Pak. J. Bot., 49(6): 2145-2151, 2017.

PROLINE APPLICATION TRIGGERS TEMPORAL REDOX IMBALANCE, BUT ALLEVIATES CADMIUM STRESS IN WHEAT SEEDLINGS

YEVHENIIA KONOTOP[1]*, MARIIA KOVALENKO[1], ILDIKO MATUŠÍKOVÁ[2], LUDMILA BATSMANOVA[1] AND NATALIYA TARAN[1]

[1]Department of Plant Biology, ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Volodymyrska Str. 64, 01601 Kyiv, Ukraine
[2]Department of Ecochemistry and Radioecology, Faculty of Natural Sciences, University of SS. Cyril and Methodius in Trnava, Nam. J.Herdu 2, SK-917 01 Trnava, Slovak Republic
**Corresponding author: golovatyuk.yevgeniya@gmail.com, +380445221427*

Abstract

Cadmium is known to be phytotoxic and as such can heavily impact crops. The adaptation of plants to different environmental factors is accompanied by the accumulation of free proline in tissues. It is declared that treatment crops in different ways with proline is favorable in terms of tolerance to environmental stresses including heavy metal pollution. In current research the effect of pre-sowing seed treatment with different concentrations of proline (0, 5, 10 and 20 mM) on mitigation of cadmium-induced damages in winter wheat seedlings is studied. It was found that proline application itself significantly influenced only intracellular proline accumulation in wheat leaves. Exposure of seedlings to cadmium stress caused dehydration of leaves and enhancement of lipid peroxidation. Pre-sowing seed treatment with proline alleviated phytotoxic effects of heavy metal due to accumulation of intracellular proline in the leaves of seedlings, which in turn helped to preserve the water content in tissues. However, further research is needed to determine the most effective proline concentration range and way of proline application to wheat.

Key words: Antioxidative enzymes, Defense, Metal stress, Proline application, *Triticum aestivum.*

Introduction

Abiotic stresses affect crop productivity, so studying of cellular and molecular mechanisms of plant adaptation to changing environment is one of the most urgent tasks. Response reaction of plant organism to stresses is formed due to the metabolic changes, controlling which allow more efficient use of production capacity and adaptation of crops. The use of substances which have contributed to increase non-specific plant resistance to a wide range of stresses, that would be safe for the environment and human health, appears to be promising. Such compounds include: osmoprotectants (proline, trehalose, glycine betaine), phytohormones (IAA, gibberellins, jasmonic and salicylic acid, brassinisteroids), antioxidants (ascorbic acid, glutathione, tocopherol), signaling molecules (NO, H_2O_2), polyamines and others (Hasanuzzaman *et al*., 2013; Abd-Allah *et al*., 2016).

The adaptation of plants to different environmental factors is accompanied by accumulation of free proline in tissues. A strong body of evidence in the literature has shown the role of proline in stress response: it is osmoprotectant, regulator of the cellular red-ox potential, quencher of oxygen radicals. For example, proline is important in stabilizing the tertiary structure of proteins; helps to activate enzymes inhibited by heavy metal stress. It is shown that in *p5cs1* mutants that are not able to stress-induced accumulation of proline, the intense generation of reactive oxygen species coincides with the inhibition of enzymes of ascorbate-glutathion cycle suggesting proline is a stabilizer of cell protective mechanisms (Hayat *et al.*, 2012). It is believed that increased levels of proline biosynthesis help in maintaining the red-ox potential and the ratio of $NAD(P)^+$ / NAD(P)H under adverse conditions (Ashraf & Foolad, 2007). Data in the literature indicate that treatment crops in different ways with proline are favorable in terms of tolerance to environmental stresses. For example, pre-sowing treatment of rice seeds with proline (1 mM) stimulated the growth of seedlings in saline conditions (Deivanai *et al*., 2011). Processing roots with proline (5 mM) stabilized superoxide dismutase by the appearance of its new isoforms, namely, Fe-SOD, in Salvia under UV irradiation (Radyukina *et al*., 2011). Proline foliar spraying was effective in maize plants under conditions of drought in the concentration of 30 mM (Ali *et al*., 2007). According to other data processing mustard with proline in a concentration of 20 mM was sufficient to reduce the destructive processes in plant in saline environment, contributed to growth and increased the antioxidative capacity of the plant (Wani *et al*., 2016). Also, treatment of cucumber leaves with proline in a concentration of 10 mM was effective to increase plant resistance to salinity, due to the stabilization of the water status and peroxidase activity (Huang *et al*., 2009). However, it has been reported, that external supply of proline is toxic to plants despite its defense functions under stress. Nanjo *et al*. (2003) demonstrated that high rate of the exogenous proline supply might be deliterious and result in plant stunting. Similarly, Dawood *et al*. (2014) showed that application of proline at a concentration 25 mM partially mitigated the phytotoxicity of diluted seawater, however the 50 mM proline treatment showed detrimental effect. Proline toxicity is believed to be connected with Δ1-pyrroline-5-carboxylate (P5C) -induced production of reactive oxygen species as shown on yeast mutant deficient in P5C dehydrogenase (Hellmann *et al.*, 2000).

