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Growth, anatomy and enzyme activity changes in maize roots induced by treatment of seeds with low-temperature plasma*

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Abstract: The seeds of *Zea mays* L. cv. KWS were exposed to low-temperature plasma (LTP) by using Diffuse Coplanar Surface Barrier Discharge (DCSBD) for 60 and 120 seconds respectively. Growth parameters, anatomy of roots and activity of some enzymes (CAT, G-POX, SOD and DHO) isolated from roots grown from the seeds treated by LTP were evaluated. Our results indicate that LTP treatment of maize seeds affects post-germination growth of seedlings and this effect depends on the duration of LTP treatment. LTP treatment in duration of 60 seconds significantly increased the length, fresh and dry weight of the roots. However, the increase in time of LTP treatment to 120 seconds had inhibitive effect on the studied growth parameters. The activities of all studied antioxidant enzymes significantly increased with the age of maize seedlings in control conditions. On the other hand the application of LTP resulted in small, mostly non significant changes in the activity of antioxidant enzymes. Significant decrease in CAT activity was observed both in 3 and 6–day old maize roots and G-POX activity in 3–day old maize roots grown from seeds exposed to LTP for 60 seconds. A small, significant increase was detected only in SOD activity in 3–day old maize roots grown from seeds treated with LTP for 120 seconds and in 6–day old maize roots treated with LTP for 60 seconds. Significantly higher DHO activity was determined in embryos isolated from seeds treated with LTP for 60 seconds. On the contrary, in roots the DHO activity decreased with the time of LTP treatment. LTP treatment of seeds did not affect the anatomy of maize roots and caused only minor changes in the isoenzyme composition of G-POX and SOD.

Key words: catalase; dehydrogenase; maize; oxidative stress; peroxidase; root; superoxide dismutase

Abbreviations: CAT, catalase; DHO, dehydrogenase; DW, dry weight; EDTA, ethylenediaminetetraacetic acid; FW, fresh weight; LTP, low-temperature plasma; G-POX, quaiacol-peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TEMED, N,N,N',N'- tetramethylethylenediamine; TTC, triphenyl tetrazolium chloride; TTF, triphenylformazane

Introduction

The application of different physical methods has revolutionized current research in the field of agricultural science (Bari et al. 2003). Physical factors such as ionizing radiation, laser, high-power light radiation, high electromagnetic field etc. were used in plant growth stimulation (Galová 1996; Martines et al. 2002; Cheen et al. 2005; Vashisth & Nagarajan 2009a; Xiong et al. 2009). Magnetic and electric fields as well as corona discharge were used widely as pretreatment for seeds to increase seed vigor and for stimulation of seed germination (Pietruszweski 1996; Palov 2003; Lynikiene et al. 2006), seedling growth (Davies et al. 1996; Carbonell et al. 2000; Gan-Mor 2003), increase of yield (Pietruszweski 1993; Ksenz & Kaciesvili 2000; Ahmet 2003) as well as stimulation of photosynthetic activity (Lebedev & Litvinenko 1977). Laser, gamma irradiation and electrostatic field pretreatments have apparently induced enzymatic activities (Hammed et al. 2008; Shabrangi & Majd 2009; Wang et al. 2009), changed thermodynamic parameters (Cheen et al. 2005), and accelerated physiological processes and metabolism (Podleceny 2002; Wang et al. 2009).

One of the next physical sources which offer a broad range of interesting industrial applications is a low-temperature plasma (LTP) (Černák et al. 2009). There are many results, which indicate positive effects of the LTP on seed germination (increase germinating power of seeds and reduction germinating time), as well as on development, growth, enzyme activity and yield of plants (Volin et al. 2000; Yin et al. 2005; Marinković & Borcean 2009; Šerá et al. 2008). The effective consequence of plasma treatment is also sterilization and destruction of microflora occurrence on the surface of plant seeds (Basaran et al. 2008; Jung et al. 2010).

In high-pressure non-equilibrium plasma discharges, reactive species are generated through various col-

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liding pathways, such as electron impact excitation and dissociation (Laroussi 2005). Reactive species play an important role in all plasma-surface interactions. For example, air plasmas are excellent sources of reactive oxygen-based and nitrogen-based species, such as O, O_2^*, O_3, $OH^\bullet$, NO, NO_2, etc. Plants have evolved protective mechanisms including enzymatic and non-enzymatic antioxidants (Foyer et al. 1994) against not only environmental stresses such as salinity, drought, temperature, pollutants, metal toxicity, but also against chemical and physical stresses (Donahue et al. 1997; Hammed et al. 2008) which generate reactive oxygen species (ROS). ROS are highly reactive in the absence of any protective mechanism, they can seriously disrupt normal metabolism through oxidative damage to membrane lipids, proteins, pigments and nucleic acids. These ROS are detoxified by the sequential and simultaneous action of a number of enzymes, including superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT). It's generally known, that the coordinated action of several antioxidant enzymes is activated when plants are exposed to different kinds of stress (Cakmak & Horst 1991; Suzuki & Mittler 2006; Agarwal 2007; Apostolova et al. 2008; Zelinová et al. 2010).

The objective of this work was the study of the LTP irradiation on maize seeds and its subsequent effect on the growth and anatomy of roots as well as on the activity of selected antioxidant enzymes and activity of DHO in roots or embryos.

