Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2003
Volume 6
Issue 2
Series BIOLOGY

CHRZANOWSKI G., CIEPIELA A. P., SPRAWKA I., SEMPRUCH C., SYTYKIEWICZ H., CZERNIEWICZ P. 2003. ACTIVITY OF POLYPHENOLOXIDASE IN THE EARS OF SPRING WHEAT AND TRITICALE INFESTED BY GRAIN APHID (*Sitobion avenae* /F./) **Electronic Journal of Polish Agricultural Universities**, Biology, Volume 6, Issue 2.
Available Online http://www.ejpau.media.pl

ACTIVITY OF POLYPHENOLOXIDASE IN THE EARS OF SPRING WHEAT AND TRITICALE INFESTED BY GRAIN APHID (*Sitobion avenae* /F./)

Grzegorz Chrzanowski, Antoni P. Ciepiela, Iwona Sprawka, Cezary Sempruch, Hubert Sytykiewicz, Paweł Czerniewicz
Department of Molecular Biology and Biophysics, University of Podlasie, Siedlce, Poland

ABSTRACT

Polyphenoloxidase (PPO) plays an important role in plant resistance to insects, and in the detoxication of phenolic compounds taken in the nutrient components. Activity of PPO was examined in the ears of spring wheat and triticale. Extraction of enzymatic protein was carried out from acetone powder with the use of 0.05M phosphoric buffer of pH 7.4. The cultivars of spring wheat have possessed higher activity of PPO than cultivars of triticale. Feeding of the grain aphid has reduced this enzyme activity in the all analysed species and cultivars.

Key words: polyphenoloxidase, spring wheat, spring triticale, *Sitobion avenae*.

INTRODUCTION

Polyphenoloxidase [EC 1.10.3.1] is a widespread enzyme found in plant cells, located in the chloroplast thylakoid membranes [3]. This enzyme is capable of dehydrogenating of o-diphenols to produce o-quinones. However, it indicates the highest activity toward hydroxylation of monophenols to diphenols [13]. In case of oxygen shortage in the PPO reaction environment it may act like peroxidase by utilizing hydrogen peroxide or ethyl as an acceptor of electrons [1].

Oxidation of phenolic compounds in plant cells is responsible for initiating the browning reaction of the tissues and is identified as presence of the pathogenetic factor [2] or of pest feeding [10,16]. Hildebrand *et al.* [9] and Felton *et al.* [6] assert that within the range of tissues damaged by feeding insects there occurs increased concentration of phenolic compounds. Moreover, PPO induces metabolization of these phenolic compounds into more toxic forms. Therefore the main aim of study was to determine polyphenoloxidase activity in the ears of spring wheat and spring triticale infested by grain aphid (*Sitobion avenae* /F./).

MATERIALS AND METHODS

The material for chemical analyses were non-infested and infested (5 wingless females per straw) ears of two spring wheat cultivars: Eta and Banti; and two spring triticale cultivars: Wanad and Migo. The cultivars selected for chemical analyses had different resistance to grain aphid. Eta and Wanad cultivars were partially resistant, whereas Banti and Migo were susceptible to this pest. The ears of spring wheat and triticale were collected when cereals were at medium milk developmental stage /G.S. 75 in Tottman and Broad scale [17]/. The ears were placed in solid carbon dioxide (dry ice) immediately after harvest and transferred to the laboratory, where it was subjected to freeze-drying. In order to do so, fresh plant tissue (150g) was homogenized with 100cm^3 of acetone (temp. -20°C). The organic solvent was removed by filtration under vacuum. Obtained precipitate was then rinsed with acetone (temp. -20°C), dried and stored in a desiccator until conducting chemical analyses.

Extraction of enzymatic protein

300 mg of acetone powder was ground with 20cm^3 of 0.05 phosphoric buffer, pH 7.4 for 10 minutes. The extract was centrifuged at 6500 g for 3 min. at 0°C. Obtained precipitate was removed, and collected supernatant was dialysed against double-distilled water at 4°C in 24 hours. The extract was centrifuged at 6500 g for 30 min after dialysis. The supernatant was used to determine PPO activity.