Accumulation of high concentrations of heavy metals, especially cadmium (Cd) in soil and water, is one of the most common antropological pollution. The causes of Cd appearance in the environment at excessive concentrations are activities of metallurgical enterprises, mining, transport (Nriagu & Pacyna, 1988). Besides, along with the main nutrients in fertilizer various impurities including heavy metals and radioactive isotopes are often present, since raw material for fertilizers (phosphates, apatite, raw potassium salts) typically already contains them up to 10^{-5} to 5% or more (Loganathan *et al*., 2008). Maximum concentration of cadmium in agricultural lands is 3 mg / kg soil, however, the actual content of toxicant is predominant that affects crop productivity (Salt *et al.*, 1998).

Among described response of plants to heavy metal pollution is accumulation of proline. Many studies are carried out to find out the influence of exogenous proline as a protective agent focusing on early-diverging plants (Siripornadulsil *et al*., 2002), or certain types of later-diverging plants, which possess high constitutive level of proline, namely, halophytes, hyperaccumulators of heavy metals (Schat *et al*., 1997; Stetsenko *et al*., 2011; Soshinkova *et al*., 2013). However, studies of the exogenous proline use for crops, including wheat, in conditions of anthropogenic impact are less presented (Song *et al*., 2013; Mahboob *et al*., 2016). Moreover, the way of proline supply is also important. There are enormous amounts of data on the effects of root or foliar application of proline in plants in alleviating the adverse effects of abiotic stresses (Ali *et al*., 2007; Islam *et al.*, 2009; Deivanai *et al.*, 2011; Wei-Tao *et al*., 2011; Song *et al*., 2013; Dawood *et al*., 2014; Mahboob *et al*., 2016), despite the effect of pre-sowing seed treatment with proline (Kamran *et al*., 2009). The current study was organized with 4 main objectives: 1) to reveal how pre-sowing treatment of wheat seeds with proline effects plant‘s growth and biochemical attributes of seedlings; 2) to evaluate the effect of Cd on seedlings parameters; 3) to examine whether pre-sowing application of seeds with proline solution could mitigate Cd stress in wheat seedlings; and 4) to follow the dynamic of influence of both factors alone and in combination.

Materials and Methods

Plant growth condition and treatments: Seeds of winter wheat, *Triticum aestivum* L., cv. Artemida, were surface-sterilized with 5% (v/v) sodium hypochlorite for 5 min, then rinsed in distilled water. Sterilized wheat seeds were pre-treated with 0, 5, 10, 20 mM solutions of proline for 6 hours. Processed seeds germinated in Petri dishes on moist filter paper in the dark at 25°C. After 3-4 days uniformly germinated seeds were transferred into the pots for hydroponic culture with distilled water under controlled conditions (illumination - 5000 lux, the duration of the light period - 16 hours, the temperature 25°C). 7-days wheat seedlings were subjected to root treatment of cadmium nitrate at a concentration of 50 mg Cd^{2+}/L. Control plants were grown in distilled water. Sampling collection for determination of plant biomass, the water content in the aerial part of seedlings, as well as for biochemical analysis was performed at 2, 24, 48 and 72 hours after root supplement with Cd.

Determination of growth parameters and water content: Fresh weight of plant was recorded using top-loading weighing balance. Fresh plants were then placed in an oven at 65°C for 5 days then dry masses were recorded and water content in each sample was calculated.

Measurement of proline content: Extraction of free proline was performed according to Bates (1973). Content of proline in plant material was determined spectrophotometrically (“Shimadzu UV-1800”) at wave length 520 nm. The proline concentration was determined from a standard curve using D-proline.

Detection of lipid peroxidation: The level of generated malonic dialdehyde (MDA) as product of lipid peroxidation was estimated according to Dhindsa & Matowe (1981). The concentration of MDA was determined in its unit equivalent using a molar extinction coefficient $155 \times 105\ mM^{-1}\ cm^{-1}$.

Measurement of total protein: Total protein was isolated from leaves according to Hurkman & Tanaka (1986). The protein content in the obtained extracts was determined by the method of Bradford (1976) spectrophotometrically at wave length 595 nm. Bovine serum albumin was used as a protein concentration standard.

Measurement of SOD activity: The activity of superoxide dismutase (SOD, EC 1.15.1.1) was assessed by inhibition of photoreduction of nitroblue tetrazolium (Giannopolitis & Ries, 1972) spectrophotometrically at wave length 560 nm.

Statistical analysis: Each experiment was performed in triplicate. The data were subjected to analysis of variance (ANOVA) with subsequent Student’s t-test or Duncan’s multiply range test at $p<0.05$. Data are expressed as means of replicates ± standard deviation.