Material and methods

Characteristic of plasma source

Treatment of maize seeds was carried out using Diffuse Coplanar Surface Barrier Discharge (DCSBD) non-thermal plasma source at atmospheric-pressure in the ambient air. The DCSBD electrode geometry consists of many parallel strip-line silver electrodes embedded 0.5 mm below the surface of 96% Al_2O_3 ceramics. The DCSBD generates on alumina plate a thin uniform layer of macroscopically homogeneous plasma. The effective thickness of the plasma layer measured using a CCD camera (Šimor et al. 2002) can be estimated to be about 0.3 mm that gives at used experimental conditions the high plasma power density of some 100 W/cm^3. The discharge was powered by 14 kHz sinusoidal voltage with amplitude of approximately 10 kV, supplied by a HV Plasma Power Supply. The electrical parameters of discharge were monitored by a current monitor Model 4100 (Pearson Electronics) and two high voltage probes Tektronix P6015A. The signals from all three electrical probes were recorded by a digitizing oscilloscope (Tektronix TDS 2014B). The input energy 370 W was calculated from measured current-voltage waveforms. The total power consumed by plasma was used for plasma processing, which allows short treatment times and the possible incorporation of the DCSBD directly in continuously working production lines (Černák et al. 2009).

By Optical Emission Spectroscopy (OES) measurements were confirmed non-equilibrium temperature character of the DCSBD air plasma generated at atmospheric pressure. As were estimated from the band of OH and from the second positive nitrogen band system of nitrogen, the rotational gas temperature was 300 ± 10 K and the vibration temperature of 2160 ± 222 K. This means that the temperature of heavy particles is relatively low, similar to room temperature, while energetic electrons of high temperature create reactive radicals and ions through collisions with neutral molecules of the background gas.

Plant material and growth conditions

Air-dried seeds of maize (*Zea mays* L.) cv. KWS were obtained from the Sedos Co. Krakovany, Slovakia. The seeds were exposed to LTP for 60 and 120 seconds. Movement of maize seeds on the electrode surface was carried out mechanically to ensure their uniform treatment. The seed samples without treatment served as the control. Control and treated seeds after 10-h imbibition in sterile distilled water were placed to germinate in Petri dishes on two filter-paper discs. The Petri dishes contained 12 mL of sterile distilled water and the seeds were germinated for 3 and 6-days in an incubator at 26 °C, 60% air humidity in the dark.

Growth parameters

Plants were harvested 3 and 6 days after sowing and separated into shoots and roots. Growth parameters, such as root length, fresh and dry weight were determined according to the standard methods 6-days after the seed treatment with LTP. The analysis of the root growth characteristics was repeated three times with 20 samples for each type of variant. Roots were oven-dried at 80 °C for 5 days until reaching a constant mass for dry weight determination. The fresh roots frozen by liquid nitrogen were sampled for next analysis.

Protein extraction and determination

Frozen roots were ground in liquid nitrogen with mortar and pestle and the powder was suspended in 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM EDTA and protease inhibitor (Protease Inhibitor Cocktail Tablets from Roche Diagnostics GmbH, Mannheim Germany) in concentration of one tablet in 50 mL of extraction phosphate buffer), filtered and centrifuged at 15,000 *g* for 20 min. Supernatant was used as an enzyme source and for the purposes of isoenzyme determinations was concentrated by ultrafiltration using Amicon (10 kDa) (Millipore, Carringtonhill, Cork). The soluble protein concentration was determined according to Bradford's method (1976) using bovine serum albumin (BSA, Sigma–Aldrich, St. Louis, MO, USA) as a standard.

Enzymes activities

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured and expressed according to Madamanchi et al. (1994) by following the inhibition of the photo-reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Merck) according to the method of Scebba et al. (2006). The reaction mixture contained: 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 2 μM riboflavin, 0.15 mM MTT and 0.150 mL of the supernatant. The reaction was initiated by placing the reacting mixture in tubes to cool fluorescent light (50 μM m^{-2} s^{-2}) for 15 min. Non-illuminated and illuminated reactions without supernatant served as calibration standards. Reaction products were measured spectrophotometrically at 560 nm (Jenway 6400, Krackeler Scientific, London, UK). The activity of SOD is the measure of MTT reduction in light without protein minus MTT reduction with protein. One unit of SOD activity is the amount of protein required to inhibit 50% initial reduction of MTT under the light.

Peroxidase (G-POX; EC 1.11.1.7) activity was determined spectrophotometrically (Jenway 6400, Krackeler Scientific, London, UK) based on the oxidation of guaiacol in the presence of H_2O_2 at 440 nm (Frič & Fuchs 1970). The assay mixture contained 0.1 M Na-acetate buffer (pH 5.2), 5 mM guaiacol, 8 mM H_2O_2, and 0.1 mL of supernatant. Specific enzyme activity was expressed as enzyme activity unit min^{-1} mg^{-1} of proteins. The enzyme activity unit is expressed as an amount of enzyme dissolved in 1 mL of solution which changed the absorbance at value 0.1 in cuvette (10 mm thick) at 25 °C during the time of 100 seconds.
Catalase (CAT; EC 1.11.1.6) activity was determined according to Hodges et al. (1997) method based on hydrogen peroxide decomposition by catalase which is expressed as absorbance decreasing of a reacting sample solution. The reaction mixture contained: 50 mM phosphate buffer (pH 7.3), 30 mM hydrogen peroxide and 0.15 mL of supernatant. Decreasing of hydrogen peroxide in sample was spectrophotometrically (Jenway 6400, Krackeler Scientific, London, UK) measured in 30 second intervals in three cycles at 240 nm. Catalase specific activity was calculated according to Claiborne (1985) and expressed to 1 mg of soluble proteins.

