ACTIVITY OF ASPARTATE AMINOTRANSFERASE AND ALANINE AMINOTRANSFERASE WITHIN WINTER TRITICALE SEEDLINGS INFESTED BY GRAIN APHID (*SITOBION AVENAE* F.)

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Abstract: Amino acid level is well known indicator of plant resistance to aphids. Our earlier studies showed that grain aphid (*Sitobion avenae* F.) infestation caused changes in the activity of the enzymes connected with amino acid biosynthesis and the transformation to defensive secondary metabolites within triticale tissues. However, there are not data on the significance of aminotransferases in these processes. The aim of our study was the quantification of changes in the activity of aspartate aminotransferase (AspAT) and alanine aminotransferase (AlaAT) in winter triticale seedlings caused by the feeding of the grain aphid. The study results showed that aphid feeding caused an increase in AlaAT activity and a decrease in AspAT activity within tissues of the triticale. The induced mechanisms of the triticale resistance to the grain aphid are discussed.

Key words: *Sitobion avenae*, aspartic aminotransferase, alanine aminotransferase, amino acids, winter triticale, aphid-plant interactions

INTRODUCTION

The amino acid content is an important factor in plant resistance to aphids, since the level of these compounds is often insufficient for pest requirements (Cole 1997; Sandström and Moran 1999). Aphids can modify amino acids accumulation within host plant tissues through redirection of its flow to the feeding spot and/or alternation of the metabolism of these amino acids (Sempruch *et al*. 2011). According to Blackmer and Byrne (1999), the free amino acid content increased within plants infested by *Aphis fabae* (Scop.), *Elatobium abitinum* (Walk.) and *Bemisia tabaci* (Gen.). Similar changes caused by the feeding of the cereal aphid *Shizaphis graminum* (Rond.) and *Diuraphis noxia* (Kord.) were systemic, since the strong increase of amino acids (especially essential) within the phloem sap of wheat and barley was not limited to the spot of the feeding, but was also observed in other parts of the infested leaves (Sandström *et al.* 2000). Moreover, an increase in the amino acid contents in the soluble protein fraction of susceptible triticale, and a decrease in relatively resistant cultivars suggests the possible participation of other metabolic pools in aphid-plant interactions (Sempruch and Ciepiela 2002).

Our earlier studies showed that activity of some enzymes catalysed the assimilation of mineral forms of nitrogen and their connection with carboxylic acids molecules during the first phase of The amino acid biosynthesis. Nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS) were altered as a result of the feeding of the grain aphid on winter triticale (Sempruch and Ciepiela 2001; Sempruch *et al.* 2007). A similar effect was observed for L-phenylalanine ammonia-lyase (PAL), arginase, ornithine decarboxylase (ODC), lysine decarboxylase (LDC) and tyrosine decarboxylase (TyDC) that transformed amino acids into defensive secondary metabolites, especially hydroxycinnamic acids, non-protein amino acids, polyamines and aromatic monoamines, and indirectly to alkaloids (Ciepiela *et al.* 1995; Sempruch *et al.* 2008, 2009, 2010). The changes in enzyme activity were dependent on aphid density and feeding duration, as well as cultivar and organ of triticale plants. However, there are no data on aminotransferases participating in biosynthesis and biodegradation of the amino acids, and in plant defense towards the aphids.

Our studies were focused on the comparison of the activity of aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) within the tissues of three winter triticale cultivars with different suitability for *S. avenae*. We also focused on the quantification of changes in the activity of the enzymes caused by the aphid feeding.

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MATERIALS AND METHODS

Plants

Three cvs. of winter triticale (*Triticosecale* Wittm. ex A. Camus): Moniko (susceptible), Presto (moderately resistant) and Ugo with different levels of suitability to the grain aphid, were used in the experiments. Seeds of the triticale cvs. were obtained from the Plant Breeding and Acclimatization Institute (Instytut Hodowli i Aklimatyzacji Roślin – IHAR) in Strzelce near Łódź (Poland).

Aphids

Parthenogenetic individuals of grain aphid (*Sitobion avenae*) were reared on winter triticale seedlings (Tornado cv.) in a climatic chamber at 24°C in the day and 18°C at night, 70% RH (relative humidity) and photoperiod 16L:8D.

Plant infestation with aphids and sampling

Seeds of the studied cvs. were germinated in a climatic chamber at 24°C in the day and 18°C at night, 70% relative humidity (RH) and photoperiod 16L:8D. Plants were grown individually in medium nutrient fine structure compost with sand, in 8.0x9.5 cm plastic pots. Plants were watered regularly.