Results and Discussion

One of the main criteria for evaluating plant tolerance to stress, especially to heavy metal’s impact, is the ability to accumulate biomass and maintain water status. Statistical analysis of the results showed that growth and water content of wheat leaves were affected by Cd. However, this effect variated when seeds were pre-treated with proline (Fig. 1A, B, Table 1). Obviously, dry weight of wheat seedlings increased with time and treatment with proline had no significant effect on the growth pattern. On the other hand Cd treatment similarly increased biomass of seedlings (Fig. 1A). The mechanisms of stimulating effect of low Cd level on plant growth are not well understood yet, however it is related to hormetic effect that may represent an “overcompensation” response to the organism’s homeostasis imbalance (Lin *et al*., 2007). Accelerated plant growth could be attributed to the impact of stress factor on cell division and proliferation. Besides, combined effect of Cd and proline treatment was admitted (Fig. 1A). Pre-sowing seed treatment with proline (10 and 20 mM) significantly contributed to an increase of seedlings above-ground mass at 72 h by 18 and 10% ($p \leq 0.001$ for both) respecting to the control even in a presence of Cd. Even more intensive growth of seedlings occurred after pre-sowing treatment with 20 mM of proline (Fig. 1A). The effect was already observed on the 48 h of the experiment, and after 72 h the wheat dry mass significantly raised compared with the corresponding control options (0 mM of proline): in the absence (15% increase) and also the presence (5% increase) of Cd (both at $p \leq 0.001$, Fig. 1A). The water content in plants remained unaffected by pre-sowing treatment with low doses of proline (Fig. 1B, Table 1). However, tissue dehydration was observed at higher proline concentrations (10 and 20 mM) after 72 h of experiment.