Native polyacrylamide gel electrophoresis and isoenzymes detection
Isoenzymes were separated by discontinuous polyacrylamide gel electrophoresis under non-denaturing conditions with a Mini-PROTEAN II (Bio-Rad, CA, USA) unit (175 V for 1 h, 4 °C). SOD isoenzymes separation according to Laemmli (1970) was performed using 3.75% stacking gel and 12% running gel. Equal amount of proteins (60 μg) from each sample were subjected for electrophoretic separation. SOD isoenzymes were detected according to Donahue et al. (1997). Gels were preincubated for 30 min at 30 °C in the dark in 50 mM phosphate buffer, pH 7.5 with 1 mM EDTA. They were incubated for 30 minutes in the dark in the same buffer containing 0.24 mM nitroblue tetrazolium chloride (NBT), 0.2% N,N,N',N'-tetramethylethylendiamine (TEMED) and 33.2 μM riboflavin. The reaction started exposing gels to white fluorescent light. For identification of individual isoformes parallel gels were treated with either 5 mM KCN (an inhibitor of Cu/Zn-SOD) or 5 mM H_2O_2 (an inhibitor of Cu/Zn-SOD) and Fe-SOD) in preincubation buffer. G-POX anionic isozymes were separated at a basic pH-value (separating gel 10%, pH 8.8; stacking gel 3.75%, pH 6.8) according to the method of Laemmli (1970). Cationic isoenzymes were separated at an acidic pH (separating gel 7%, pH 4.3; stacking gel 3.75%, pH 6.8) according to Reisfeld et al. (1962). Equal volumes of the protein (60 μg anionic and 20 μg cationic, respectively) were subjected to electrophoretic separation. Peroxidase isoenzymes were detected by incubating the gels for 5–10 min in a reaction mixture containing 50 mM guaiacol and 50 mM H_2O_2 in 50 mM Na-acetate buffer, pH 5.2 according to Tamás et al. (2003).

Dehydrogenase activity (DHO)
Dehydrogenase activity in roots and embryos was measured according to Yin et al. (2005) method by using triphenyl tetrazolium chloride (TTC) as an artificial electron acceptor. Reduction of TTC and formation of red triphenyl formazan (TTF) was quantified after acetone extraction spectrophotometrically (Jenway 6400, UK) at 480 nm.

Isolation of embryos from seeds soaked for 20 hours at 26 °C in distilled water was performed with a scalpel. To 1 g of embryos or roots placed into test tubes 10 mL of reaction solution (in equal volume with 0.4% (w/v) 2,3,5-triphenyl tetrazolium chloride (TTC) and 0.1 M phosphate buffer pH 7.5) was added. Samples were incubated in the dark at 37 °C for 1 h, then 2 mL 2N H_2SO_4 was added to stop the reaction. The red TTF from roots and embryos was extracted using 100% acetone and determined spectrophotometrically.

Root anatomy
Selected morphological parameters (root perimeter; root diameter; area of the whole root; epidermis; outer cortex – combined exodermis and mesodermis; endodermis; and stele) on cross-sections of 6-days old primary roots were measured. The samples were collected from the roots in the distance of 50 mm from the root apex to investigate the influence of LTP on the plant anatomy in the bright field (microscope – Axioskop 2 plus, Carl Zeiss). The captured digital images (digital camera – DP 72, Olympus) were analyzed by software analysis (Lucia, v. 4.8, LIM).

Statistical analysis
Data were analysed using of Student's t-test (Microsoft Excel) and analysis of variance (ANOVA) and comparisons between the mean values were made by the least significant difference (LSD) test at $P < 0.05$, and a standard error (SE) was calculated.

Results and discussion

Growth parameters
Based on our previous results (Henselová et al. 2011), the positive influence of LTP treatment on germination and early growth of seedlings were found in the time range of 40–80 seconds with optimum at 60 seconds. Growth characteristics of 6-day old seedlings grown from seeds treated with by LTP for 60 and 120 seconds are shown in Table 1. LTP treatment of seeds for 60 seconds resulted in significant increase of the growth parameters in comparison to the control plants. This increase represented 21% in length, 10% in fresh

Table 1. Root parameters of 6-day old maize roots grown from seeds treated with LTP for 60 and 120 seconds. Values are means ± SE ($n = 3$). For each parameter, means with the different letters are statistically different at ($P < 0.05$).

Parameters	Control	Exposure dose of LTP (seconds)	
		60	120
Root length (cm)	17.42 ± 0.77^b	21.02 ± 0.72^c	14.67 ± 0.62^a
Root fresh weight (mg)	502.70 ± 9.60^b	551.17 ± 4.29^c	460.07 ± 12.28^a
Root dry weight (mg)	29.78 ± 0.83^b	34.08 ± 0.79^c	25.92 ± 1.06^a

and 14% in the dry weight of the roots. On the other hand an increased exposition of seeds to LTP for 120 seconds significantly inhibited all measured parameters in comparison with untreated seeds (Table 1). From our unpublished results it seems that LTP treatment of seeds has a stronger impact on root growth than shoot growth (data not published). The mechanism of LTP effect is still not well known. According to some authors (Chaomei & Yanlin 1993; Volin et al. 2000; Dubinov et al. 2000; Henselová et al. 2011) the plasma induces not only changes on the seed surface but also in some biochemical parameters.

