS shoots at the 10^{-7} M Cd-stress, and decrease at the 10^{-3} M Cd treatment. Cadmium content of wheat seedlings exceeded the 3 mg/kg limit with several order of magnitudes, where cadmium toxicity symptoms appear in plants.

Practical application of the results

Results concerning cadmium tolerance well complete data of the drought tolerance of the investigated *Triticum aestivum* L. cv. Chinese Spring and Cappelle Desprez test cultivars. Beside other metals (e.g. Cu), the chosen genotypes showed differing tolerance also in the case of cadmium: the more drought-tolerant Chinese Spring better manages metal toxicity, too. Chromosomes playing role in metal tolerance can be localised using the substitution lines of the two genotypes, and the results allows to understand the mechanism of metal tolerance, which can lead to the related genes. Long-term plan could be the application of metal tolerant plants modified with cadmium transporter in phytoremediation, which makes possible enhanced cadmium transport to the shoots, similarly to zinc transporter already working in barley.

References

Chance, B., Maehly, A.C. (1955): Assay of catalases and peroxidases. Methods Enzymol., **2**, 764-817.

Nakano, Y., Asada, K. (1981): Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol., **22**, 867-880.

Pandolfini, T., Gabrielli, R., Comparini, C. (1992): Nickel toxicity and peroxidase activity in seedlings of *Triticum aestivum* L. Plant Cell Envir. **15**, 719-725.

Singleton V.L., Rossi J.A. (1965): Colorymetry of total phenolics with phosphomolybdic-phosphotunstic acid reagents. Am. J. Enol. Vitic., **16**, 144-158.

Smith, I.K., Vierheller, T.L., Thorne, C.A. (1988): Assay of gluthationee reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). Anal. Biochem., **175**, 408-413.

Publications

1. Hegedűs, A., **Leskó, K.**, Vajda, K., Tóth, E., Simon-Sarkadi, L., Stefanovits-Bányai, É., Pais, I. (2003): Titán-aszkorbát hatása a kadmiumkezelt árpa (*Hordeum vulgare* L.) csíranövények antioxidáns védekező rendszerére (Effect of titanium on the antioxidant defense system of cadmium treated barley seedlings (*Hordeum vulgare* L.) In: Mikroelemek a táplálékláncban (Trace Elements in the Food Chain) Konferenciakötet, Szerk.: (Eds.) Simon, L., Szilágyi, M., Bessenyei György Könyvkiadó, Nyíregyháza, pp.107-115., ISBN:963 9385 81
2. Stefanovits-Bányai, É., Kerepesi , I., Sárdi, É., Simon-Sarkadi, L., **Leskó, K.**, Hegedűs, A., Pais, I. (2003): A titán-aszkorbát hatása a kadmium stressz okozta biokémiai változásokra búza (Triticum aestivum L.) csíranövényekben (Effect of titanium-ascorbate on biochemical changes of wheat seedlings (Triticum aestivum L.) during cadmium stress) In: Mikroelemek a táplálékláncban (Trace Elements in the Food Chain) Konferenciakötet, Szerk.: (Eds.) Simon, L., Szilágyi, M., Bessenyei György Könyvkiadó, Nyíregyháza, pp.203-212., ISBN:963 9385 81