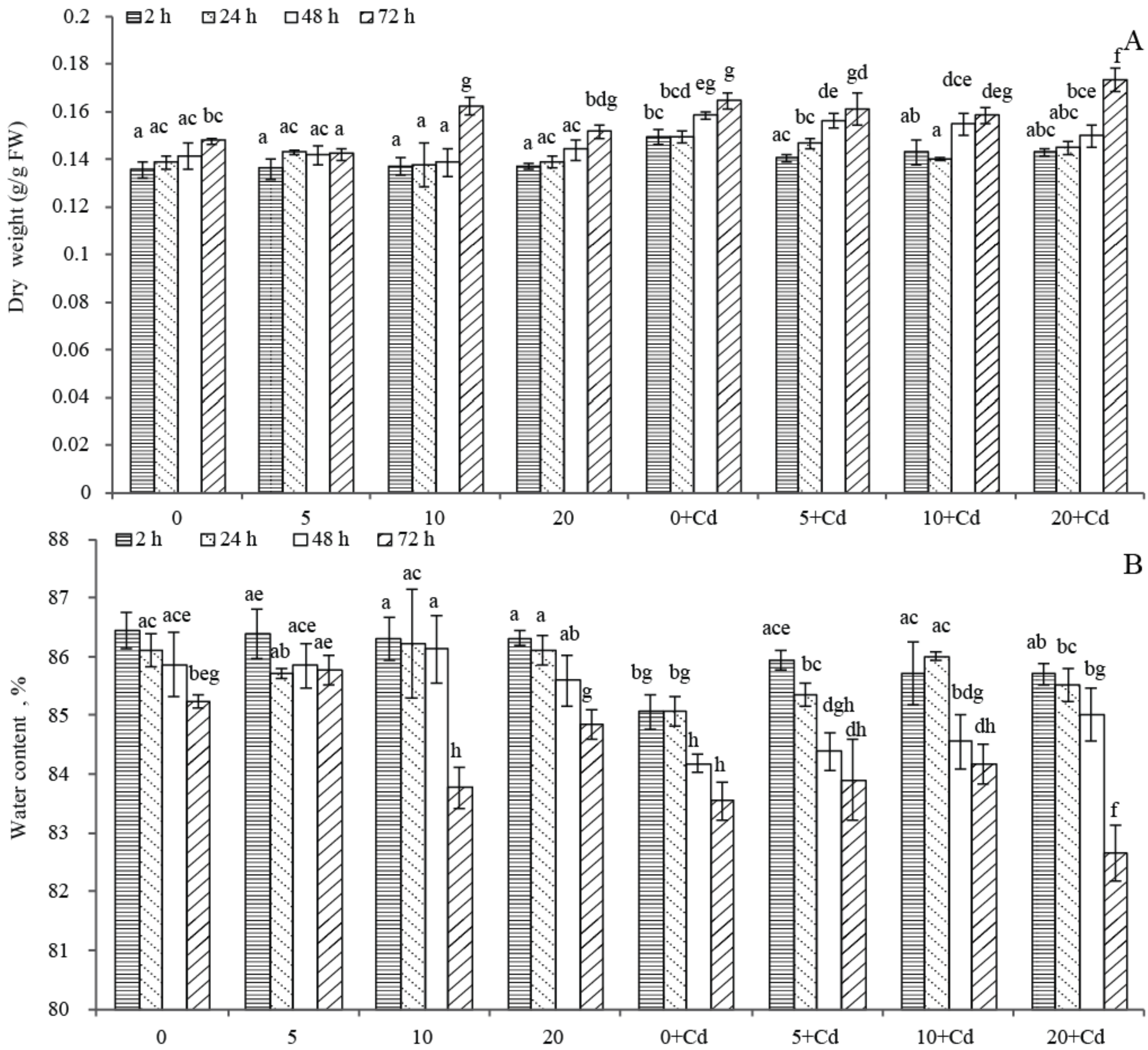

Fig. 1. Growth and water state of experimental wheat seedlings. Effect of pre-sowing treatment of wheat seeds with proline (0, 5, 10 and 20 mM) on dry weight (A) and water content (B) of seedlings in absence and presence (+Cd) of cadmium at different time points (2, 24, 48 and 72 hours). Hereinafter: the values labelled with different letters were significantly different at $p<0.05$ according to Duncan's multiply range criterion.

Table 1. Significance of differences (according to ANOVA) between values of growth, water status, some biochemical attributes of wheat seedlings (*Triticum aestivum* L.) subjected to pre-sowing seed treatment with proline (0, 5, 10, 20 mM), Cd stress, and their combined effect during exposure time

Source of variation	Dry weight	Water content	Intracellular proline	Malonic dialdehyde	Superoxide dismutase
Proline	ns	ns	**	ns	ns
Cd	***	***	***	ns	*
Time	***	***	***	***	***
Proline x Cd	***	***	***	***	ns
Proline x time	***	***	***	ns	ns
Cd x time	***	***	***	***	**
Proline x Cd x time	***	***	***	**	ns

ns – not significant; *, **, *** - significant at 0.05, 0.01, and 0.001 levels, respectively

As already mentioned some researches revealed phytotoxicity of exogenously applied proline (Hellmann *et al*., 2000; Nanjo *et al*., 2003; Dawood *et al*., 2014). Similarly, Cd caused water loss from seedling's tissues of different plant species (Perfus-Barbeoch *et al*., 2002). This effect appeared to be more obvious in time-depended manner. Reducing the water content in plant tissues under the influence of heavy metals is associated mainly with impaired growth, which in turn leads to a reduction in leaf area and the number and size of stomata on them. In this way Cd causes reduction of water potential and plant stunting (Seregin & Ivanov, 2001; Parrotta *et al*., 2015). After penetration of Cd ions in the plant multiple defense mechanisms are induced: alteration of chemical composition of the cell wall and hormone balance, increase of activity of enzymes, antioxidants, synthesis of metal-binding proteins and peptides (Seregin & Ivanov, 2001; Hall, 2002; Konotop *et al*., 2012; Parrotta *et al*., 2015). It is noted that proline is a key defense metabolite that accumulated in plant tissues in response to metal stress, and other abiotic as well as biotic factors (Schat *et al*., 1997; Szabados & Savoure, 2010). Pre-sowing seed treatment with proline in all researched concentrations contributed to the preservation of sustainable water content in the seedlings during the first 24 hours of Cd stress, however, did not seem to be effective later (Fig. 1B).

Other studies on the role of exogenous proline have proved its contribution to reduction the level of reactive oxygen species in fungi and yeast thus preventing apoptosis, protecting human cells from oxide stress of carcinogenic origin, declining the intensity of lipid peroxidation in algae (Szabados & Savoure, 2010). It was reported that treatment of vascular plants with exogenous proline prevented oxidative damage caused by the toxicity of Hg^{2+} and Ni^{2+} ions (Wang *et al*., 2009; Stetsenko *et al*., 2011). Other researchers believed proline accumulation is merely a sign of stress (Ashraf & Foolad, 2007), or have attributed proline's beneficial function to the processes of its metabolism (activation of synthesis, inhibition of degradation or release of macromolecules) rather than to the proline molecule itself (Sharma & Dietz, 2006; Wei-Tao *et al*., 2011).

In present study application of proline in seedlings had no obvious effect on proline accumulation in tissues but the observed differences can be attributed to time factor. In contrast, Cd ions caused increasing of endogenous proline level in leaves by 70% already after 2 hours of exposure ($p \leq 0.01$), keeping such a tendency to grow with time (Fig. 2, Table 1). Similarly, other plants species, such as mustard (Asgher *et al*., 2013) and wheat (Khan *et al*., 2015), accumulated proline in tissues while growing in Cd contaminated environment, that allowed researchers to suggest that proline is usually generated to help organism in dealing with Cd stress. This effect was stronger in seedlings pre-treated with proline.