The quality and vigour of seeds are very often based on the estimation of the viability of seeds with the tetrazolium test using dehydrogenase systems (Jensen et al. 1951; Smith 1952). Generally, the higher DHO activity was determined in embryo, than in root, what might depend on the respiratory or enzymatic activity at an early stage of the germination process. In embryos significantly higher DHO activity was determined only after their isolation from seeds treated with LTP for 60 seconds (Fig. 1). In roots both doses of LTP treatment decreased DHO activity. This inhibition represented 18.5% at 60 seconds and 27% at 120 seconds LTP treatment in comparison to untreated control

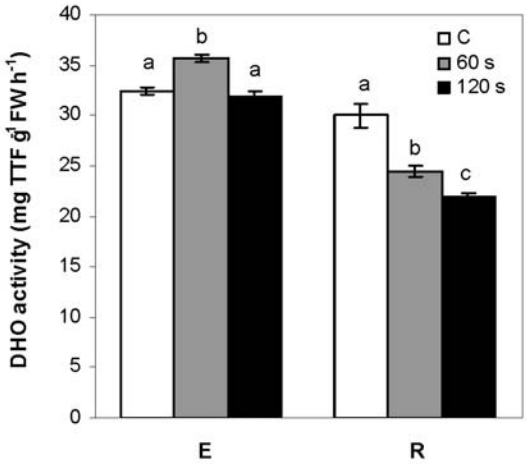

Fig. 1. Dehydrogenase activity in roots (R) and embryos (E) of maize seedlings grown from seeds treated with LTP for 60 and 120 seconds. C – Untreated control seeds. Values are the means ± SE of three replicates. Bars with different letters are statistically different at ($P < 0.05$).

(Fig. 1). According to Yin et al. (2005), the intensity of TTC reduction in tomato roots increased with the increase of the current ranging of magnetized plasma,

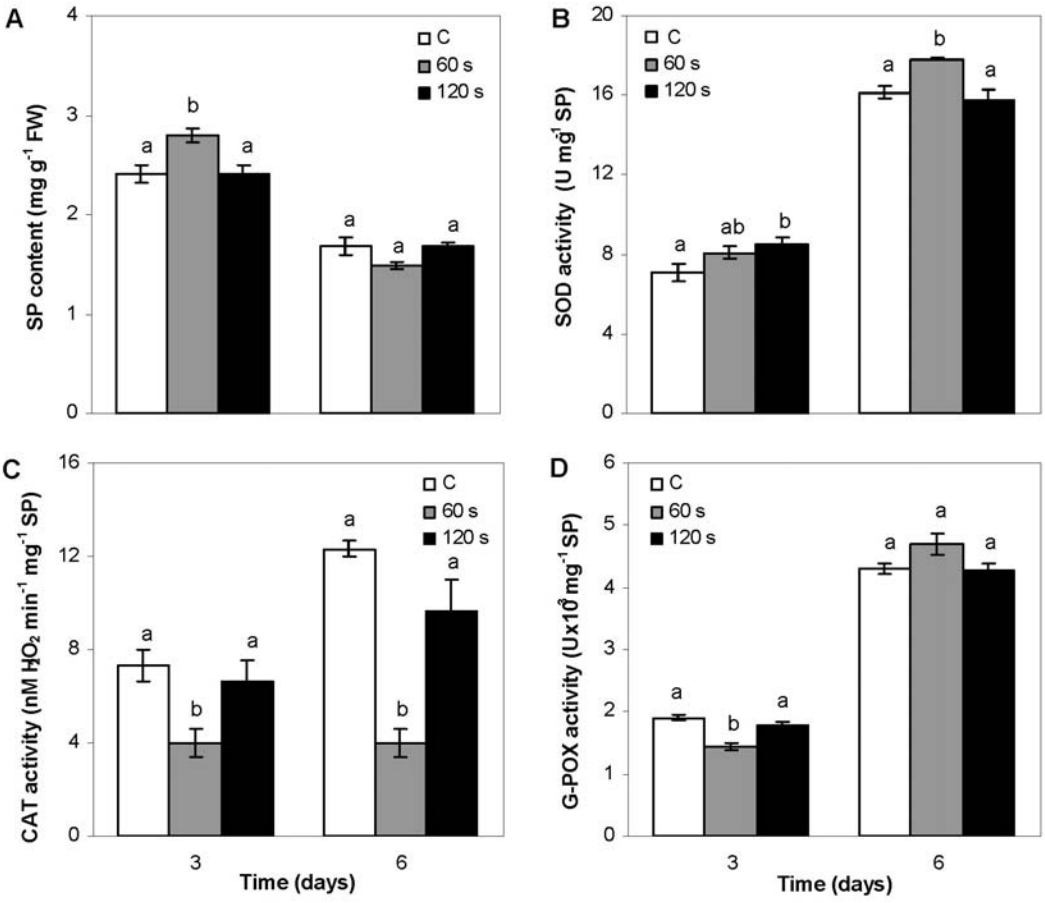

Fig. 2. Total soluble protein content (A) and enzyme activities in extracts from roots of 3– and 6–days old maize seedlings grown from seeds treated with LTP for 60 and 120 seconds. C – untreated control seeds; B – SOD activity; C – CAT activity; D – G-POX activity. Values are the means ± SE of three replicates. Bars with different letters are statistically different at ($P < 0.05$).

