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Changes in the activity of glutamine synthetase in thissues of winter triticale seedlings caused by *Sitobion avenae* (F.) feeding

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Introduction

Glutamine synthetase (GS; EC 6.3.1.2) is an enzyme that is responsible for the ammonium ion joining the molecule of the glutamine acid, with a generation of a glutamine molecule. Together with the glutamine synthetase (GOGAT), in GS-GOGAT cycle it catalyzes the transfer of amino group from glutamine on to 2-oxoglutaran, with a generation of two molecules of the glutamine acid. These reactions next to transformation regulated by glutamine dehydrogenase (GDH) play a key role in the assimilation of NH_4+.

GS is common both in above-ground parts as well as in the roots of many plant species. Glutamine is the product of the reaction which is catalysed by this enzyme. Apart from playing a vital role in the plant nitrogen distribution, the glutamine also influences indirectly their nutrition value for phytophags. Glutamine and then glutamine acid, asparagine and serine were recorded to dominate in the amino-acid composition of the phloem sap of *Loium perrenne* (L.) (Amiard *et al.,* 2004). According to Sandström (1998) the phloem sap of the primary and secondary hosts of *Ropalosiphum padi* (L.) and *Aphis fabae* (Scop.) implied overwhelming dominance of the following amino-acids: glutamine, glutamic acid, asparagine, aspartic acid and homoserine. These aminoacids were present also in the highest concentration in the sieve vasculars of host plants of *Sitobion avenae* (F.), *Drepanosiphum platanoides* (Schr.), *Hyalopterus pruni* (Geoffrey) and were used by

aphids or their endosymbionts for biosynthesis of the remaining amino-acids (DOUGLAS, 1993).

The so far published papers have provided relatively extensive data on the amino-acids share in interactions between cereal aphids and their host plants (CIEPIELA & SEMPRUCH, 1993; CIEPIELA *et al.,* 1991; LESZCZYŃSKI *et al.,* 1999; SEMPRUCH & CIEPIELA, 1998; 2004). No information concerning the course of biosynthesis of these compounds in plant tissues that were attacked by aphids has been provided as yet. Hence the aim of this research is to determine the impact of *S. avenae* feeding on the GS activity in seedlings of winter triticale.

Material and methods

Two cultivars of winter triticale which were used for the research (*Triticosecale*, Wittm. Ex A. Camus) were obtained from the Institute of Breeding and Adaptation of Plants (Instytut Hodowli i Aklimatyzacji Roślin-IHAR) in Strzelce near the city of Łódź: Tornado and Witon. *S. avenae* individuals, which were used for the research, came from a rearing maintained by the Department of Biochemistry and Molecular Biology of the University of Podlasie in the town of Siedlce.

The feeding behaviour of cereal aphid on seedlings of the studied triticale variety was analysed with the use of the EPG (electrical penetration graphs) technique according to the procedure described by LESZCZYŃSKI & TJALLINGI (1994). Average time of subsequent patterns of feeding was measured: non probing – Np, total pathay – ABC, sieve element salivation – E_1, w ingestion of phloem sap – E_2, ingestion of xylem sap – G. Ten-hour-long registers were carried out with the use of 10 wingless females of *S. avenae* which were feeding on 10 different seedlings of the studied winter triticale varieties.

The changes in enzyme activity caused by *S. avenae* feeding were researched in the tissues of 7-day-old seedlings of the triticale varieties, artificially settled by five wingless females and isolated for two weeks. Analogously the control plants (with no aphids) were prepared. Both, the attacked and the control seedlings were collected after 24 hours, 48 hours and 1 and 2 weeks of aphid feeding, each time determining the number of *S. avenae* on 25 seedlings.

GS was extracted from fresh plants by means of homogenization in 0.02 M buffer Tris-HCl pH 7.5, with 1 mM 2-merkaptoethanol and 2mM ethylenediaminetetraacetic acid disodium salt (EDTA). The suspension was centrifuged whirled by 18 000 x g for 30 minutes and the obtained supernatant was used to identify the enzyme activity by a method described by KANAMORI & MATSUMOTO (1972). The content of protein in enzyme extracts was determined by the method of LOWRY *et al.* (1951). The activity of GS was stated in γ -glutamyl hydroxamate /mg protein/ hour.