The intensity of lipid peroxidation was evaluated in leaves of wheat seedlings to study the effect on generation of oxidative stress (Fig. 3). The data showed that MDA production remained unaffected by proline pre-treatment regardless the dose. The effect of Cd alone was rather small at early stages of the experiment (2 h), however, increased with time and significant changes were detected after 72 h (Fig. 3). Application of proline in presence of Cd resulted in suppression of MDA level in leaves on the late stage of the exposure time. Although proline is generally considered as antioxidant (Szabados & Savoure , 2010) under some circumstances it can be a cause of oxidative stress. Oxidative effect of proline in plants is associated with activation of proline dehydrogenase. In this case molecules of proline are oxidated in mitochondria and the generated excess of electrons leads to disruption of red-ox balance and accumulation of reactive oxygen species in these organelles (Elton & Stewart, 1982; Cooper *et al*., 2008; Soshynkova *et al*., 2013). Temporal disbalance of MDA content that was observed at the early stage of experiment, can be interpreted as a signal of cellular status that subsequently is managed by pre-existing antioxidant defense mechanisms of plant (Møller *et al*., 2007).

The obtained result showed that pre-sowing treatment of wheat seedlings with proline did not promote significant changes in SOD activity (Fig. 4, Table 1). There was a general trend to a sharp increase of enzyme activity at 24 h of experiment with its further decline to control values. This pattern could be attributed to relatively low research concentrations (up to 20 mM) or the way of application. Cd itself suppressed the SOD activity in wheat leaves at 72 h of exposure time. However, the combined application of proline and Cd did not seem to be significant for alteration of SOD activity (Table 1).

Activation of oxidative processes in wheat tissues at the given concentration of Cd and the stimulation of seedlings growth we observed in the present study is conformable with data of Erofeeva (2014). The author affirmed that the same toxicant concentrations can cause normalization of some parameters of plant, but a disruption of others. Significant imbalance in the organism's state is harmful for its normal functioning. Therefore, regulatory systems are activated to prevent them. As long as the organism is able to normalize its parameters, the changes will be moderate. Under the action of sublethal toxicant concentrations, however, the compensatory capacity of the organism is depleted and significant impairment of its parameters is observed.

Conclusions

The results of the present study showed that pre-sowing seed treatment with proline did not have significant effect on researched parameters of wheat seedlings. The proline application significantly influenced only the intracellular proline accumulation. Exposure of seedlings to Cd stress caused dehydration of leaves and enhancement of lipid peroxidation. Pre-sowing seed treatment with proline alleviated phytotoxic effects of heavy metal due to accumulation of intracellular proline in the leaves of seedlings, which in turn helped to preserve the water content of the above-ground parts of plant. In addition, it suppresses MDA level. Re-establishing of the red-ox homeostasis, however, does not seem to occur via SOD action. The time factor appeared to be important for making relevant conclusions, since temporal changes (especially at early time point of experiment) do not necessarly reflect the true outcome of metal toxicity and plant defence interactions. Further studies focusing on the determination of the most effective proline concentration range and way of proline application (pre-sowing seeds, through root supply or foliar spraying) to wheat plants are needed.

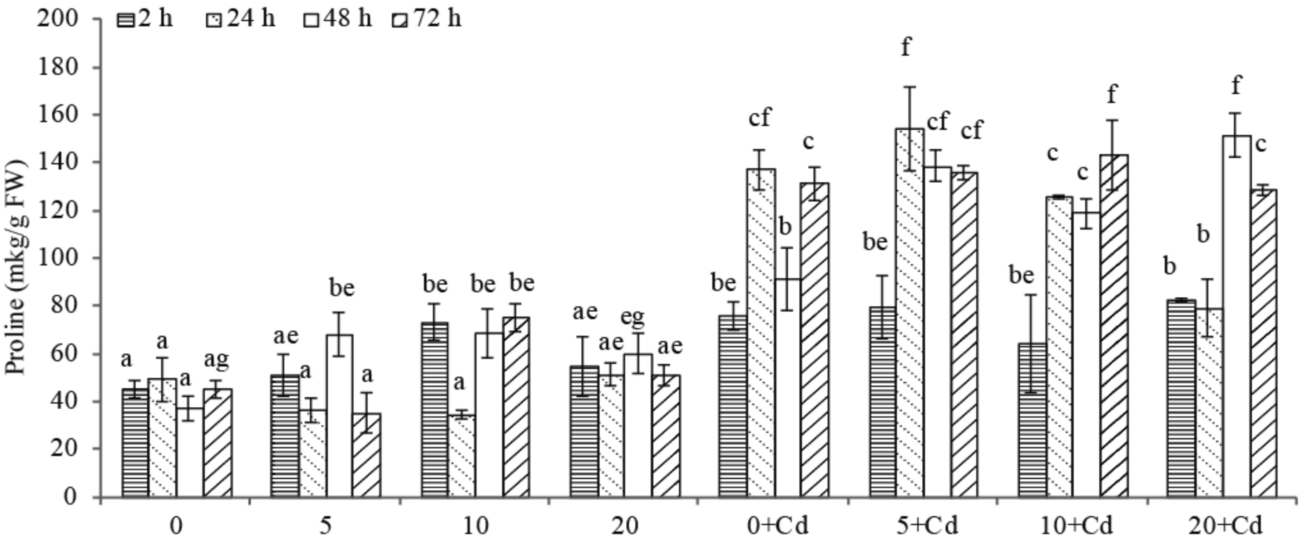

Fig. 2. Intracellular proline accumulation in leaves of wheat seedlings after pre-sowing seed treatment with proline (0, 5, 10 and 20 mM) in absence and presence (+Cd) of cadmium at different time points (2, 24, 48 and 72 hours).

