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Activity of cereal aphid enzymes towards scavenging hydrogen peroxide

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Introduction

Reactive oxygen species (ROS) such as superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2) are involved in the defense of plants against pathogens attack (Mittler 2002). During such response ROS are produced by the activity of plant NADPH oxidases, amine oxidases and cell-wall-bound peroxidases (Grant & Loake, 2000; Hammond-Kosack & Jones, 2000). Some of the plant pro-oxidant compounds upon photochemical or metabolic activation may also generate reactive forms of the oxygen (Barbehenn *et al.*, 2003). ROS can react with biomolecules such as DNA, RNA, proteins and lipids causing alterations within their structures.

Among the generated ROS, hydrogen peroxide plays a central role in plant defense responses (Wu *et al.*, 1995; Mehdy *et al.*, 1996; Kuźniak & Urbanek, 2000). However, herbivores insects possess antioxidant mechanisms composed of catalase and ascorbate peroxidase that destroy toxic H_2O_2 (Summers & Felton, 1993; Mathews *et al.*, 1997; Łukasik, 2007). The inefficiency of catalase at low hydrogen peroxide concentrations and lack Se-dependant glutathione peroxidase, a key enzyme for the removal H_2O_2 in mammals, suggests that ascorbate peroxidase may play a crucial role in protecting herbivores against H_2O_2 toxicity (Mathews *et al.*, 1997).

The aim of the present paper is to compare the level of the hydrogen peroxide and activity of the catalase and ascorbate peroxidase within tissues

monophagous grain aphid, *Sitobion avenae* (F.) and oligophagous bird cherry-oat aphid, *Rhopalosiphum padi* (L.) fed on winter triticale.

Material and methods

The experiment was conducted on *apterae* and *larvae* of the grain aphid *Sitobion avenae* (Fabricius, 1775) and the bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus, 1758). Insects came from the aphid stock cultures kept on winter triticale cv. 'Tornado' (susceptible) and 'Witon' (less-susceptible) at the University of Podlasie at Siedlce.

The collected aphids were placed in 50 mM K-phosphate buffer pH 7.0 and then homogenized for 5 min at 0°C. The homogenates were filtered through two layers of cheesecloth and centrifuged at 3000 x g for 15 min. The pellets were discarded and the supernatants were used to assay the hydrogen peroxide content and activity of catalase and ascorbate peroxidase.

The level of the hydrogen peroxide was determined according to GREEN & HILL (1984), based on the reaction of 4-aminoantipyrine and phenol with H_2O_2, catalyzed by peroxidase. Thereby the colored product (chinonimin) was formed that was determined spectrophotometrically at 510 nm.

The activity of catalase was determined by monitoring spectrophotometrically the degradation of hydrogen peroxide at 240 nm (AEBI, 1984). The activity of ascorbate peroxidase was measured as described by ASADA (1984). This assay was based on the disappearance of the ascorbate (measured by its absorbance at 290 nm) as it is oxidized to dehydroascorbate by hydrogen peroxide.

The content of proteins within homogenates of the studied aphids was determined by the BRADFORD method (1976).

Results and discussion

The carried out experiments showed quite clear differences in the content of the hydrogen peroxide within tissues of the cereal aphid morphs. Generally the higher H_2O_2 concentration was recorded for *apterae* adults of both aphid species. In case of the bird cherry-oat aphid, the level of hydrogen peroxide within wingless females was 5-7-fold higher in comparison to *larvae* morphs (Fig. 1). This is in agreement with studies considering level of another biochemical marker of oxidative stress, lipid peroxidation products (TBARS) (ŁUKASIK *et al.*, unpublished). These results may point out more effective antioxidant mechanisms that protect *larvae* from damages caused by oxidative stress. It is

supported by results of our earlier studies where *larvae* possesed higher level of non-enzymatic as well as enzymatic antioxidants than *apterae* adults (Łukasik, 2006; Łukasik, 2007; Łukasik & Goławska, 2007).

On the other hand, the host-plant clearly affected accumulation of H_2O_2 within the cereal aphid tissues. The studied morphs fed on 'Witon' cv. had a higher content of hydrogen peroxide than which occurred on 'Tornado' cv., with the exception of the bird cherry-oat aphid *larvae* which showed similar H_2O_2 level on both triticale cultivars (Fig. 1). These differences may be associated with different susceptibility of the studied winter triticales towards the cereal aphids, since 'Witon' cv. was more resistant to the aphids infestation than 'Tornado' cv. Electrical penetration graphs (EPG) recordings showed that *S. avenae* females fed on 'Witon' cv. seedlings were characterized by longer duration of no probing, total pathways and shorter time of salivation into sieve element and phloem sap ingestion that *apterae* adults fed on the 'Tornado' cv. (Sempruch *et al.*, 2008). The Russian wheat aphid, *Duraphis noxia* (Kurdjumov, 1913) infestation caused a higher generation of hydrogen peroxide within resistant wheat than in susceptible wheat (Moloi & Westhuizen, 2006). Plants may use hydrogen peroxide not only as inducer of the defensive metabolic processes but rather as defense mechanism itself (Lamb & Dixon, 1997).