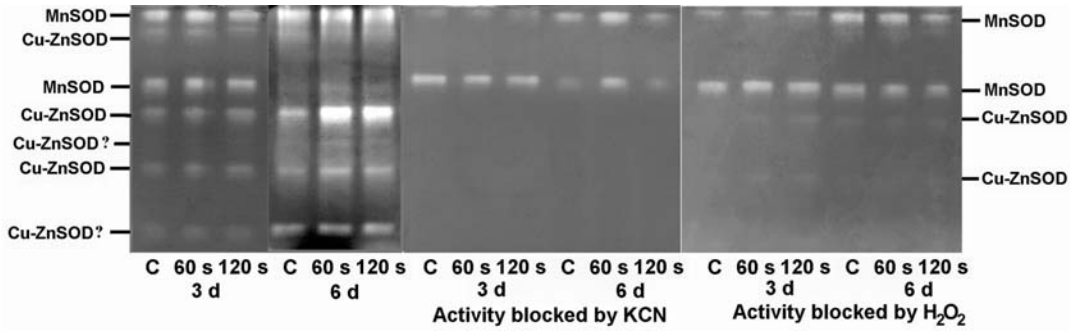

Fig. 3. Native PAGE of SOD isoforms activity in extracts from roots of 3- and 6-days old maize seedlings grown from seeds treated with LTP for 60 and 120 seconds. C – untreated control seeds. Identification of particular enzyme isoforms was made by incubation of gels in buffer containing hydrogen peroxide and KCN.

nevertheless, the root activity began to decrease when the current was higher than 1.5 A, which corresponds with our results.

Soluble protein contents

Application of LTP on maize seeds for 60 seconds significantly increased the root soluble protein content only in extracts from 3-day old seedlings (ca 15%). In roots of 6-day old seedlings both doses of LTP caused slight but non significant decrease in soluble proteins content (Fig. 2A). Hammed et al. (2008) in experiments with gamma irradiation of two chickpea cultivars found that the total protein content depends on dose of irradiation and also genotype specific changes were observed.

Activity of antioxidant enzymes

High metabolic activity of germinating seeds under the influence of LTP and early seedlings growth can be accompanied by a generation of reactive oxygen species (ROS) (Laroussi 2005). To prove this statement we analysed the activity of three ROS scavenging enzymes CAT, G-POX and SOD in roots grown from LTP treated maize seeds. In young 3-day old seedlings both doses of LTP induced a slight increase of SOD activity and similar increase was detected also in roots of 6-day old plants exposed to lower (60 seconds) dose of LTP. SOD catalyzes the dismutation of superoxide to hydrogen peroxide (Sandalio et al. 2001), and stimulation of SOD activity in response to stresses is possibly attributed to the *de novo* synthesis of the enzymatic protein (Slooten et al. 1995). Analyses of SOD by native PAGE (Fig. 3) showed changes both in isoenzyme patterns and in their activity in dependence on age of seedlings. It is known that many particular SOD isoforms play a key role in the removal of superoxide from several cell compartments and provide enhanced tolerance to oxidative damage. In maize roots, there are active isoenzyme MnSOD (in mitochondria) and Cu-ZnSOD in cytoplasm. Increase in exposition time of LTP treatment induced quantitative changes in the activity of different isoenzymes in which the most active was Cu-ZnSOD. This isoenzyme is abundant in cytoplasm. The differences in SOD isoenzymes activity can be related to their subcellular localisation. Impact of elevated oxidative stress caused increase in production of hydrogen peroxide which can inhibit the activity of Cu-ZnSOD which is sensitive to hydrogen peroxide (Sandalio et al. 2001). Therefore, we can assume that SOD will be the main enzyme which has a dominant role in quenching of oxidative stress caused by an application of LTP on maize seeds. On the other hand, the activity of another antioxidant enzyme CAT in 3 and 6-day old roots showed a similar tendency. CAT activity significantly decreased in roots after 60 seconds of LTP treatment reaching 50% reduction in 3-day and 75% reduction in 6-day old seedlings. In roots grown from seeds treated with LTP for 120 seconds CAT activity was also reduced but this reduction was not as substantial (Fig. 2C). Similar results were obtained in maize seeds exposed to specific static magnetic field (Vashisth & Nagarajan 2009b).

G-POX activity (Fig. 2D) increased with the age of the roots. In extracts from the 3-day old roots treated with LTP for 60 seconds a significant decrease in G-POX activity (ca 25%) was observed. In 6 day-old roots, the activity of G-POX was not significantly influenced with LTP treatment. Soaking of maize seeds with different concentration of hydrogen peroxide, which produced higher amount of oxidative stress also caused a decrease of G-POX activity but an increase of ascorbate peroxidase activity in comparison with a control seeds (Gondim et al. 2010). On the other hand, the gamma irradiation treatment of chickpea seeds increased the activity of peroxidase two fold in comparison to control plants (Hameed et al. 2008). Authors concluded that peroxidases act as compensation mechanisms which participate in detoxication of free radicals accumulated after gamma irradiation of seeds. Besides the antioxidant activity peroxidases are also involved in cell expansion influencing cell-wall strengthening (Zelinová et al. 2010). More detailed picture about distribution of G-POX isoenzymes isolated from roots grown from LTP treated maize seeds are presented in Fig. 4. In 3-day old roots, four anodic isoenzymes were detected with the similar activity as in control roots. In extracts from 6-day old roots 7 isoenzymes were detected and their activity according to strength of bands was much slighter but similar as in control roots. More abundant were cathodic isoenzymes (10 bands) activity of which increased with the age of roots. Application of LTP

