BIOLOGIA PLANTARUM 54 (3): 597-600, 2010

BRIEF COMMUNICATION

The effect of 2,4-dichlorophenol and pentachlorophenol on antioxidant system in the leaves of *Phalaris arudinacea*

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Abstract

The purpose of this work was to evaluate the effect of 2,4-dichlorophenol (2,4-DCP) and pentachlorophenol (PCP) on the activity of antioxidative system and lipid peroxidation in the leaves of reed canary grass (*Phalaris arudinacea*). The activity of catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR) and glutathione S-transferase (GST) as well as the content of glutathione, ascorbate and phenolic compounds were determined. An induced-increase in the APX, CAT, GPX and GR activities was stronger for PCP, while a significant increase in the GST activity was noted only for 2,4-DCP. Both compounds increased the content of phenolic compounds, oxidized and reduced glutathione as well as the content of ascorbic acid. PCP induced stronger increase in lipid peroxidation than 2,4-DCP. The observed changes revealed that chlorophenols induce oxidative stress and oxidative damage in the leaves of reed canary grass.

Additional key words: reed canary grass, chlorinated phenols, enzymatic antioxidants, lipid peroxidation, non-enzymatic antioxidants.

Chlorinated phenols are introduced into the environment because they are used as the precursors or components of synthetic polymers, chemical reagents, drugs or cosmetics (Michałowicz and Duda 2007). 2,4-Dichlorophenol (2,4-DCP) is used as a herbicide (Cernakowa and Zemanovicova 1998). It is also one of the main transformation products of 2,4-dichlorophenoxyacetic acid – one of the most commonly used pesticides in the world (Różański 1998). Pentachlorophenol (PCP) is used as a pesticide (Laine and Jorgensen 1996) in wood, textile and skin impregnation. PCP is also formed during degradation of other pesticides such as hexachlorocyclohexane, hexachlorobenzene, pentachlorobenzene and pentachloronitrobenzene (Michałowicz and Duda 2007).

The effect of xenobiotics on the cell leads to a formation of reactive oxygen species including superoxide radical, hydrogen peroxide and hydroxyl radical, which may provoke damage in cells including lipid peroxidation (Baťková *et al*. 2008). The plants have developed a defensive mechanism that employs both enzymatic antioxidants like catalase, CAT (Polidoros and Scandalios 1999), ascorbate peroxidase, APX (Rhizsky *et al*. 2002) or guaiacol peroxidase, POX (Herman *et al*. 1998) and non-enzymatic antioxidants, like ascorbate, ASA (Asada 1999), glutathione, GSH (Noctor *et al*. 1998) and phenolic compounds (Pasqualini *et al*. 2003). Detoxification of xenobiotics is also executed by their conjugation with glutathione with the participation of glutathione S-transferase, GST (Zhao and Zhang 2006). In the previous work we reported that reed canary grass, long-lived perennial plant commonly used in phytoremediation, absorbed 4-chlorophenol from medium and simultaneously increased GST activity and glutathione content (Urbanek *et al*. 2005). In the present work we have focused on the evaluation of the oxidative effect of chlorophenols like 2,4-DCP and potentially more toxic PCP on the activity of catalase, guaiacol peroxidase, ascorbate peroxidase, glutathione reductase and glutathione S-transferase and the contents of glutathione, ascorbate and free phenols.

Specimens of reed canary grass (*Phalaris arudinacea*) were taken from the botanic garden (Institute of Biology and Environmental Protection, University of Łódź). Plants were cultivated in a naturally lit greenhouse at

Received 12 April 2009, *accepted* 17 October 2009.

Abbreviations: APX - ascorbate peroxidase; ASA - ascorbate; CAT - catalase; 2,4-DCP - 2,4-dichlorophenol; GPX - guaiacol peroxidase; GR - glutathione reductase; GSH - glutathione; GST - glutathione S-transferase; PCP - pentachlorophenol.