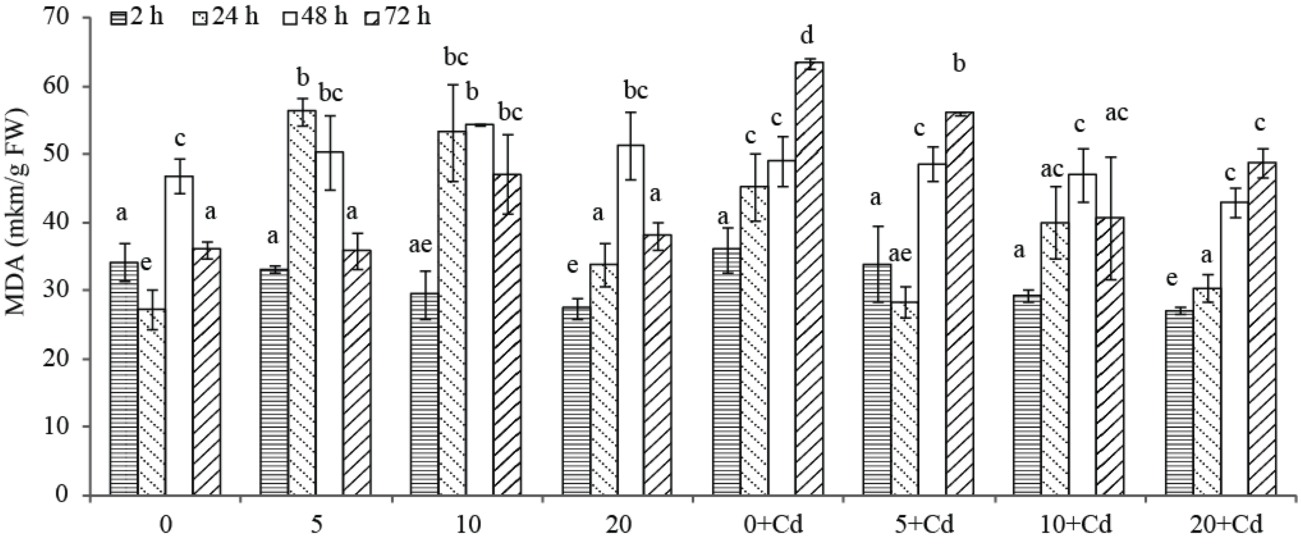

Fig. 3. MDA content in leaves of wheat seedlings after pre-sowing seed treatment with proline (0, 5, 10 and 20 mM) in absence and presence (+Cd) of cadmium at different time points (2, 24, 48 and 72 hours).

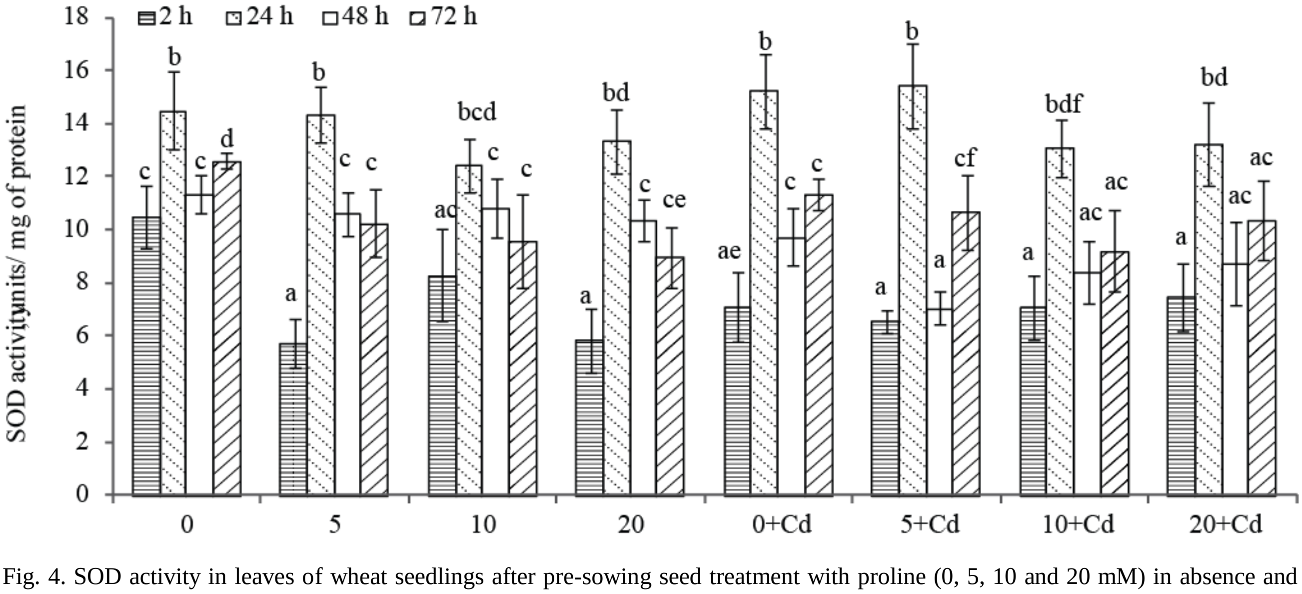

Fig. 4. SOD activity in leaves of wheat seedlings after pre-sowing seed treatment with proline (0, 5, 10 and 20 mM) in absence and presence (+Cd) of cadmium at different time points (2, 24, 48 and 72 hours).