3. **Leskó K.**, É. Stefanovits-Bányai, I. Pais, L. Simon-Sarkadi (2002): Effect of cadmium and titanium-ascorbate stress on biological active compounds in wheat seedlings. J. Plant Nutr., **25, (11)** pp. 2571-2581. IF 0,543
4. **Leskó K.**, L. Simon-Sarkadi, É. Stefanovits-Bányai, Z. Végh, G. Galiba (2004): OPLC analysis of the polyamines in wheat seedlings under cadmium stress, Journal of Planar Chromatography, 17, 435-437. *IF 0,879 (2003)*
5. **Leskó, K.**, É. Stefanovits-Bányai, L. Simon-Sarkadi (2002): Effect of cadmium and magnesium on free amino acids, polyamines and peroxidase enzyme activity in wheat seedlings. Cereal Research Communications, **30, (Nos. 1-2)** pp. 103-110. IF 0,235
6. **Leskó, K.**, Simon-Sarkadi, L. (2002): Effect of Cadmium Stress on Amino acid and Polyamine Content of Wheat Seedlings. Periodica Polytechnica. Chemical Engineering, **46, (1-2)** pp.65-71.
7. **Leskó K.**, Stefanovits-Bányai É., Pais I., Simon-Sarkadi L. (2001): Kadmium és titán-aszkorbát okozta stressz hatása a búzanövények biológiailag aktív komponenseire. Növénytermelés, **Tom. 50. No. 1.** pp. 71-81. IF 0,274
8. **Leskó, K.**, Stefanovits-Bányai, É., Simon-Sarkadi, L. (2002): Effect of magnesium on free amino acid and polyamine content in wheat seedling exposed to cadmium stress. 7th Hungarian Congress on Plant Physiology, Acta Biologica Szegediensis, Proceedings of the 7th Hungarian Congress on Plant Physiology, Szeged, 2002. June 24-27. **46, (3-4)**, pp. 109-111. IF 0,282
9. **Leskó, K.**, É. Stefanovits-Bányai, I. Pais, L. Simon-Sarkadi (2001): Change of free amino acids and peroxidase activity by cadmium and titanium ascorbate treatments on wheat seedlings. In: Proceedings of the 9th International Trace Elements Symposium "on New perspectives in the research of hardly known trace elements and the importance of the interdisciplinary cooperation", Budapest, 2000. Aug. 31.-Sept. 2. (Ed. by Prof. Dr. Istvan Pais) pp. 173-181.
10. **Leskó, K.**, É. Stefanovits-Bányai, I. Pais, L. Simon-Sarkadi (2000): Effect of cadmium and titanium-ascorbate on polyamine content of wheat seedlings. In: Proceedings of the 20. Arbeitstagung Mengen- und Spuren-Elemente, Friedrich-Schiller-Universität, Jena, 2000. Dec. 1-2. (Ed. by Prof. Dr. Dr. h.c. Manfred Anke) pp. 819-824.
11. Stefanovits-Bányai, É., L. Simon-Sarkadi, **K. Leskó**, I. Kerepesi, É. Sárdi and I. Pais (2001): Effects of titanium-ascorbate on heavy metal induced stress-effects in plants. In Proceedings book of the 3rd International Symposium on trace elements in human: New perspectives. (Ed. by Dr. S. Pollet) Athens (Greece), 2001. Oct. 4-6. pp. 139-148. (előadás)
12. Simon-Sarkadi, L., G. Kocsy, **K. Leskó**, Á. Várhegyi, Z. Végh (2003): Investigation of the effect of drought stress on polyamine accumulation in soybean by OPLC. In Proceedings of the International Symposium on Planar Separations, Planar Chromatography 2003, (Ed. by Sz. Nyiredy) Budapest (Hungary), 2003. June 21-23. pp. 415-421. (poszter)
13. **Leskó K.**, Hegedűs A., Stefanovitsné Bányai É., Simonné Sarkadi L., Galiba G. (2004) Antioxidáns enzimek aktivitásváltozása eltérő szárazságtűrésű búza-növényekben kadmiumstressz hatására, Proceedings of the 11th Symposium on Analytical and Environmental Problems (ISBN 963 217 147 0), SZAB, Szeged, Hungary (Ed. Zoltán Galbács), 2004. szeptember 27. pp. 96-100.