Polyphenoloxidase activity assay

Polyphenoloxidase activity was determined using the colorimetrical method (Laurem *et al.* 1985). The reactive mixture contained: 1.0cm^3 of 0.4% catechol in 0.05M phosphoric buffer, pH 7.4 and 2.0cm^3 of enzymatic extract. The mixture was incubated at 30°C for 60 min.; then 0.5 cm^3 of 10% H_2SO_4 was added. The value of absorbance of the analysed samples was measured with the spectrophotometer HP 8453 at wavelength 460 nm compared to the blank sample that had the same content as the analysed sample (but 10% H_2SO_4 was added before the enzymatic extract). Enzyme activity was expressed as $\Delta A \cdot h^{-1} \cdot$mg protein $^{-1}$.

Protein content was determined in the same extract with use of Folin-Ciocalteu's phenol reagent [12].

All chemical analyses were repeated three times. Significant differences between their means were calculated using Duncan multiple range test at $P \leq 0.05$.

RESULTS

Results concerning activity of polyphenoloxidase in ears of examined varieties and cultivars not infested by *S. avenae* are presented in figure 1. Statistically significant differences between the cultivars in PPO activity have been determined. PPO activity in Eta wheat, which is partially resistant to this species of pest, was higher than in the susceptible Banti cultivar. However, in the case of the triticale inverse interdependence was ascertained. The Migo cultivar, which was classified as susceptible had higher activity of the enzyme in comparison with the relatively resistant Wanad cultivar. Furthermore, feeding of the grain aphid wingless female decreased polyphenoloxidase activity in the all examined cultivars (fig. 2).

Fig. 1. Activity of polyphenoloxidase in the ears of spring wheat (Eta, Banti) and triticale (Wanad, Migo) non-infested by the grain aphids

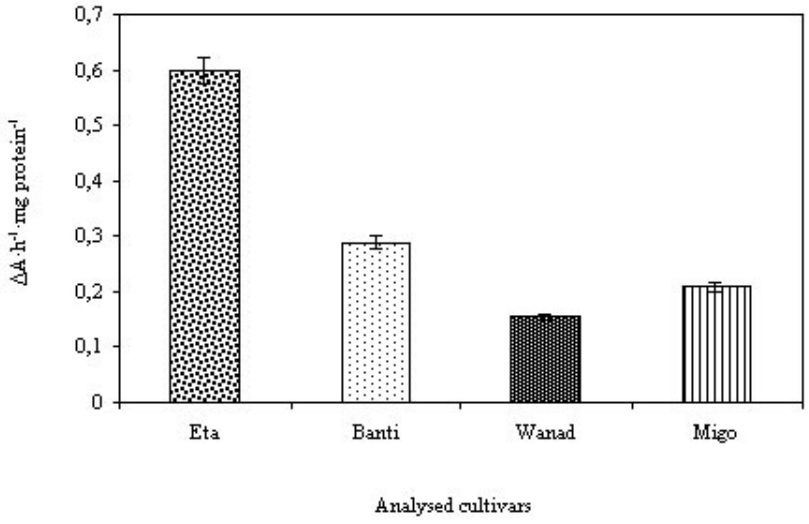
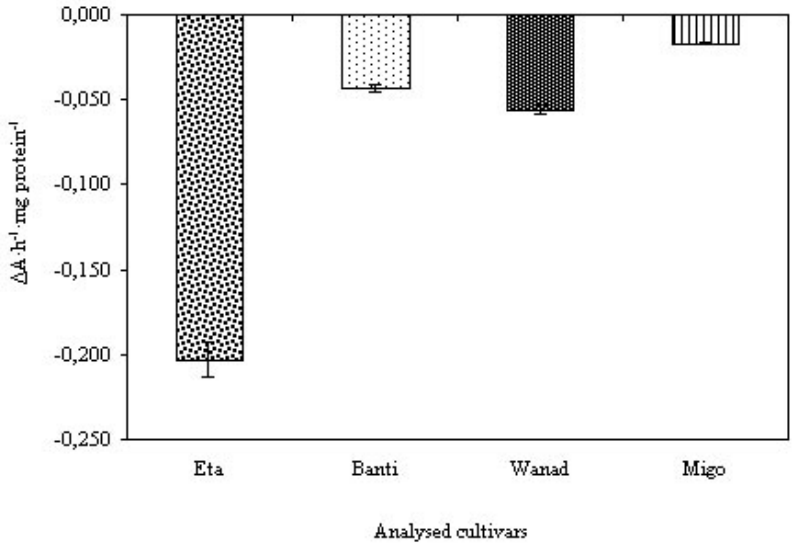


