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Effect of powdery mildew infection on the antioxidant enzyme activities in different lines of Thatcher-based wheat

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ABSTRACT Changes of antioxidant enzyme activities (glutathione reductase, glutathione-S-transferase, catalase, ascorbate peroxidase and guaiacol peroxidase) were studied in four near-isogenic Thatcher-based wheat lines following powdery mildew infection. The activities of ascorbate peroxidase and guaiacol peroxidase were increased significantly after 7 days of infection in all lines. Significant increases were observed in glutathione-S-transferase activity in the lines carrying Lr33, Lr26 and Lr19 genes, furthermore in catalase and glutathione reductase activity in the case of lines with Lr26 and Lr19 genes on the 7th day of infection. Changes in enzyme activities, caused by aging, were also detected in all lines. Four peroxidase isozymes, induced by infection, were found by gelelectrophoresis. **Acta Biol Szeged 55(1):99-100 (2011)**

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Biotic stressors cause several changes leading to altered functions of some biochemical pathways in plants. One of the most important molecular differences in plants is the changing of oxidation-reduction state, known as oxidative stress. During this a plasma membrane-localized NADPH oxidase is induced that results to formation directly of superoxide anion ($O_2^{\cdot-}$) and indirectly of hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS), which can be transformed to each other (Vranová et al. 2002). When balance between the formation of ROS and their detoxification is disrupted, plant cells are damaged in varying degrees (Asthir et al. 2009). To avoid destroying non-enzymatic and enzymatic antioxidative systems decrease the levels of ROS. Enzymatic system contains for example catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (POD, EC 1.11.1.7) and the key enzymes of ascorbate-glutathione cycle, such as ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.5.4), which can remove excess H_2O_2 from cells (Kocsy et al. 2011). These antioxidant enzymes may have role in different signal pathways and other defensive mechanisms, too.

Several papers describe the effects of biotic stress in wheat. Leaf rust infection caused CAT and glutathione-S-transferase (GST) activation in resistant wheat genotypes, by contrast in susceptible genotypes these enzymes were inhibited at the same time (Ivanov et al. 2005). Stripe rust infection leads to stimulation of peroxidase, CAT and other antioxidant enzymes along with increase in polyamine content, while the contents of non-enzymatic antioxidants, namely ascorbate and

chlorophyll decreased in susceptible cultivar and no visible symptoms appeared in resistant cultivar (Asthir et al. 2010).

The aims of this study are to reveal changes in the activities of antioxidant enzymes during powdery mildew infection in four wheat lines, furthermore to investigate correlation between these changes and the level of resistance to powdery mildew in order to the better understanding of the role of antioxidants in biotic and oxidative stress.

Materials and Methods

Four near-isogenic lines of Thatcher-based wheat (carrying Lr33, Lr26, Lr19 and Lr9 leaf rust resistance genes, respectively) were grown in greenhouse, 2 plants/pot in a 2:1 mixture of soil and sand. Inoculation was carried out using a mixture of powdery mildew (*Blumeria graminis* f. sp. *tritici*) pathotypes with a known virulence spectrum (determined on a differentials carrying genes Pm0, 1, 2, 3a, 3b, 3c, 3d, 3f, 4a, 4b, 5, 6, 7, 8, 17, 2+6, 2+4b+8, 1+2+9, 2+Mld). Plants were inoculated at adult stage (GS45) by shaking conidia onto the leaf surface of the test plants. Throughout the experiment the temperature in the greenhouse was 16–22°C and the relative humidity of the air under the isolation boxes was above 90%. Half of the plants were the controls, the others were the infected. Phenotypic comparison and leaves sampling were carried out on the 3rd and 7th days of infection. Enzyme extraction and the spectrophotometric determination of the activity of the various antioxidant enzymes (GR; GST; CAT; APX and POD) took place as reported by Janda et al. (1999) and Ádám et al. (1995). The isozymes of POD were separated on 10% non-denaturing polyacrylamide gels by the modified method described Janda et al. (1999).

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Results

The phenotypic comparison of the different lines showed that lines carrying Lr33 and Lr26 were susceptible, while lines carrying Lr19 and Lr9 were resistant against powdery mildew infection. Slight increases were observed in GR activities in all investigated wheat lines due to the aging, but this increase was only significant in the case of line with Lr19. Powdery mildew infection could only cause significant increase of GR activity in the line carrying Lr26 and Lr19 after 7 days. The activity of GST decreased only in the line with Lr33 with aging. After infection GST activity already increased in the line with Lr33 after 3 days, while in lines with Lr26 and Lr19 only increased after 7 days. Aging alone increased CAT activity in the line carrying Lr26. After infection CAT activity slightly increased in all lines on the 7th day but this increase was only significant in the lines with Lr26 and Lr19. APX activity was decreased in the line carrying Lr9 by aging. Powdery mildew infection caused increased APX activity after 3 days only in line with Lr26, while after 7 days the activities were significantly increased in all lines. Aging could hardly affect POD activity in the line with Lr33 and Lr9, but results showed significant increases in other lines. It was 2-fold higher in the line with Lr19 and 3-fold higher in the line with Lr26. After infection the POD activity was drastically increased in 3d samples, its rate was particularly high in the lines carrying Lr26 and Lr19. In 7d samples changes after infection were significant too, but their magnitudes were lower than in 3d samples.

Four peroxidase isozymes (POD1, POD2, POD3 and POD4) were found by gelelectrophoresis. POD1 with the lowest electrophoretic mobility was observed in all lines. POD2 with higher electrophoretic mobility was appeared in the control line carrying Lr26 and with higher intensity in all infected samples. Bands of POD3 and POD4 were not so strong than the bands of POD1 and POD2. POD3, with higher electrophoretic mobility than POD2, showed higher activity in controls, especially in line carrying Lr19, and lower activity in infected samples. POD4 with the highest electrophoretic mobility, was presented in both the control and the infected lines carrying Lr33, Lr19 and Lr9, but was missed in line carrying Lr26.

Discussion

We found that the four investigated Thatcher-based wheat lines showed phenotypic differences after infection with powdery mildew. These differences have not been manifested in the activities of antioxidant enzymes as similar results were obtained in both the phenotypically resistant and phenotypically susceptible genotypes. Powdery mildew induced the enzymatic antioxidant defence mechanism in all investigated wheat lines.

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