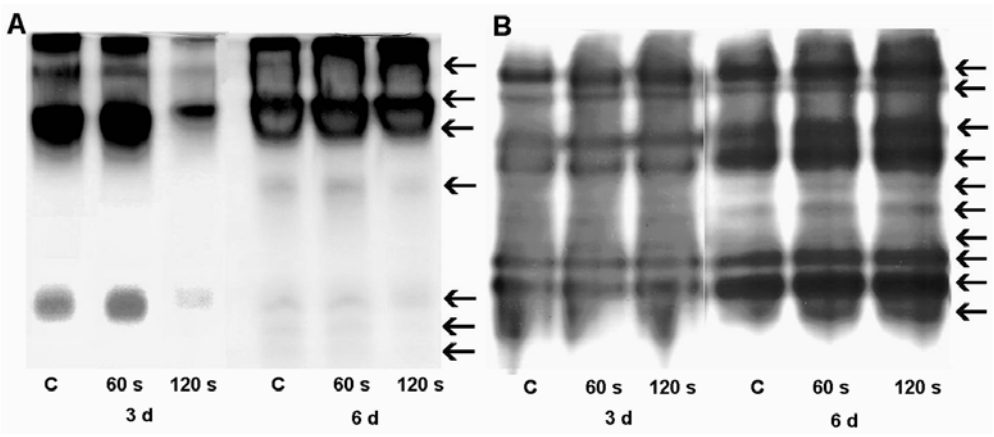

Fig. 4. Native PAGE of isoperoxidases in extracts from roots of 3- and 6-day old maize seedlings grown from seeds treated with LTP for 60 and 120 seconds. A – anodic isoperoxidases; B – cathodic isoperoxidases were detected with guaiacol and hydrogen peroxide as substrates. Arrows indicate position of isoenzyme bands.

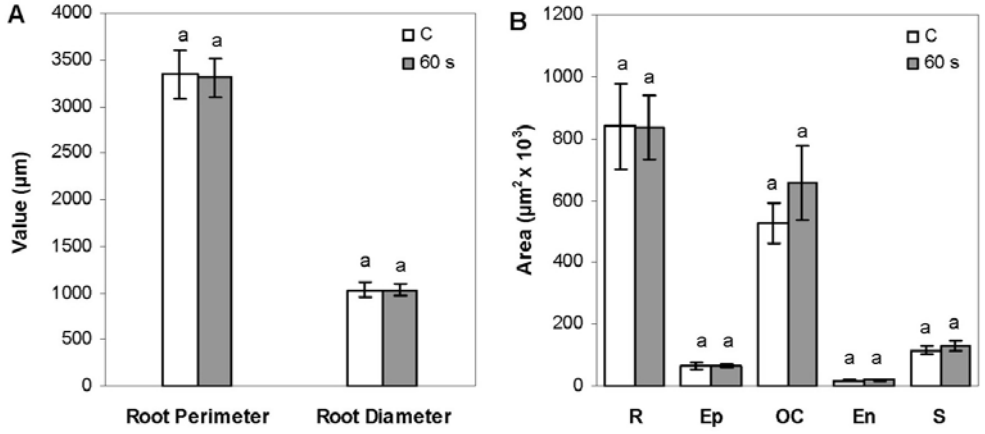

Fig. 5. Morphological parameters of the primary seminal roots of 6-day old maize seedlings grown from seeds treated with LTP for 60 seconds. A – root perimeter and diameter; B – root area, epidermis, outer cortex, endodermis and stele. The samples were collected from the cross sections 50 mm behind the root apex. Values are the means ± SE of three replicates.

treatment in duration of 120 seconds resulted in a decrease of the colour intensity of the individual bands. It was probably caused by growth retardation of maize plants after treatment of seeds with this higher dose of LTP. There were also differences in the amount of proteins which were needed for successful separation of anodic (60 µg) and cathodic (20 µg) peroxidase isoenzymes. It is known that the accumulation of cationic peroxidase isoenzymes starts at the beginning of the germination (Gaspar et al. 1985; Faivre-Rampant et al. 1998). The anodic isoenzymes have been shown to be involved in the lignifications process (Christensen et al. 2001). We suppose that the antioxidant function had possibly cathodic isoenzymes which activity increased with exposition time of LTP application. The same pattern of isoenzyme bands of POD was observed in tomato roots (Yin et al. 2005) after treatment of seeds by magnetized plasma and in *Nicotiana debneyi* and *N. tabacum* after gamma irradiation treatment (Wada et al. 1998).

Anatomy of roots

The potential influence of LTP treatment on maize seeds on the anatomy of the primary roots was investigated only at a 60 second exposition. In a control variant the root perimeter and diameter was 3,343.55 ± 258 µm and 1,031.92 ± 85 µm, respectively (Fig. 5A). The area of the whole root system was 841,391.90 ± 138,503 µm^2, from which the epidermis represented 65,330.19 ± 9,475 µm^2, the outer cortex 526,293.54 ± 66,965 µm^2, the endodermis 18,523.65 ± 3,452 µm^2 and the stele 115,751.57 ± 14,710 µm^2 (Fig. 5B). LTP treatment of maize seed for 60 seconds induced slight but not significant changes in the area of the outer cortex and the stele. According to the results from the investigated morphological parameters of the maize primary seminal roots from the control and LTP 60 seconds treatment we can assume that the treatment of seeds with LTP does not affect the anatomy of the root.

Based on our results we can conclude that the application of LTP on maize seeds affects post-germination growth of primary roots (length, fresh and dry weight production) but does not significantly affect root anatomy and morphology. Strong negative effect of LTP treatment have been observed in DHO activity of roots, however such equivocal effects was not confirmed in the

other antioxidant enzymes (G-POX, CAT, SOD).

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References

Agarwal S. 2007. Increased antioxidant activity in *Cassia* seedlings under UV-B radiation. Biol. Plant. **51:** 157–160.

Ahmet E. 2003. Effects of magnetic fields on yield and growth in strawberry. J. Hort. Sci. Biotec. **78:** 145–147.

Apostolova P., Yordanova R. & Popova L. 2008. Response of antioxidative defence system to low temperature stress in two wheat cultivars. Gen. Appl. Plant Physiol. **34:** 281–294.

Bari M.L., Nazuka E., Sabina Y., Todoriki S. & Isshiki K. 2003. Chemical and irradiation treatments for killing *Escherichia coli* O157:H7 on alfalfa, radish, and mung bean seeds. J. Food Protec. **66:** 767–774.