Fig. 2. Changes of polypheoloxidase activity in the ears of analysed species and cultivars of cereals caused by grain aphid feeding, calculated as difference between activity PPO in infested and non-infested plants



DISCUSSION

Significant quantities of phenolic compounds and oxidases oxidizing these compounds indicate their participation in metabolic conversion of phenols [4]. Results of previous studies showed that most plants resistant to feeding insects have higher polyphenoloxidase activity. However, research conducted by Hedin *et al.* [8] has proved that corn cultivars resistant to *Diatracea grandiosella* had lower activity of the enzyme by 25% in comparison with susceptible cultivars. Harborn [7] explains that this differentiation of plants by pest feeding exists due to increased adaptation and capability of instigating the defense mechanism. On the basis of obtained results it was ascertained that grain aphid feeding markedly decreased PPO activity, yet this reaction was peculiar to each variety and cultivar of examined cereals. The Eta wheat, which had higher PPO activity in non-infested plants than Banti was proved to have decreased activity of the enzyme when infested by *S. avenae*. In

the case of spring triticale cultivars the relation was contrary to the one of spring wheat. According to Felton *et al.* [5] reactions to pest feeding in plants are different for various host-plant relations. Even though conversion of monomeric phenols to quinones causes detoxication of secondary metabolites, Miles and Oertli [14] believe that too high a degree of phenol oxidation may induce an excessively rapid loss of toxicity by further transformation into harmless polymers. Changes of polyphenoloxidase activity in infested plants may also be induced by insertion of oxidases with aphid's saliva into tissues of the host plant [10]. Salivary enzymes inserted into the host plant may neutralise the harmful effect of phenolic compounds on aphid [18,19], and also may transform them into compounds stimulating feeding [15]. Therefore plants can defend themselves against aphid in two ways. On the one hand, they can accumulate large quantities of phenolic compounds (obtained as a result of metabolic transformations); in this way they prevent infestation. And on the other hand, by oxidizing phenols of low toxicity they induce synthesis of compounds with immense antibiotic capability.

CONCLUSIONS

Conducted research has proved that spring wheat cultivars have considerably more intense polyphenoloxidase activity than the spring triticale cultivars. It has also been ascertained that grain aphid feeding induces decrease of oxidizing activity of the enzymes in all analysed cultivars.

High level of wheat's constitutional resistance to grain aphid is connected with intense polyphenoloxidase activity, and low PPO activity in case of triticale. Induced resistance of both varieties is linked with decreased activity of this enzyme.