The results underwent one-way analysis of variance (ANOVA). The significance of the difference between the enzyme activity in control plants and aphid-attacked plants was estimated by means of a t-Student test with $p \leq 0.05$ and $p \leq 0.01$.

Results and discussion

The EPG tests proved that *S. avenae* females showed different behaviour when feeding on the researched cultivars of the triticale (Fig. 1.). On the 'Witon' cultivar a higher percentage share in the total feeding time of the pest constituted the following: non probing (pattern Np), total pathway (ABC), salivation into sieve elements (E_1) and in particular the ingestion of xylem sap (G). Aphids feeding on the 'Tornado' cultivar were taking the phloem sap in definitely for a longer time (pattern E_2). Close results were obtained previously by WÓJCICKA *et al.* (1999) and WÓJCICKA & LESZCZYŃSKI (2003). They found out that in the *S. avenae* individuals feeding on the less susceptible cereals the non probing or piercing of the peripheral tissues took more time and the proper feeding in the phloem was much shorter than in plants that were better accepted by aphids.

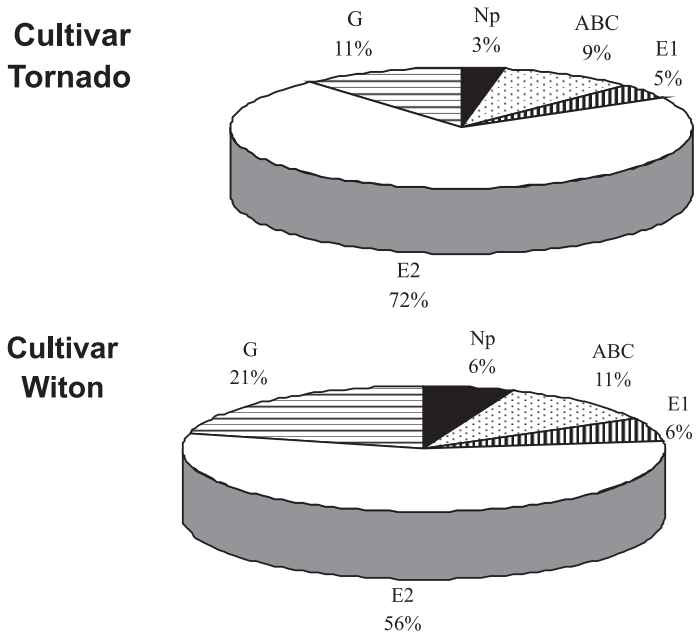

Fig. 1. Percentage share of analysed EPG patterns in integral time of *Sitobion avenae* feeding on the observed cultivars of winter triticale seedlings: Np non probing, ABC total path way, E1 salivation into sieve elements, E2 the ingestion of phloem sap, G the ingestion of xylem sap

The presented results point out to a diversified susceptibility of the studied cultivars of triticale in relation to *S. avenae*. According to LESZCZYŃSKI & TJALLINGII (1994) the EPG is an unusually useful method to detect mechanisms of natural resistance in wild and arable plants. A diversification of the course of aphid feeding on plants which are different with respect to the degree of susceptibility to these pests is linked also with the frequency as well as with the time that particular patterns take, and in particular with the length of nutrient intake from sieve elements. On cultivars with lower susceptibility these insects in general take a long time to move about the plant and prior to the feeding they take the nutrient in for a short time with many intervals, while their stylets often do not even reach the phloem elements at all.