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25 °C and 16-h photoperiod as described previously (Urbanek *et al*. 2005). The young culms with 5 leaves were cut off and rooted in water. The rooted culms were put into pots containing soil and after 3 weeks the soil was treated with 2,4-DCP and PCP in a concentration of 0.5 mg kg^{-1} (the compounds were dissolved in 1 cm^3 of ethanol and then in deionized water). The suitable concentration was set in preliminary experiments. Plants growing in soil without chlorophenols were used as controls. The leaves were harvested after 3, 6 and 12-d treatment. Chlorophenols were extracted from the leaves with methanol, adsorbed on octadecyl (C18) solid phase and then determined by gas chromatograph type *5890* connected with a quadruple mass spectrometer type *5972* (*Hewlett-Packard*, Palo Alto, CA, USA). The enzyme extractions were carried out at 4 °C. The leaves (0.5 g) were homogenized with 0.1 M Tris-HCl (pH 7.8) or 0.1 M of potassium phosphate buffer (pH 7.0) containing 1 mM of EDTA, 0.2 mM of insoluble polyvinylpyrrolidone (PVPP) and 1 mM of dithiothreitiol (DTT). The catalase (CAT, EC 1.11.1.6) activity was assayed according to the procedure of Aebi (1984). The measurement of the activity of ascorbate peroxidase (APX, EC 1.11.1.11) was performed according to Nakano and Asada (1981). The guaiacol peroxidase (POX, EC 1.11.1.7) activity was determined according to the method of Chance and Maehly (1956) with modifications. Glutathione S-transferase (GST, EC 2.5.1.13) activity was determined by the method of Navari-Izzo and Izzo (1994) and glutathione reductase (GR, EC 1.6.4.2) activity was as assayed using the method of Beutler (1975). Phenolic compounds were analyzed by the use of Folin reagent, glutathione content was measured by the method of Griffith (1980), and ascorbate content was determined with α,α'-bipirydyl according to Okamura (1980). The content of thiobarbi-turic acid reactive substances (TBARS) as marker of lipid peroxidation was assayed according to Dhindsa *et al*. (1981). The protein concentration was determined according to Bradford (1976). The mean value was calculated from 5 to 6 individuals, whereas for each individual an experimental point was a mean value of three replications. The significance of differences between control and each treatment was analysed using Student's *t*-test.

Chromatographic analysis proved that plants after 12 d accumulated in the leaves low concentrations of PCP (0.143 ± 0.014 μg g^{-1}) and slightly higher concentrations of 2,4-DCP (0.183 ± 0.014 μg g^{-1}). In control samples none chlorophenols were detected. Catalase (CAT) and ascorbate peroxidase (APX) are capable of destroying hydrogen peroxide in the cells (Rhizsky *et al*. 2002; Ammar *et al*. 2008). Statistically significant changes in the activity of these enzymes in the leaves of plants exposed both to 2,4-DCP and PCP were observed after 3, 6 and 12 d of the experiment. PCP strongly enhanced the activity of these enzymes, which was particularly observed on the 6th d of the exposure of plants to this xenobiotic. The increased activities of catalase and ascorbate peroxidase observed at the same time showed that these enzymes functioned concurrently to remove H_2O_2 from cells. The increase in the activity of guaiacol peroxidase (POX) has been observed in plants exposed to chlorinated xenobiotics (Roy *et al*. 1992). In our study GPX activity was increased in plants treated with 2,4-DCP and PCP during the whole time of the study, and particularly on the 12th day of the treatment. Phenolic compounds participate in elimination of numerous oxidants, thus their content is usually increased during oxidative stress (Pasqualini *et al*. 2003). We observed that both 2,4-DCP and PCP increased free phenols content in the leaves of reed canary grass. Moreover, we observed that PCP increased the content of free phenols in the leaves of the plants between the 3rd and the 6th day more strongly than 2,4-DCP. An increase in GSH content was shown in plants under various stresses (Tausz *et al*. 2004). The counteraction of uncontrolled destructive oxidation of cell components caused by ROS may be due to the conversion of reduced GSH to oxidized glutathione (GSSG), the essential reaction in ascorbate-glutathione cycle. The increase of GSSG content induced by 2,4-DCP and PCP was observed during the whole time of our experiment. A higher increase in the content of reduced glutathione in comparison to its oxidized form, allowed keeping advantageous redox state of GSH in the leaves of the plants studied. Glutathione reductase (GR) plays an important role in the regeneration of reduced GSH (Tsai *et al*. 2004). Surely, the maintenance of the favourable redox state of glutathione was mainly due to the high GR activity that was observed in our study. It was also noted that GR activity increased with the time of the exposure of plants to both chlorinated phenols, but more in the case of PCP. The involvement of GST in the conjugation of herbicides and hexachlorobenzene has been observed in aquatic plants (Roy *et al*. 1992, Mars 1996). In present experiments, an essential increase in the activity of GST was observed only for 2,4-DCP after 3 and 6 d of the experiment, which may suggest that conjugation (detoxification) of 2,4-DCP and/or its metabolites was executed by this enzyme. The changes in ascorbic acid/dehydroascorbate (ASA/DHA) contents are important indicators of the redox status of the cell and are one of the first signs of oxidative stress (Lechno *et al*. 1997). In our study, a statistically significant increase in ASA content was noted both for 2,4-DCP and PCP-treated plants in the 3rd and the 6th day of the study. We also observed that in plants exposed to 2,4-DCP, the rise of the ASA concentrations was correlated with a depletion in DHA content. Lipid peroxidation is considered to be a marker of oxidative damage in cells exposed to toxic compounds (Saladin *et al.* 2003). An increase in the lipid peroxidation (TBARS content) was statistically significant in the whole period of the experiment with a stronger effect observed for PCP. Moreover, we observed that the extent of lipid damage was consequently reduced in the leaves of reed canary grass after 6 d and in particular after 12 d of the exposure of the plants to both chlorophenols (Table 1). The induction of lipid peroxidation was also

Table 1. Changes in enzymatic and non-enzymatic antioxidants and lipid peroxidation (TBARS content) in the leaves of reed canary grass (*Phalaris arudinacea*) exposed to 0.5 mg kg^{-1}(soil) of 2,4-DCP and PCP for 3, 6 and 12 d. Means of five to six separate experiments ± SE; **, * - the values statistically different from control at $P < 0.01$ and $P < 0.05$, respectively, according to *t*-test.

