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Activity of arginase within winter triticale seedlings attacked by grain aphid (*Sitobion avenae* F.)

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Abstract: Arginase (EC 3.5.3.1) catalyses biodegradation of L-arginine to L-ornithine and urea. The aim of the study was to determine changes in arginase activity within seedlings of winter triticale caused by *Sitobion avenae* feeding. Obtained results showed an increase in the arginase activity within shoots of winter triticale seedlings susceptible Tornado cv and decrease in less susceptible Witon cv after 24 h of the grain aphid feeding. Further aphid feeding caused decrease in activity of the enzyme within shoots of the both studied cultivars and final induction after two weeks of feeding. In the roots of Tornado cv the enzyme activity decreased during the first week of the aphid feeding and slightly increased after two weeks. However, in analogous parts of the Witon cv seedlings settled by *S. avenae* an increase in arginase activity was observed after 24 h and decrease after one week was noticed. Importance of the arginase in interactions between the winter triticale and *S. avenae* is discussed.

Keywords: arginine, ornithine, amino acids metabolism, insect-plant interactions, plants responses to aphids attack

INTRODUCTION

Arginase (L-arginine aminohydrolase; EC 3.5.3.1) catalyses degradation of L-arginine to L-ornithine and urea, by elimination of guanidine group from arginine molecule [1, 2]. In plant tissues this enzyme play various physiological and metabolic role, i.e. nitrogen mobilization during development of fruits, bulbs and tubers [3-5], seeds germination [6], biosynthesis of glutamine and polyamines [7] and biodegradation of canavanine to canaline and urea [8].

Arginase may also participate in plants responses to herbivorous insects since it catalyses transformation essential protein amino acid – arginine to nonprotein ornithine. In our previous study [9] high content of protein amino acids (especially essential) was important in susceptibility of winter wheat and triticale to grain aphid. On the other hand Weibull [10] stated that higher concentration of arginine within phloem sap of oat caused its preference as a host plant by *Rhopalosiphum padi* L. However, nonprotein amino acids also belong to secondary metabolites and act as deterrents or toxic compounds to aphids [11]. Ciepiela and Sempruch [12] showed negative relations between ornithine and 3,4-dihydroxyphenylalanine (DOPA) level within winter wheat tissues and values of intrinsic rate of natural increase (r_m) of the *S. avenae*. The aim of the study was to assess changes in arginase activity within seedlings of winter triticale (*Triticosecale,* Wittm. ex A. Camus) caused by the grain aphid feeding.

MATERIAL AND METHODS

Triticale cultivars and aphids

Two cultivars of winter triticale Tornado and Witon with different level of susceptibility to the grain aphid were used in experiments. Seeds of the both cultivars were obtained from the Plant Breeding and Acclimatization Institute (IHAR) at Strzelce near Łódź (Poland). The parthenogenetic clone of *S. avenae* was reared at Department of Biochemistry and Molecular Biology (University of Podlasie, Siedlce) on winter triticale seedlings (Lamberto cv) in environmental chamber at 24 °C at day and 18 °C at night, 70% r.h. and photoperiod 16L:8D.

Population tests

The population tests were carried out in environmental chamber. The adult apterous females were pleaced individually on abaxial surfaces of leaves of seven days old seedlings of the triticale cultivars. The cultivars were grown in a medium nutrient fine structure compost with sand (UMEX), in 8.0 x 9.5 cm plastic pots, and regularly watered. Seedlings with aphids were isolated with Plexiglass cages with a cheese cloth cover (10 x 30 cm). After 24 h, one nymph remained on each single plant and other offsprings and the adult were removed. The experiments were conducted in 25 independent replicates for each cultivar. On the basis of daily observations prereproductive period and daily fecundity were estimated [13]. An intrinsic rate of natural increase (r_m) and mean time of generation development (T) were calculated using the following equations after Wyatt and White [14]:

$$r_m = 0.738 \frac{\ln Md}{d},$$

$$T = \frac{d}{0.738},$$

were d is the length of prereproductive period, Md – the number of larvae born during the reproduction period which equals the d period, 0.738 – the correction factor.

Influence of the grain aphid feeding on arginase activity

The seven days old seedlings of the studied cultivars were artificially infested with five wingless females of *S. avenae* and isolated with Plexiglass cages with a cheese cloth cover, and control plants (without aphids) were similarly prepared. Infested and control seedlings were collected after 24 h, one week and two weeks of the grain aphid feeding. Aphid number was determined on five randomly selected shoots during the plant material collection. Obtained results were calculated as an average of the aphid number on a single seedling. Collected plants were divided into shoots and roots and used immediately for the enzyme assay.

Assay of arginase activity

The enzyme extraction and determination of arginase activity were conducted according to Kang and Cho [15]. Estimation of protein quantity within the enzymatic extracts was performed according to Lowry et al. [16]. All chemical analyses were performed in three replicates.

Statistic analysis

Differences in performance of *S. avenae* on seedlings of studied triticales were calculated with Kolomogorov-Smirnov test.

RESULTS AND DISCUSSION

Results of population tests showed differences in biology of *S. avenae* on the studied triticale cultivars (Table 1). The aphids on Witon cv were characterized by significantly lower values of daily fecundity and intrinsic rate of natural increase than individuals occurred on Tornado cv. Obtained results confirmed earlier data of Ciepiela et al. [17], who showed that antibiotic properties of host

plants reduced the growth, development and fecundity of the grain aphid and caused resistance of triticale to *S. avenae*. Antibiosis of host plants to insects is often connected with nutritive value of plant tissues. Abbot et al. [18] suggest, that changes of nutritive value was an important factor of non-host resistance of plants to herbivores. Usefulness of plant food to herbivores may be modified by various classes of secondary metabolites [19] and/or primary metabolites such as protein amino acids [20, 21].