Presentations

1. Stefanovits-Bányai, É., L. Simon-Sarkadi, **K. Leskó**, I. Kerepesi, É. Sárdi and I. Pais (2001): Effects of titanium-ascorbate on heavy metal induced stress-effects in plants. Abstracts of the 3rd International Symposium on trace elements in human: New perspectives. Athens (Greece), 2001. Oct. 4-6. 17-18. (oral)
2. **Leskó, K.** (2000): A nehézfém stressz vizsgálata búzanövényeken. Tudományos Diákköri Konferencia, BME Vegyészmérnöki Kar, Budapest, 2000. nov. 10. (oral)
3. Stefanovits-Bányai, É., **Leskó, K.**, Simon-Sarkadi, L. (2002): A magnézium szerepe a kadmium okozta stressz csökkentésére búza növényekben. A Magyar Magnézium Társaság előadói ülése, Budapest, 2002. október 16. (oral)
4. Simonné Sarkadi L., **Leskó K.**, Stefanovits-Bányai É. (2003): A nehézfémek okozta stressz hatása a búzanövény biológiailag aktív komponenseire. KÉKI Kollokvium, Budapest, 2003. november 14. (oral)
5. **Leskó K.**, Simonné Sarkadi L. (2003): A nehézfémstressz vizsgálata búzanövényekben. MKE Analitikai Szakosztályának Szerves Analitikai Szakcsoportjának előadóülése, Budapest, 2003. november 25. (oral)
6. **Leskó K.**, Simonné Sarkadi L., Stefanovitsné Bányai É. (2003): A kadmium okozta stressz hatása a búzanövényekre. MKE Analitikai Szakosztály Agrár- és Élelmiszeranalitikai Szakcsoport, Agrár- és Élelmiszeranalitikai Nap, Budapest, 2003. december 12. (oral)
7. **Leskó, K.**, L. Simon-Sarkadi, É. Stefanovits-Bányai, I. Pais (2000): Effects of cadmium and titanium ascorbate on free amino acids in wheat seedlings. 12th Congress of the Federation of European Societies of Plant Physiology, Budapest, 2000. Aug. 21-25. (poster)
8. **Leskó, K.**, É. Stefanovits-Bányai, I. Pais, L. Simon-Sarkadi (2000): Change of free amino acids and peroxidase activity by cadmium and titanium ascorbate treatments on wheat seedlings. 9th International Trace Elements Symposium "on New perspectives in the research of hardly known trace elements and the importance of the interdisciplinary cooperation" with international partcipation, Budapest, 2000. Aug. 31.-Sept. 2. (poster)
9. Stefanovits-Bányai, É., **K. Leskó**, S. A. Kiss, É. Sárdi, L. Simon-Sarkadi (2000): Effect of cadmium and magnesium on free amino acids in wheat seedlings. 9th International Magnesium Symposium, Vichy (France), 2000. Sept. 10-15. (poster)
10. **Leskó, K.**, É. Stefanovits-Bányai, I. Pais, L. Simon-Sarkadi (2000): Effect of cadmium and titanium-ascorbate stress on polyamine content of wheat seedlings, Macro and Trace Elements 20th Workshop, The biological essentiality of macro and trace elements Jena (Germany), 2000. Dec. 1-2. (poster)
11. **Leskó, K.**, Stefanovits-Bányai, É. (2001): Changes of peroxidase enzyme activities of five Hungarian grapevine cultivars in different phenological phases. 9th International Conference of Horticulture. Lednice (Czech Republic), 2001. Sept. 3-6. (poster)
12. Simon-Sarkadi, L., G. Kocsy, **K. Leskó**, Á. Várhegyi, Z. Végh (2003): Investigation of the effect of drought stress on polyamine accumulation in soybean by OPLC. Planar Chromatography 2003 In honour of Prof. Dr. E. Tyihák on the occasion of his 70th birthday, Budapest (Hungary), 2003. June 21-23. (poster)

13. Stefanovits-Bányai, É., **K. Leskó** (2000): Preliminary experiments on the changing of grapevine peroxidase enzyme activity by pesticide treatments, Lippay János és Vas Károly Tudományos Ülésszak, Budapest, 2000. nov. 5-6. (poster)
14. **Leskó, K.**, Stefanovits-Bányai, É., Simon-Sarkadi, L. (2002): Effect of magnesium on free amino acid and polyamine content in wheat seedling exposed to cadmium stress. 7th Hungarian Congress on Plant Physiology, Szeged (Hungary), 2002. June 24-27. (poster)
15. **Leskó K.**, M. Fodor, A. Hegedűs, É. Stefanovits-Bányai, L. Simon-Sarkadi (2003): Investigation of zirconium treated wheat seedlings by amino acid analyser. 5th Balaton Symposium on high-performance separation methods, Siófok (Hungary), 2003. Sept. 3-5. (poster)
16. Stefanovits-Bányai É., **Leskó K.** (2003): Azonos termőhelyről származó szőlőfajták peroxidáz enzimaktivitásának változása a különböző fenológiai fázisokban, Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, Budapest, 2003. nov. 6-7. (poster)
17. **Leskó K.**, Simon-Sarkadi L., Stefanovits-Bányai É., Galiba G. (2004): A szabad aminosav-tartalom változása különböző stressz-érzékenységű búzafajtákban nehézfémstressz hatására, Vegyészkonferencia 2004, Balatonföldvár, 2004. június 30-július 2. (poster)
18. **Leskó K.**, Simon-Sarkadi L., Stefanovits-Bányai É., Végh Z., Galiba G. (2004): A kadmiumstressz okozta poliamin-tartalom változásának nyomon követése búzanövényekben OPLC-vel, Elválasztástudományi Vándorgyűlés 2004 in memoriam Horváth Csaba, Hévíz, 2004. szeptember 22-24. (poster)
19. **Leskó K.**, Hegedűs A., Stefanovitsné Bányai É., Simonné Sarkadi L., Galiba G. (2004): Antioxidáns enzimek aktivitásváltozása eltérő szárazságtűrésű búzanövényekben kadmiumstressz hatására, 11th Symposium on Analytical and Environmental Problems, SZAB, Szeged, Hungary 2004. szeptember 27. (poster)