Acknowledgment

This work was supported by the Ministry of Education and Science of Ukraine under the contract number 0116U004779. Contribution of IM was supported by the Slovak Research and Development Agency under contract number APVV-15-0051. Financial support for the stay of YK at UCM was provided by the Slovak Academic Information Agency (SAIA, n.o.).

References

Abd-Allah, E.F., A.A. Alqarawi, A. Hashem, D.A.W. Soliman and M.A. Alghamdi. 2016. Selenium ameliorates cadmium stress-induced damage by improving antioxidant defense system in *Chlamydomonas reinhardtii. Pak. J. Bot*., 48(6): 2223-2231.

Ali, Q., M. Ashraf and H.U.R. Athar. 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. *Pak. J. Bot*., 39(4): 1133-1144.

Asgher, M., M.I.R. Khan, N. Iqbal, A. Masood and N.A. Khan. 2013. Cadmium tolerance in mustard cultivars: dependence on proline accumulation and nitrogen assimilation. *J. Funct. Environ. Bot*., 3: 30-42.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. *Environ. Exp. Bot*., 59: 206-216.

Bates L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*.,39: 205-207.

Bradford, M.M. 1976. A rapid and sensitive method for the quantation of microgram quantities of protein utilizing the principle of protein-dye binding. *Annal. Biochem*., 72: 248-254.

Cooper, S.K., J. Pandhare, S.P. Donald and J.M. Phang. 2008. A novel function for hydroxyproline oxidase in apoptosis through generation of reactive oxygen species. *J.Biol. Chem*., 283(16): 10485-10492.

Deivanai, S., R. Xavier, V. Vinod, K. Timalata and O.F. Lim. 2011. Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. *J. Stress Physiol. Biochem*., 7(4): 157-174.

Dawood, M.G., H.A.A. Taie, R.M.A. Nassar, M.T. Abdelhamid, and U. Schmidhalter. 2014. The changes induced in the physiological, biochemical and anatomical characteristics of *Vicia faba* by the exogenous application of proline under seawater stress. *South Afr. J. Bot*., 93: 54-63.

Dhindsa, R.S. and W. Matowe. 1981. Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. *J. Exp. Bot.,* 32: 79-91.

Elton, T.E. and C.R. Stewart. 1982. Proline oxidation in corn mitochondria. *Plant Physiol.*, 70: 567-572.

Erofeeva, E.A. 2014. Hormesis and paradoxical effects of wheat seedling (*Triticum aestivum* L.) parameters upon exposure to different pollutants in a wide range of doses. *Dose-Response*, 12: 121-135.

Giannopolitis, C.N. and S.K. Ries. 1972. Superoxide dismutase I. Occurrence in higher plants. *Plant Physiol*., 59: 309-314.

Hall, J.L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot*., 53(366): 1-11.

Hasanuzzaman, M., K. Nahar and M. Fujita. 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad, P., M.M. Azooz and M.N.V. Prasad (Eds.) // *Ecophysiology and responses of plants under salt stress.* Springer Science + Business Media, LLC., pp. 25-87.

Hayat, S., Q. Hayat, M.N. Alyemeni, A.S Wani., J. Pichtel and A. Ahmad. 2012. Role of proline under changing environments. *Plant Signal. Behav.*, 7(11): 1456-1466.

Hellmann, H., D. Funck, D. Rentsch and W.B. Frommer. 2000. Hypersensitivity of an *Arabidopsis* sugar signaling mutant toward exogenous proline application. *Plant Physiol*., 123: 779-790.

Huang, Y., Z. Bie, Z. Liu, A. Zhen and W. Wang. 2009. Protective role of proline against salt stress is partially related to the improvement of water status and peroxidase enzyme activity in cucumber. *Soil Sci. Plant Nutr.*, 55: 698-704.

Hurkman, W.J. and C.K. Tanaka. 1986. Solubilization of plant membrane proteins for analysis by two-dimensional gel electrophoresis. *Plant Physiol.*, 81(3): 802-806.

Islam, M.M., M.A. Hoque, E. Okuma, M.N.A. Banu, Y. Shimoishi, Y. Nakamura and Y. Murata. 2009. Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. *J. Plant Physiol*., 166: 1587-1597.

Kamran, M., M. Shahbaz, M. Ashraf and N.A. Akram. 2009. Alleviation of drought-induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as a pre-sowing seed treatment. *Pak. J. Bot.*, 41(2): 621-632.