Table 1. Values of the population parameters ($\bar{x} \pm SE$) of the grain aphid on the studied winter triticale cultivars

Cultivar	Parameters			
	Prereproductive period (days)	Daily fecundity per female	Mean time of generation development (T) (days)	Intrinsic rate of natural increase (r_m)
Tornado	7.84 ±0.32	3.97 ±0.34	10.64 ±0.44	0.3129 ±0.0033
Witon	8.32 ±0.40	2.50 ±0.32	11.24 ±0.53	0.2508 ±0.0040
D_{25}	0.12	0.44	0.12	0.92
P	> 0.05	< 0.01	> 0.05	< 0.01

Comparison of population parameters for grain aphid on the triticale cultivars with test of Kolomogorv and Smirnov.

Obtained results showed that the grain aphid feeding during initial period caused an increase in the arginase activity within shoots of seedlings of more susceptible Tornado cv and decrease in less susceptible Witon cv (Figure 1). Increase in number of the grain aphid on above ground parts of both studied cultivars during first week of infestation caused a decrease of the enzyme activity. Further feeding induced arginase activity followed after two weeks of the aphid attack. Such variation in the arginase activity under the aphid attack suggests that these interactions are dependent not only on triticale genotype but also on the size of aphid population. It is possible that increase of the enzyme activity after two weeks of *S. avenae* feeding step on of the maximum population occurred at one week after start of the experiments. It is also possible that two weeks of the grain aphid feeding caused a nonspecific response of triticale to tissue damage during feeding of various aphids number. According to Goggin [19] mechanical damage of plant tissues during transiently puncture of epidermal, mesophyll, and parenchyma cells by aphid stylets may cause the metabolic response. An example of such responses, may be an increase of the arginase activity in shoots of the analysed cultivars two weeks after grain aphid infestation. As a result, an

increase of ornithine content and/or decrease in arginine level may be occurred. Such disturbances in equilibrium between level of essential amino acid arginine and nonprotein one ornithine can limit a nutritive value of triticale seedlings for *S. avenae*.

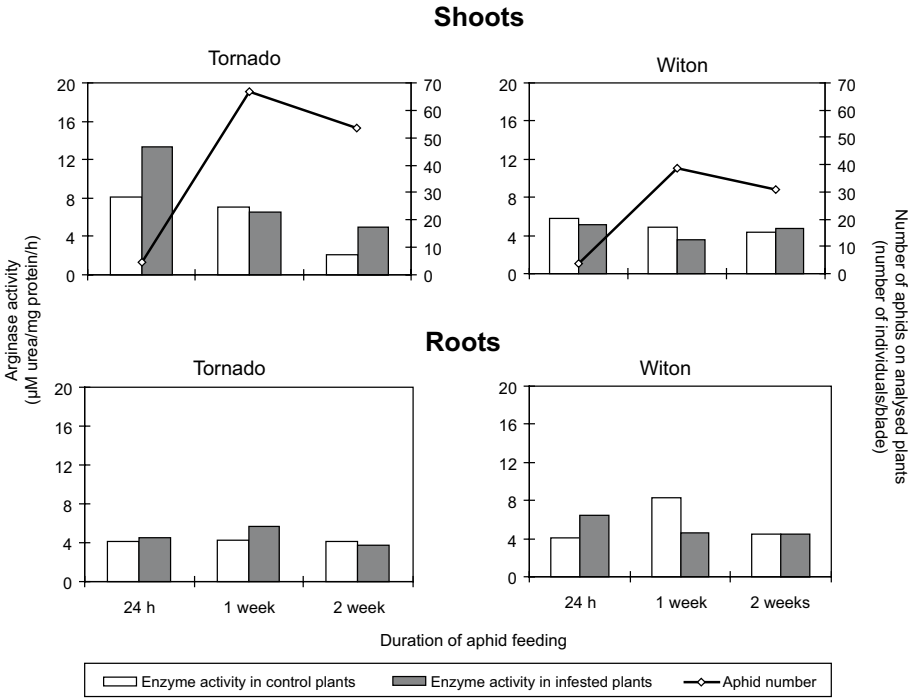

Figure 1. Influence of the grain aphid feeding on arginase activity within tissues of the studied triticale cultivars.

Moreover, it was stated that *S. avenae* feeding on above-ground parts of triticale seedlings caused changes in arginase activity in the roots (Figure 1). In Tornado cv enzyme activity decreased during the first week of the aphid feeding and slightly increased after two weeks. However, in seedling roots of Witon cv the arginase activity increased after the first 24 h of the grain aphid feeding and decreased later. The presented results also proved that changes in arginase activity in winter triticale seedlings infested with grain aphid had a systemic character.

In conclusion, we can state that biodegradation of arginine to ornithine and urea is a part of triticale response to the grain aphid attack. Changes in arginase activity caused by *S. avenae* feeding had systemic character and were dependent on triticale genotype, aphids density, and duration of pest feeding. Further

study should prove if the mechanism of responses also induced the polyamines biosynthesis as a result of the triticale infestation with the grain aphid.

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