Ten, seven-day-old seedlings were artificially infested; each with ten wingless females of *S. avenae*. The seedlings were isolated in Plexiglass cages. The cages had a cheese cloth cover. The control plants (without aphids) were similarly prepared. The aphid density was kept at a constant level of ten individuals per blade during the experiment – through daily removal of the new born larvae. All infested and control seedlings were collected after one week of infestation. The aphids were removed from the infested plants. Aerial parts of the triticale seedlings were divided into three samples and next homogenized with cool acetone to obtain the enzyme preparations.

Enzyme assays

Activity of both aspartate aminotransferase and alanine aminotransferase was assayed in the enzyme preparations according to the Reitman and Frankel method (1957). The diagnostic kit supplied by Sigma Chemicals was used.

In the case of AspAT, 0.5 g enzyme preparation was homogenized with 10 cm^3 0.05 M buffer Tris-HCl pH 8.5, and the obtained suspension was filtered through two layers of cheese cloth and centrifuged at 2,800 x g for 20 min. All steps of the extraction were conducted at 4°C. The incubation mixture contained 1 cm^3 of the substrate (1.8 mM α-oxoglutaric acid and 0.2 M aspartic acid in phosphate buffer pH 8.5) incubated at 30°C for 10 min. Enzyme extract (0.2 cm^3) was added and the mixture was incubated for 60 min, and then mixed with 1 cm^3 20 mg% solution of 2,4-dinitrophenylhydrasine in 1 M HCl. After 20 min, the reaction was stopped by the addition of 10 cm^3 of 0.4 M NaOH, and absorbance was measured on a Hewlett Packard UV-Vis Spectrophotometer type 8,453 at 505 nm. The pyruvate content was quantified on the basis of the freshly prepared standard curve.

The analogical procedure was used for assays of AlaAT activity, however, 0.05 M buffer Tris-HCl pH 7.25 was applied to the enzyme extraction and the substrate was composed of 1.8 mM a-oxoglutaric acid and 0.1 M alanine in phosphate buffer pH 7.25.

Activity of AspAT was calculated in Sigma-Frankel units (SF) assuming that 20 SF is appropriate to 0.15 mM pyruvic acid formed as a result of the enzymatic reaction, 55 SF – 0.30 mM, 95 SF – 0.45 mM, 148 SF – 0.60 mM and 216 SF – 0.75 mM. In the case of the AlaAT, 23 SF was equal to 0.15 mM pyruvate, 50 SF – 0.30 mM, 83 SF – 0.45 mM and 125 SF – 0.60 mM. Activity of both enzymes was finally converted into International Units (U) through multiplication of the SF values by 0.48 coefficients.

Statistics

All analyses were conducted in three replications, and the arithmetical means with standard errors (SE) are presented in the tables. The data were subjected to Kruskal-Wallis analysis of variance by ranks after rejection of the normality when the chi-square test was used. Differences between the enzymes activity within tissues of triticale seedlings infested by the aphids, and the control ones, were confirmed by the Mann-Whitney's U-test. The acceptance level of statistical significance was $p \leq 0.05$; and all statistical analyses were conducted with Statistica software for Windows ver. 9.0 (StatSoft Inc. 2010)

RESULTS

Statistical analyses with the use of the Kruskal-Wallis test proved significant differences between activity of AspAT ($H_{(5, N = 18)} = 12.11$ at $p = 0.03$) and AlaAT ($H_{(5, N = 18)} = 11.81$ at $p = 0.04$) within analysed triticale cvs. In the control plants, AspAT was the most active within tissues of Moniko cv. and the least active in the case of Ugo cv. (Tables 1, 2). AlaAT showed the highest activity in the Presto cv. and the lowest in tissues of the Moniko cv. But, these differences

Table 1. Changes in aspartate aminotransferase activity of the studied winter triticale cultivars caused by aphids feeding on the grain

Triticale cultivar	Aspartate aminotransferase activity (international unit – U)		$U_{3'}$ p
	the control plants $\bar{x}$ ±SE	aphid infested plants $\bar{x}$ ±SE	
Moniko	48.05±2.07	40.10±1.18	0.00, 0.05
Presto	43.16±1.85	37.04±1.80	1.00, 0.13
Ugo	41.93±1.16	37.65±1.03	0.00, 0.05

Mann-Whitney's U-test: comparisons of the enzyme activity within tissues of plants infested by the aphids and the enzyme activity within tissues of the control ones; SE – standard error; x – arithmetical mean; p – significance level

Table 2. Changes in alanine aminotransferase activity caused by aphids feeding on the grain of the studied winter triticale cultivars

Triticale cultivar	Alanine aminotransferase activity (international unit – U)		U_3, p
	the control plants $\bar{x} \pm SE$	aphids infested plants $\bar{x} \pm SE$	
Moniko	28.74±0.57	32.45±1.30	0.00, 0.05
Presto	30.34±0.91	37.90±1.14	0.00, 0.05
Ugo	29.28±0.91	31.40±1.20	1.00, 0.13

Mann-Whitney's U-test: comparison of the enzyme activity within tissues of plants infested by the aphids and the enzyme activity within tissues of the control ones; SE – standard error; x – arithmetical mean; p – significance level

were not statistically confirmed ($H_{(2, N = 9)} = 4.62$ at $p = 0.10$ for AspAT and $H_{(2, N = 9)} = 1.87$ at $p = 0.39$ for AlaAT).