Khan, M.I.R., F. Nazir, M. Asgher, T.S. Per and N.A. Khan. 2015. Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *J. Plant Physiol*., 173: 9-18.

Konotop, Ye., P. Mészáros, N. Spieß, V. Mistríková, B. Piršelová, J. Libantová, J. Moravčíková, N. Taran, P. Hauptvogel and I. Matušíková. 2012. Defense responses of soybean roots during exposure to cadmium, excess of nitrogen supply and combinations of these stressors. *Mol. Biol. Rep*., 39: 10077-10087.

Lin, R., X. Wang, Y. Luo, W. Du, H. Guo and D. Yin. 2007. Effect of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). *Chemosphere*, 69(1): 89-98.

Loganathan, P., M.J. Hedley and N.D. Grace. 2008. Pasture soils contaminated with fertilizer-derived cadmium and fluorine: Livestock effects. *Rev. Environ. Contam. Toxicol*., 192: 29-66.

Mahboob, W., M. Athar Khan and M.U. Shirazi. 2016. Induction of salt tolerance in wheat (*Triticum aestivum* L.) seedlings through exogenous application of proline. *Pak. J. Bot.*, 48(3): 861-867.

Møller, I.M., P.E. Jensen and A. Hansson. 2007. Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol*., 58: 459-481.

Nanjo, T., M. Fujita, M. Seki, T. Kato, S. Tabata and K. Shinozak. 2003. Toxicity of free proline revealed in an *Arabidopsis* T-DNA-tagged mutant deficient in proline dehydrogenase. *Plant Cell Physiol*., 44(5): 541-548.

Nriagu, J.O. and J.M. Pacyna. 1988. Quantitative assessment of worldwide contamination of air, water and soils with trace metals. *Nature*, 333: 134-139.

Parrotta, L., G. Guernierro, K. Sergeant, G. Cai and J.-F. Hausman. 2015. Target or barrier. The cell wall of early- and later-diverging plants vs. cadmium toxicity: differences in the response mechanisms. *Front. Plant Sci*., 6: 1-16.

Perfus-Barbeoch, L., N. Leonhardt, A. Vavasseur and C. Forestier. 2002. Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.*, 32(4): 539-548.

Radyukina, N.L., A.V. Shashukova, S.S. Makarova and V.V. Kuznetsov. 2011. Exogenous proline modifies differential expression of superoxide dismutase genes in UV-B-irradiated *Salvia officinalis* plants. *Russ. J. Plant Physiol.*, 58(1): 51-59.

Salt, D.E., R.D. Smith and I. Raskin. 1998. Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 49: 643-668.

Schat, H., S.S. Sharma and R. Vooijs. 1997. Heavy metal-induced accumulation of free proline in a metal-tolerant and a non-tolerant ecotype of *Silene vulgaris*. *Physiol. Plantarum*., 101: 477-482.

Seregin, I.V. and V.B. Ivanov. 2001. Physiological aspects of cadmium and lead toxic effects on higher plants. *Russ. J. Plant Physiol.*, 48: 523-544.

Sharma, S.S. and K.-J. Dietz. 2006. The significajnce of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot*., 57(4): 711-726.

Siripornadulsil, S., S.Traina, D.P.S. Verma and R.T. Sayre. 2002. Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. *Plant Cell*., 14: 2837-2847.

Song, M., W.J. Xu, X.Y. Peng and F.H. Kong. 2013. Effects of exogenous proline on the growth of wheat seedlings under cadmium stress. *Chi. J. Appl. Ecol*., 24(1): 129-34.

Soshinkova, T.N., N.L. Radyukina, D.V. Korolkova and A.V. Nosov. 2013. Proline and functioning of the antioxidant system in *Thellungiella salsuginea* plants and cultured cells subjected to oxidative stress. *Russ. J. Plant Physiol.*, 60: 41-54.

Stetsenko, L.A., N.I. Shevyakova, V.Y. Rakitin and V.V. Kuznetsov. 2011. Proline protects *Atropa belladonna* plants against nickel salt toxicity. *Russ J Plant Physiol*., 58: 337-343.

Szabados, L. and A. Savoure. 2010. Proline: a multifunctional amino acid. *Trends Plant Sci*., 15(2): 89-97.

Wani, A.S., A. Ahmad, S. Hayat and I. Tahir. 2016. Is foliar spray of proline sufficient for mitigation of salt stress in *Brassica juncea* cultivars. *Environ. Sci. Pollut. Res*., 23: 13413-13423.

Wang, F., B. Zeng, Z. Sun and C. Zhu. 2009. Relationship between proline and Hg^{2+}-induced oxidative stress in a tolerant rice mutant. *Arch. Environ. Contam. Toxicol*., 56(4): 723-731.

Lv, W.T., B. Lin, M. Zhang and X.J. Hua. 2011. Proline accumulation is inhibitory to arabidopsis seedlings during heat stress. *Plant Physiol.*, 156: 1921-1933.

(Received for publiation 11 January 2016)