The research proved that tissues of both roots and shoots of the 'Witon' cultivar, which were not infested by aphids (control) were conspicuous for a decidedly higher GS activity in comparison with the analogous organs of the 'Tornado' cultivar (Fig. 2.). The *S. avenae* feeding caused a great increase in the enzyme activity in the above-ground parts of the 'Tornado' cultivar seedlings after 24 hours, which remained unchanged in the first week of the experiment. In the aphid-infested shoots of the 'Witon' cultivar, the GS activity also kept rising to a small degree after 24 hours, after which it declined as the feeding prolonged. The two researched cultivars characterized by a lowering of enzyme activity after two weeks of *S. avenae* feeding. In tissues of roots of the 'Tornado' cultivar seedlings on which the cereal aphid fed, the GS activity increased extensively after 24 hours and 48 hours and lowered after two weeks of the experiment. In the analogous organs of the 'Witon' cultivar the pest feeding caused a decline in enzyme activity except for one week after which the tempo of GS functioning did not change substantially in terms of statistics. The results point out to the fact that the changes in GS activity during the first 24 hours of *S. avenae* attack were genetically conditioned. During this time, with the same number of aphids (5 individuals/blade) slight differences in the reaction of both researched cultivars to aphid feeding were registered. In the two subsequent terms the researched cultivars also differed in terms of direction and intensity of changes in GS activity prompted by *S. avenae* feeding, which may have resulted from the diversified number of the pest. The higher number of *S. avenae* specimens on seedlings of the 'Tornado' cultivar after 48 hours and 7 days was followed by an induction in GS activity in above-ground parts, while in the analogous plant organs of the 'Witon' cultivar with lower number of aphids the enzyme activity tended to decrease. After two weeks of *S. avenae* feeding there was a reduction in GS activity in both the shoot and root tissues of both triticale cultivars, though it was strongly marked in the less aphid infested 'Witon' cultivar. The results suggest that the *S. avenae* feeding systemically influences the GS functioning because the changes in enzyme activity were not restricted to the directly damaged by aphids above-ground parts but

also involved the root tissues. Similar results were provided by SANDSTRÖM *et al.* (2000), who claimed that the changes in amino acid composition of the phloem sap of wheat and barley leaves caused by *Schizaphis graminum* (Rond.) and *Diuraphis noxia* (Mordvilko) had a systemic character.

The presented results prove that the changes in GS activity caused by cereal aphid feeding take part in the formation of interactions between winter triticale and *S. avenae*. The increase in enzyme activity which was observed in most cases of the researched variants of the experiment in tissues of the above-ground parts and roots of plants of the susceptible 'Tornado' cultivar may contribute to the increase of tempo of glutamine biosynthesis. The lowering of GS activity in the less susceptible 'Witon' cultivar may inhibit the pest development. According to DOUGLAS (1993) and SANDSTRÖM (1998) the high content of glutamine is characteristic for the phloem sap of plants susceptible to *S. avenae*, *D. plantanoides*, *H. pruni*, *R. padi* and *A. fabae*. This compound moreover, along with glutamic acid, asparagine aspartic, acid and homoserine, may be used by aphids and their endosymbionts for biosynthesis of the remaining protein amino-acids as a donor of the amino group in transamination reactions. It has to be taken into consideration that the course of the considered reactions may have been dependent on both the cultivar and individual parts of triticale seedlings as well as on the time of feeding and the number of cereal aphids.