The wingless females of oligophagous species *R. padi*, which change the hosts between trees and grasses, showed higher concentration of hydrogen peroxide than *apterae* of monophagous species *S. avenae*. However, *R. padi* had asignificant higher activity of the ascorbate peroxidase that decompose toxic hydrogen peroxide with the help of ascorbic acid (Fig. 2). It was not so clear in the case of the catalase, where *apterae* adults of both studied species had comparable activity of CAT, but the *larvae* of the bird cherry-oat aphid possessed higher catalase activity than the grain aphid ones (Fig. 3). Diverse results were obtained for two species of grasshoppers, where polyphagous *Mellanoplus sanquinipes* had comparable activity of antioxidant enzymes as the graminovorous *Aucolara ellioti* (Barbehenn, 2002).

In contrary to hydrogen peroxide content, no differences in the activity of the studied enzymes were found between aphids fed on both triticale cultivars, with exception of the bird cherry-oat aphid females that showed higher APOX activity on 'Tornado' cv. (Fig. 2 and 3). Loayza *et al.*, (2000) noted the lack significant effect of wheat cultivar on catalase activity within the grain aphid. It contrasts with results obtained by Figueroa *et al.* (1999) where twofold increase of CAT activity in *S. avenae* morphs treated with DIMBOA was observed. On the other hand, dietary o-dihydroxyphenols decreased catalase and ascorbate peroxidase activity within herbivorous insects (Ahmad, 1992; Łukasik, 2007). Thus the level of antioxidant enzyme activities within cereal aphid tissues may be affected by the quantitative composition and pro-oxidant status of their host plants. The higher content of hydrogen peroxide within the wingless *apterae* fed

on 'Witon' cv. without the greater antioxidant potential may lead to free oxygen radicals cascade and damages of the aphid main macromolecules, e.g. nucleic acids and proteins.

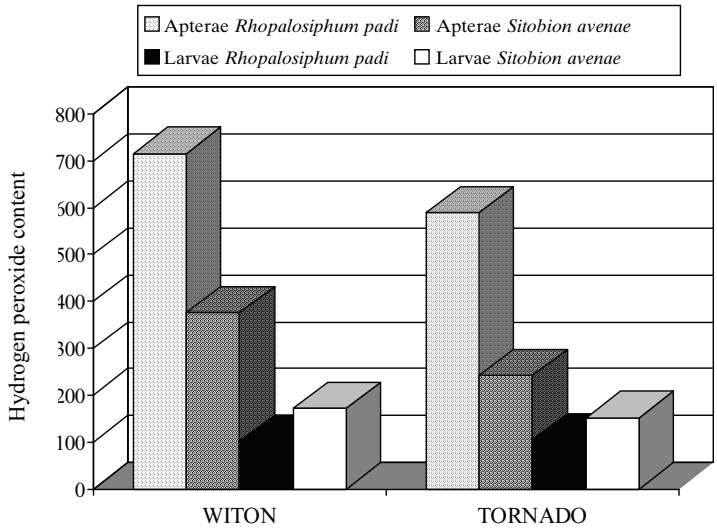

Figure 1. Content of hydrogen peroxide (nmol/mg protein) within tissues of the grain aphid and the bird cherry-oat aphid fed on the winter triticale cultivars