REFERENCES

1. Asada K., 1992.Ascorbate peroxidase – α-hydrogen peroxide – scavenging enzyme in chloroplast. Physiol. Plant., 84, 75-81.
2. Boss P.K., Gardner R.C., Janssen B.J., Ross G.S., 1995. An apple polyphenoloxidase cDNA is upregulated in wounded tissue. Plant Mol. Biol., 18, 193-215.
3. Chazarra S., Cabanes J., Escribano J., Garcia-Carmona F., 1997. Kinetic study of the suicide inactivation of latent polyphenoloxidase from iceberg lettuce (*Lactuca sativa*) induced by 4-tert-butylcatechol in the presence of SDS. Biochim. Biophys. Acta, 1339, 297-303.
4. Esteban-Carasco A., Lopez-Serrano M., Zapata J.M., Sabater B., Martin M., 2001. Oxidation of phenolic compounds from *Aloe barbadensis* by peroxidase activity: Possible involvement in defence reactions. Plant Physiol. Biochem., 39, 521-527.
5. Felton G. W., Broadway R.M., Duffey S.S., 1989. Inactivation of protease inhibitor activity by plant – derived quinines: complications for host – plant resistance against noctuid herbivore. J. Insect. Physiol., 35, 981-990.
6. Felton G.W., Donato K.K., Broadway R.M., Duffey S.S., 1992. Impact of oxidized plant phenolics one the nutritional quality of dietary protein to a noctuid herbivore. J. Insect. Physiol., 38, 277-285.
7. Harborn J.B., 1997. Koewolucyjny wyścig zbrojeń: obrona roślin i reakcja zwierząt. (In:) Ekologia biochemiczna. Wydawnictwo Naukowe PWN Warszawa, 215-241.
8. Hemin P.A., Davies F.M., Williams W.P., Salin M.L., 1984. Possible factors of leaf-feeding resistance In Horn to the south western Horn borer. J. Agric. Food Chem., 32, 262-267.
9. Hildebrand D.F., Rodriquez J.G., Brown G.C., Luu K.T., Volden C.S., 1986. Peroxidative responses of leaves in two soybean genotypes injured by two spotted spider mite (*Acari: Tetranychidae*). Appl. Ent. Zool., 20, 348-349.
10. Jiang Y., Miles P.W., 1993. Responses of compatible Lucerne variety to attack by spotted alfalfa aphid: changes in the redox balance in affected tissue. Entomol. Exp. Appl., 67, 263-274.
11. Laurem S., Varis A.L., Miettinen H., 1985. Studies on enzymes in the salivary glands of *Lygus rugulipensis* (*Hemiptera: Miridae*). Insect Biochem., 15, 211-224.
12. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265-275.
13. Meyer A.M., 1987. Polyphenol oxidases in plants – recent progress. Phytochemistry, 26, 11-20.
14. Miles P.W., Oertli J.J., 1993. The significance of antioxidants in the aphid – plant interaction: the redox hypothesis. Entomol. Exp. Appl., 67, 275-283.
15. Peng Z., Miles P.W., 1991. Oxidases in the gut of an aphid Macrosiphum rosae /L./ and their relation to dietary phenolics. J. Insect Physiol., 37, 779-787.
16. Tomczyk A., 1992. Changes in phenolic compounds in cucumber leaves infested by the two-spotted spider mite (*Tetranychus urtice*). (In:) Proc. 8th Int. Symp. Insect – Plant Relationships, Dordecht, 309-310.
17. Tottman D.R., Broad H., 1987. The decimal code for the growth stages of cereals, with illustrations. Ann. Appl. Biol., 93, 221-234.
18. Urbańska A., Tjallingii W.F., Dixon A.F.G., Leszczyński B., 1998. Phenol oxidizing enzymes in the grain aphid's saliva. Entomol. Exp. Appl., 86, 197-203.
19. Urbańska A., Leszczyński B., Tjallingii W.F., Matok H., 2002. Probing behaviour and enzymatic defence of the grain aphid against cereal phenolics. EJPAU, Biology, Vol. 5, Issue 2.

Grzegorz Chrzanowski, Antoni P. Ciepiela, Iwona Sprawka, Cezary Sempruch,
Hubert Sytykiewicz, Paweł Czerniewicz
Department of Molecular Biology and Biophysics
University of Podlasie
ul. Prusa 12, 08-110 Siedlce, Poland
e-mail: grzegorzc@ap.siedlce.pl