Parameters	3 d			6 d			12 d		
	control	2,4-DCP	PCP	control	2,4-DCP	PCP	control	2,4-DCP	PCP
Catalase	1266	1637	1755	1233	1504	1937	1264	1583	1695
[U mg^{-1}(protein)]	±106	±156**	±173**	±102	±104**	±231**	±178	±174*	±197*
Ascorbate peroxidase	12.0	16.2	18.3	12.0	17.1	21.8	12.1	15.7	18.5
[U mg^{-1}(protein)]	±1.3	±1.8*	±2.5*	±1.5	±2.5*	±1.4**	±1.2	±2.0*	±2.2**
Guaiacol peroxidase	520	696	681	503	715	719	538	704	907
[U mg^{-1}(protein)]	±50	±62**	±37**	±27	±62**	±72**	±40	±45**	±92**
Free phenols	22.2	28.1	30.3	21.9	25.2	29.7	22.4	25.6	25.5
[mg g^{-1}(f.m.)]	±0.9	±1.5**	±2.1**	±0.8	±0.8**	±2.8**	±0.7	±0.9*	±1.2*
Bound phenols	6.1	6.8	6.9	6.3	7.3	7.5	6.5	8.6	8.5
[mg g^{-1}(f.m.)]	±0.8	±0.8	±0.6*	±0.8	±0.6	±0.3*	±0.7	±1.0**	±0.7**
Reduced glutathione	147.8	177.7	231.7	202.4	270.2	259.8	208.3	249.5	245.3
[µmol g^{-1}(f.m.)]	±11.8	±7.5*	±7.5**	±7.3	±11.7**	±11.7*	±4.8	±9.5*	±9.5*
Oxidized glutathione	30.7	58.1	65.1	48.1	56.9	53.0	45.2	53.9	49.4
[µmol g^{-1}(f.m.)]	±2.7	±3.7**	±3.7**	±1.8	±3.2**	±3.2*	±3.8	±5.2**	±5.2*
Glutathione reductase	24.1	35.4	39.8	26.3	42.0	54.7	26.4	47.0	60.8
[U mg^{-1}(protein)]	±1.4	±6.1*	±7.1*	±6.0	±5.5**	±1.5**	±1.7	±7.0**	±4.1**
Glutathione S-transferase	61.0	81.7	73.7	56.1	84.0	56.1	56.6	63.5	54.5
[U mg^{-1}(protein)]	±4.7	±5.8**	±6.4*	±11.2	±9.4*	±11.2	±9.3	±5.2	±8.8
Ascorbic acid	28.6	30.2	32.4	29.4	35.2	32.7	30.1	33.1	32.2
[µmol g^{-1}(f.m.)]	±2.4	±1.4*	±1.2*	±1.7	±1.4**	±1.2*	±1.5	±2.0	±3.2
Dehydroascorbate	11.7	10.5	13.1	10.9	6.7	10.9	11.7	9.4	11.6
[µmol g^{-1}(f.m.)]	±2.0	±2.4	±1.0	±2.0	±1.6*	±1.0	±0.8	±1.5*	±3.0
TBARS (MDA)	3.6	7.0	9.1	3.6	7.2	8.0	3.6	6.4	6.7
[µmol g^{-1}(f.m.)]	±0.30	±0.62	±0.88	±0.34	±0.29	±0.87	±0.32	±0.37	±0.95

observed in the leaves of wheat exposed to chlorophenols (Michałowicz *et al*. 2009), however, this process was not decreased with time of the exposure of plants to these compounds. This finding may suggest that wild plants are more resistant to lipids damage provoked by chlorinated compounds including chlorophenols in comparison to cultivated plants.

To sum up, the increase in antioxidant enzymes activities and also the rise in ascorbate, glutathione and free phenols contents suggest that oxidative burst was provoked by 2,4-DCP and PCP in the leaves of reed canary grass. The obtained results also revealed that the toxicity of chlorophenols towards reed canary grass rises with the increase in the number of chlorine atoms as stronger changes in the examined parameters were observed for PCP. The considerable increase in lipid peroxidation showed that chlorophenols are able to provoke oxidative damage in the cells of the leaves of the investigated plant species, however, the extent of these changes decreased with time, which showed that plants were able to reduce noxious effects exerted by 2,4-DCP and PCP.

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