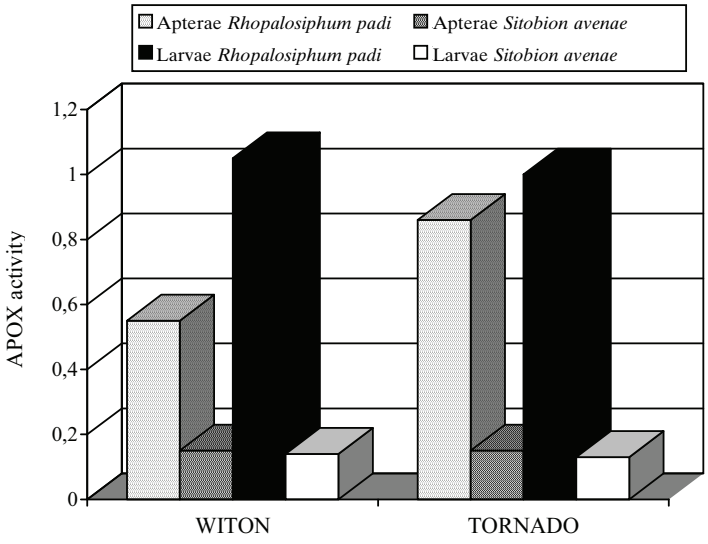

Figure 2. The activity of ascorbate peroxidase (mol oxidized ascorbate/min/mg protein) within tissues of the grain aphid and the bird cherry-oat aphid fed on the winter triticale cultivars

The results presented here demonstrated that the host plant feeding might cause the oxidative stress within the cereal aphid tissues what was evidenced by accumulation of H_2O_2. On the other hand, the cereal aphids have antioxidant enzymes system that allow them to decompose toxic hydrogen peroxide. The *larvae* of the bird cherry-oat aphid seems to possess the most efficiency of hydrogen peroxide scavenging mechanisms since they had the higher level of catalase and ascorbate peroxidase activity in comparison to other morphs.

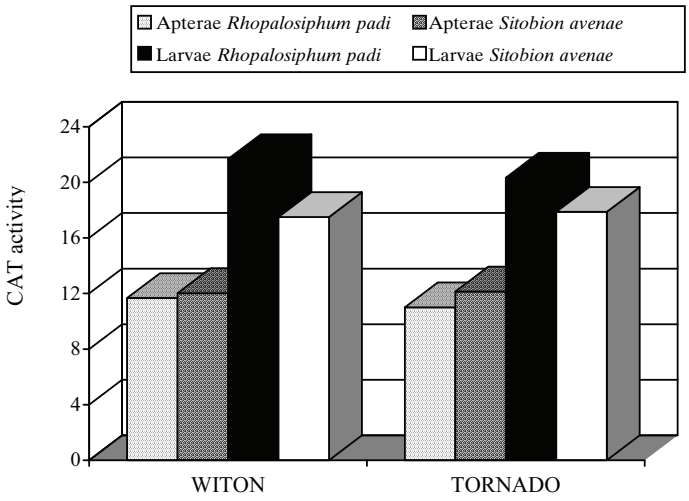

Figure 3. The activity of catalase (mol decomposed H_2O_2/min/mg protein) within tissues of the grain aphid and the bird cherry-oat aphid fed on the winter triticale cultivars

Conclusions

1. Among the studied triticale cultivars 'Witon' showed a stronger effect on the content of the hydrogen peroxide within tissues of the cereal aphids.
2. The cereal aphids possess the activity of catalase and ascorbate peroxidase that decompose the toxic hydrogen peroxide.
3. The studied aphid species showed similar catalase activity but the bird cherry-oat aphid had a several times higher level of ascorbate peroxidase. It points out a very important role of this enzyme in antioxidant system of *R. padi.*

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Aktywność enzymów neutralizujących nadtlenek wodoru w tkankach mszyc zbożowych

Streszczenie

W prezentowanej pracy porównano poziom nadtlenku wodoru (H_2O_2) w tkankach mszycy czeremchowo-zbożowej *Rhopalosiphum padi* (L.) i mszycy zbożowej *Sitobion avenae* (F.), żerujących na dwóch odmianach pszenżyta ozimego, różniących się podatnością na badane gatunki mszyc. Oznaczono również aktywność katalazy i peroksydazy askorbinianowej, enzymów neutralizujących toksyczne działanie H_2O_2. Przeprowadzone badania wykazały, że żerowanie mszyc na mniej podatnej odmianie 'Witon' powodowało wyższą kumulację nadtlenku wodoru w ich tkankach. W tkankach bezskrzydłych samic (*apterae*) zaobserwowano wyższy poziom H_2O_2 w porównaniu z larwami. Badane gatunki mszyc charakteryzowały się podobnym poziomem aktywności katalazy, natomiast gatunek oligofagiczny *R. padi* wykazywał kilkakrotnie wyższą aktywność peroksydazy askorbinianowej. Nie zaobserwowano natomiast wyraźnych różnic w poziomie aktywności enzymów usuwających nadtlenek wodoru w tkankach mszyc żerujących na badanych odmianach pszenżyta. Wyjątek stanowiły bezskrzydłe samice *R. padi*, dla których zanotowano wyższą aktywność peroksydazy askorbinianowej podczas żerowania na odmianie 'Tornado'.