Moreover, the obtained results proved that the grain aphid caused a decrease in AspAT activity within the tissues of all analysed triticales, and these changes were statistically significant as concerns the Moniko and Ugo cvs. (Table 1). On the other hand, AlaAT activity was induced, and significant differences were statistically confirmed for Moniko and Presto cvs. (Table 2).

DISCUSSION

Rozbicka *et al.* (1994) stated the highest values of relative growth rate (RGR) were typical for *S. avenae* individuals developed on seedlings of Moniko cv., and the lowest on seedlings of Presto. A similar tendency was observed in the prereproductive period, although aphid mortality was the highest on Ugo cv. and the lowest on Moniko. The level of susceptibility of the triticales was compatible with differences in AspAT activity in tissues of the control plants and incompatible with AlaAT activity. The results suggest the high level of constitutive resistance of winter triticale towards the grain aphid may be connected with higher AlaAT activity and lower AspAT activity. Since, the tendency was not statistically confirmed, we conclude that further study is needed using more triticale cvs.

There are no data about the role of the aminotransferases as part of insect-plant chemical interactions. Nonetheless, it was stated that AlaAT participate in plant responses to some abiotic stressors. According to Sousa and Sodek (2003), alanine content and AlaAT activity increased within the roots of soybean under hypoxia conditions. These changes are important since alanine is one of main products of anaerobic plant metabolism, when it is synthesised *via* AlaAT activity. An increase of AlaAT within *Arabidopsis thaliana* (L.) roots during hypoxia was regulated at the transcriptional level through induction of *AlaAT1* and *AlaAT2* genes (Miyashita *et al.* 2007). Both genes were expressed predominantly in vascular tissues and *AlaAT1* isozyme was primarily responsible for the break down of alanine excess. Gajewska *et al.* (2009) observed that nickel-stressed wheat roots were characterised by an induction of AlaAT activity but the response was not observed in the case of AspAT. Of special interest in our research are the reports focused on the importance of aminotransferases in plant responses to pathogens. These reports state that molecular pattern is like that of the plant responses to sucking-piercing insects (Goggin 2007; Stern 2008). Taler *et al.* (2004) showed that resistance of *Cucumis melo* (L.) towards *Pseudoperonospora cubensis* (Berkeley & M. A. Curtis) was connected with two 45-kDa proteins characterised by aminotransferase activity named AT1 and AT2. The proteins were highly similar to serine-glyoxylate aminotransferase (SGT) from *Fritilaria agrestis* (Stinkbells) and to Arabidopsis alanine-glyoxylate aminotransferase (AGT1). These peroxisomal enzymes participate in the generation of glycine during photorespiration. According to Eckardt (2004), two other Arabidopsis aminotransferases AGD2 and ALD1, localised in chloroplasts and cytoplasm respectively, are responsible for the resistance to *Pseudomonas syringae* infection. Based on studies with Arabidopsis mutants, it was concluded that AGD2 catalyses synthesis of an amino acid-derived molecule that supress disease resistance, whereas ALD1 generates related amino acid derivatives activating defence signalling.

Our results pointed out that changes in the activity of the aminotransferases (especially AlaAT) within triticale tissues might result not only from abiotic stresses and pathogen infections, but are also caused by the feeding of the grain aphid. A similar pattern of changes in the case of the all triticale cvs. may suggest a nonspecific response to wounding of plant tissues during the aphid penetration of the plant tissues. Thus, it is possible that activity changes in response to aphid attack could disturb amino acid transformations; the intracellular system of protons transport between cytosol and chloroplasts as well as peroxisomes; photorespiration and transport of metabolites during C_4 photosynthesis. It could lead to further modification of the nutritive value of the host plant tissues for the grain aphid. Further study is needed, which focuses on the mechanism of the changes and its consequence for aphid growth and development on the susceptible and moderately resistant cultivars.

In conclusion, we can state that attack by *S. avenae* caused an increase of the AlaAT activity and a decrease of the AspAT activity within tissues of the all triticale cvs. The observed changes in activity of the aminotransferases (especially AlaAT) might result from herbivory stress caused by the feeding on the plant by the aphids.

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