Basaran P., Basaran-Akgul N. & Oksuz L.2008. Elimination of *Aspergilus parasiticus* from nut surface with low pressure cold plasma (LPCP) treatment. Food Microbiol. **25:** 626–632.

Bradford M.M. 1976. A rapid a sensitive method for the quantification of micrograms quantities of protein utilising the principle of protein dye bindings. Analyt. Biochem. **72:** 248–254.

Cakmak I. & Horst W.J. 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). J. Plant Physiol. **83:** 463–468.

Carbonell M., Martinez E. & Raya A. 2000. Effects of 125 mT stationary magnetic field in the initial stages of growth of wheat. Agric. Eng. Res. Papers **32:** 5–10.

Černák M., Černáková Ľ., Hudec I., Kováčik D. & Zahoranová A. 2009. Coplanar surface barrier discharge and its applications for in-line processing of low-added-value materials. Eur. Phys. J. Appl. Phys. **47:** 22806 (p1–p6).

Claiborne A. 1985. Catalase activity, pp.283–284. In: Greenvald R.A. (ed.), Handbook of methods for oxygen radical research, Boca Raton CRC Press.

Chaomei Z. & Yanlin M. 1993. Irradiation induced changes in enzymes of wheat during seed germination and seedling growth. Acta Agric. Nucleatae Sinica **7:** 93–97.

Cheen Y., Yuea M. & Wanga X. 2005. Influence of He-Ne laser irradiation on seeds thermodynamic parameters and seedlings growth of *Isatis indogotica*. Plant Sci. **168:** 601–606.

Christensen J.H., Overney S., Rohde A., Diaz W.A., Bauw G., Simon P., van Montagu M. & Boerjan W. 2001. The syringaldazine-oxidizing peroxidase PXP 3–4 from poplar xylem: cDNA isolation, characterization and expression. Plant Mol. Biol. **47:** 581–593.

Davies M.S. 1996. Effects of 60 Hz electromagnetic fields on early growth in three plant species and replication of previous results. Bioelectromagnetics **2:** 154–161.

Donahue J.L., Okpodu C.M., Cramer C.L., Grabau E.A. & Alscher R.G.1997. Responses of antioxidants to paraquat in pea leaves. Plant Physiol. **113:** 249–257.

Dubinov A.E., Lazarenko E.M. & Selemir V.D. 2000. Effect of glow discharge air plasma on grain crops seed. IEEE Transactions Plasma Sci. **28:** 180–183.

Faivre-Rampant O., Kevers C., Bellini C. & Gaspar T. 1998. Peroxidase activity, ethylene production, lignification and growth limitation in shoots of a nonrooting mutant of tobacco. Plant Physiol. Biochem. **36:** 873–877.

Foyer Ch., Lelandais M. & Kunert K.J. 1994. Photooxidative stress in plants. Physiol. Plant. **92:** 696–717.

Frič F. & Fuchs W.H. 1970. Veränderungen der Aktivität einiger Enzyme im Weizenblatt in Abhängigkeit von der temperaturlabilen Verträglichkeit für *Puccinia graminis tritici*. Phytopathol. Z. **67:** 161–174.

Galová Z. 1996. The effect of laser beams on the process of germinating power of winter wheat grains. Roczniki AR, Poznań, CCCLXXXVI, ser.Rol. **49:** 39–43.

Gan-Mor G. 2003. Electrostatic pollen application development and tests for almond, kiwi, date and pistachio – an overview. Appl. Eng.Agric. **19:** 119–124.

Gaspar T., Penel C., Castillo F. & Greppin H. 1985. A two-step control of basic and acidic peroxidases and its significance for growth and development. Physiol. Plant. **64:** 418–423.

Gondim F.A., Gomes-Filho E., Lacerda C.F., Prisco J.T., Azavedo Neto A.D. & Marques E.C. 2010. Pretreatment with H_2O_2 in maize seeds: effects on germination and seedlings acclimation to salt stress. Braz. J. Plant Physiol. **22:** 103–112.

Hammed A., Shah T.M., Atta B.M., Haq M.A. & Sayed H. 2008. Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in Desi and Kabuli chickpea. Pak. J. Bot. **40:** 1033–1041.

Henselová M., Zahoranová A. & Černák M. 2011. Effect of low-temperature plasma on the germination and ontogenetic development of corn (*Zea mays* L.), pp. 166–169. In: Blaha J.(ed), Proceeding from Conference " Vliv abiotických a biotických stresorů na vlastnosti rostlin 2011", Praha 9.-10.3. 2011. (In Slovak)

Hodges D.M., Andrews C.J., Johnson D.A. & Hamilton R.I. 1997. Antioxidant enzyme responses to chilling stress in differentially sensitive inbred maize lines. J. Exp. Bot. **48:** 1105–1113.

Jensen C.O., Sacks W. & Baldauski F.A. 1951. The reduction of triphenyltetrazolium chloride by dehydrogenases of corn embryos. Science **113:** 65–66.

Jung H., Kim D.B., Gweon B., Moon S.Y. & Choe W. 2010. Enhanced inactivation of bacterial spores by atmospheric pressure plasma with catalyst TiO_2. Appl Catalysis B: Environ. **93:** 212– 216.

Ksenz N.V. & Kaciesvili S.V. 2000. Electrostatic field and productivity of cereals (in Russian). Mechanization and Electrification in Agriculture **6:**18–10.

Laemmli U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage *T4*. Nature **227:** 680–685.

Laroussi M. 2005. Low temperature plasma-based sterilization: Overview and state-of-the-art. Plasma Process. Polym. **2:**391–400.