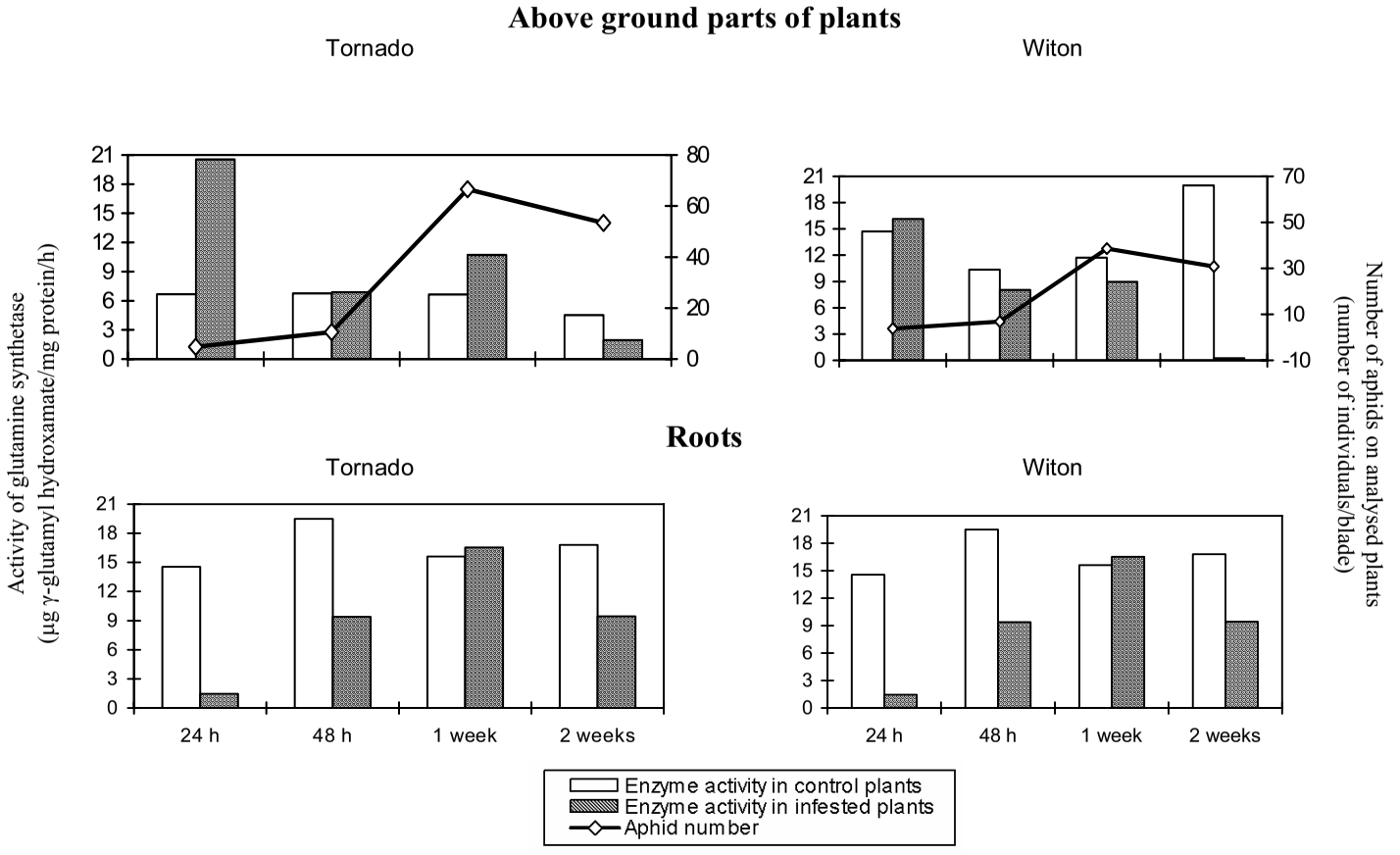

Fig. 2. Impact of *Sitobion avenae* feeding on glutamine synthetase activity in the tissues of triticale cultivars
*differences between plants with- and without aphids in p≤0.05 (t-Student test), ** differences signifficant at p≤0.01

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Zmiany w aktywności syntetazy glutaminowej w tkankach siewek pszenżyta ozimego wywołane żerowaniem mszycy zbożowej, *Sitobion avenae* (F.)

Streszczenie

Celem pracy było określenie wpływu żerowania *Sitobion avenae* na aktywność syntetazy glutaminowej w tkankach korzeni i części nadziemnych siewek dwóch odmian pszenżyta ozimego, o zróżnicowanej podatności na tego szkodnika. W częściach nadziemnych podatnej odmiany Tornado żerowanie mszyc powodowało wzrost aktywności enzymu w okresie pierwszego tygodnia trwania eksperymentu oraz jej spadek po dwóch tygodniach. W pędach mniej podatnej odmiany Witon aktywność GS zwiększała się po 24 godz. oraz obniżała po dłuższym czasie żerowania. Tkanki korzeni odmiany Tornado charakteryzowały się wzrostem aktywności enzymu po 24 i 48 godz. oraz jej spadkiem po dwóch tygodniach, podczas gdy w przypadku odmiany Witon żerowanie *S. avenae* wywoływało spadek aktywności enzymu. Otrzymane wyniki dyskutuje się w kontekście znaczenia GS w interakcjach między pszenżytem ozimym a mszycą zbożową.