Lebedev I.S. & Litvinenko L.T. 1977. After effect of a permanent magnetic field on photochemical activity of chloroplast. Soviet Plant Physiol.**24:** 394–395.

Lynikiene S., Pozeliene A. & Rutkauskas G. 2006. Influence of corona discharge field in seed viability and dynamics of germination. Inter. Agrophysics **20:** 195–200.

Madamanchi N.R., Donahue J.L., Cramer C.L., Alscher R.G. & Pedersen K. 1994. Differential response of Cu, Zn superoxide dismutases in two pea cultivars during a short term exposure to sulphur dioxide. Plant Mol. Biol. **26:** 95–103.

Marinković D. & Borcean I. 2009. Effect of cold electron plasma and extremely low frequency electron-magnetic field on wheat yield. Res. J. Agric. Sci. **41:** 96–101.

Martines, E., Carbonell M.V. & Florez M. 2002. Magnetic biostimulation of initial growth stages of wheat. Electromagn. Biol. Med. **21:** 43–53.

Palov I. 2003. Research influence of electromagnetic impact on maize seed and plants (in Bulgarian). Machinery in Agriculture, XL. **1:** 10–15.

Pietruszweski S. 1993. Effects of magnetic seed treatment on yields of wheat. Seed Sci. Technol. **21:** 621–626.

Pietruszewski S. 1996. Effects of magnetic biostimulation of wheat seeds on germination, yield and proteins. Inter. Agrophysics **10:** 51–55.

Podleceny J. 2002. Effect of laser irradiation on the biochemical changes in seeds and the accumulation of dry matter in the faba bean. Inter. Agrophysics **16:** 209–213.

Reisfeld R.A., Lewis V.J. & Williams D.E. 1962. Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. Nature **195:** 281–283.

Sandalio L.M., Dalurzo H.C., Gómez M., Romero-Puertas M.C. & del Río L.A. 2001. Cadmium-induced changes in growth

and oxidative metabolism of pea plants. J. Exp. Bot. **52:** 2115–2126.

Shabrangi A. & Majd A. 2009. Effects of magnetic fields on growth and antioxidant system in plants. Progr. Electromagnet. Res. **29:** 1089–1094.

Scebba F., Arduini I., Ercoli L. & Sebastiani L. 2006. Cadmium effects on growth and antioxidant enzymes activities in *Miscanthus sinensis*. Biol. Plant. **50**: 688–692.

Slooten L., Capiau K., van Camp W., van Montagu M., Sybesma C. & Inze D. 1995. Factors affecting the enhancement of oxidative stress tolerance in transgenic tobacco over expressing manganese superoxide dismutase in the chloroplasts. Plant Physiol. **107:** 373–380.

Smith F.G. 1952. The mechanism of the tetrazolium reaction in corn embryos. Plant Physiol. **27:** 445–456.

Suzuki N.R. & Mittler R. 2006. Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. Physiol. Plant. **126:** 45–51.

Šerá B., Straňák V., Šerý M., Tichý M. & Špatenka P. 2008. Germination of *Chenopodium album* in response to microwave plasma treatment. Plasma Sci. Technol. **10:** 506–511.

Šimor M., Ráheľ J., Vojtek P., Brablec A. & Černák M. 2002. Atmospheric-pressure diffuse coplanar surface discharge for surface treatments. Appl. Phys. Lett. **81:** 2716–2718.

Tamás L., Huttová J. & Mistrík I. 2003. Inhibition of Al-induced root elongation and enhancement of Al-induced peroxidase activity in Al-sensitive and Al-resistant barley cultivars are positively correlated. Plant Soil **250:** 193–200.

Vashisth A. & Nagarajan S. 2009a. Germination characteristic of seeds of maize (*Zea mays* L.) exposed to magnetic field under accelerating ageing condition. J. Agric. Physics **9:** 50–58.

Vashisth A. & Nagarajan S. 2009b. Characterization of water distribution and activities of enzyme during germination in magnetically-exposed maize (*Zea mays* L.) seeds. Ind. J. Biochem. Biophys. **47:** 311–318.

Volin J.C., Denes F.S., Young R.A. & Park S.M.T. 2000. Modification of seed germination performance through cold plasma chemistry technology. Crop Sci. **40:** 1706–1718.

Wada H., Koshiba T., Matsui T. & Sato M. 1998. Involvement of peroxidase in differential sensitivity to irradiation in seedlings of two *Nicotiana* species. Plant Sci. **132:** 109–119.

Wang G., Huang J., Gao W., Lu J., Li J., Liao R. & Jaleel Ch.A. 2009. The effect of high-voltage electrostatic field (HVEF) on aged rice (*Oryza sativa* L.) seeds vigor and lipid peroxidation of seedlings. J. Electrostatics **67:** 759–764.

Xiong Q., Xing Z., Feng Z., Tan Q. & Bian Y. 2009. Effect of ^{60}Co γ-irradiation on postharvest quality and selected enzyme activities of *Pleurotus nebrodensis*. Food Sci. Technol. **42:** 157–161.

Yin M.Q., Huang M.G., Ma B.Z. & Ma T.C. 2005. Stimulating effects of seed treatment by magnetized plasma on tomato growth and yield. Plasma Sci. Technol. **7:** 3143–3147.

Zelinová V., Mistrík I., Paľove-Balang P. & Tamás L. 2010. Peroxidase activity against guaiacol, NADH, chlorogenic acid, ferulic acid and coniferyl alcohol in root tips of *Lotus japonicus* and *L. corniculatus* grown under low pH and aluminium stress. Biologia **65:** 279–283.

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