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Original Research

Changes in Activity of Triticale Tyrosine Decarboxylase Caused by Grain Aphid Feeding

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Abstract

The aim of our study was to quantify changes in the activity of tyrosine decarboxylase (TYDC) within tissues of two cultivars of winter triticale: *Triticosecale* (Wittm. ex A. Camus), caused by grain aphid, and *Sitobion avenae* (F.). Obtained results show that Witon cv was less acceptable by *S. avenae* than Tornado cv and that antibiosis was an important part of the acceptability. The grain aphid feeding on aerial parts of more acceptable Tornado cv caused fluctuations in TYDC activity instead of on less acceptable Witon cv, and a constant increase in enzyme activity was observed. Changes in the TYDC activity within the triticale tissues had a systemic character, since they were present not only in aerial parts directly attacked by the pest, but also within root tissues. It suggests that induction of the enzyme activity is related to an antibiotic response of the triticale towards the grain aphid.

Keywords: triticale, *Sitobion avenae*, tyramine, hydroxycinnamic acid amides, insect-plant interactions

Introduction

Tyrosine decarboxylase (TYDC; EC 4.1.1.25) catalyze biosynthesis of tyramine and dopamine within plant tissues by elimination of carboxylic group from tyrosine and 3,4-dihydroxyphenylalanine (DOPA), respectively [1,2]. It also participates in biosynthesis of hydroxycinnamic acid amides (HCAAs) of tyramine and other secondary metabolites, i.e. hydroxyphenylethanol glycoside-varbascoside in *Siringa vulgaris*, simple alkaloid phytoalexin-hordinine in *Hordeum vulgare* and benzylisoquinoline alkaloids in five plant families [1, 3]. On the other hand, an increase in activity of the enzyme and elevated level of tyramine, coumaroyltyramine (CT) and feruloyltyramine (FT) within tissues of transgenic tobacco plants, wounded by herbivorous insects, was found [4]. Goggin [5] and Groppa and Benavides [6] maintrained that mechanical damage of epidermis, mesophyll, and parenchyma cells by aphid stylets during probing may cause a plant response such as an increase of free amines and its HCAAs. The HCAAs are accumulated as signaling substances (i.e. jasmonate) that induce cascades of plant responses to pathogen and/or herbivore attacks [7]. The tyramine-HCAAs are polymerized within the cell wall by oxidative enzymes and create a chemical barrier against pathogens and herbivores [8].

Cereal tissues contain a number of the HCAAs, including derivatives of agmatine, putrescine, spermidine, spermine and tyramine [9, 10]. A highly diversified group of soluble HCAAs are avenanthramides that are accumulated within oat tissues in response to biotic stress [11]. According to Fixon-Owoo et al. [12], many plant HCAAs are structurally similar to neurotoxical acylpolyamines founded in venoms of spiders and wasps. Such biomolecules are highly selective and potent ligands for specific

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ionotropic receptors, particularly certain glutamate subtype receptors and nicotinic and acetylcholine receptors [13]. HCAA derivatives induced paralysis of numerous insects by binding to quisqualte-type glutamate receptors on exoskeletal muscles, and blocking synaptic transmission [14]. Such abilities suggest that plant-derived phenolic polyamines might serve as natural bioinsecticides.

Our previous studies showed that the TYDC activity was changed within triticale tissues in response to grain aphid feeding [15]. However, the problem needs further detailed investigations, thus the present paper focuses on the influence of the pest number and feeding duration on activity of the enzyme within triticale tissues.

Experimental Procedures

Plant Material

Two cultivars of winter triticale (*Triticosecale*, Wittm. ex A. Camus), Tornado and Witon, (varied in acceptability by the grain aphid) were used in the experiments. Seeds of both cultivars were obtained from the Plant Breeding and Acclimatization Institute (IHAR) in Strzelce near Łódź, Poland.

Aphids

Parthenogenetic individuals of *S. avenae* were reared on winter triticale seedlings (Lamberto cv) in an environmental chamber at 24°C at day and 18°C at night, 70% r.h. and photoperiod 16L:8D.

Field Experiments

Field experiments were carried out at the Agricultural Experimental Station in Zawady near Siedlce (east-central Poland). Abundance of the grain aphid on the triticale was estimated in random block arrangements in three replicates for both studied cultivars. The plots' area were 2 x 9 m, and distances between plots were 3 m.

The grain aphid density on the studied triticales was estimated, according to the method described earlier by Wratten et al. [16] and Lykouressis [17]. The observations were carried out from the aphid arrival on the cereals, until its disappearance (G.S.52 – 88; Tottman and Broad [18]), in one-week intervals. Technique of counting the aphids on 50 randomly selected plants, diagonally across the field was applied and population dynamics of *S. avenae* on the studied cultivars was performed.

Laboratory Experiments

Seeds of the studied cultivars were germinated in a climatic chamber at 24°C during the day and 18°C at night, 70% r. h. and photoperiod 16L:8D. Plants were grown in a medium nutrient fine structure of compost with sand, in 8.0 x 9.5 cm plastic pots, and regularly watered.

Population Tests

The adult apterous females were placed individually on the abaxial surfaces of seven-day-old seedlings of the triticale cultivars. Seedlings with aphids were isolated with Plexiglas cages with a cheese cloth cover (10 cm x 30 cm). After 24 h, one nymph remained on each single plant and other offsprings and the adult were removed. The experiment was run in 25 independent replicates for each studied triticale. The aphid's prereproductive period (time from birth until maturity of female) and daily fecundity were estimated. 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 [19]:

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

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

...where 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.

EPG Tests

Feeding behaviour of the grain aphid on the studied cultivars was monitored by an electrical penetration graphs (EPG) technique after Tjallingii [20]. Apterous adult females were connected to DC EPG amplifier (type Giga 4) by 2 cm gold wire, 20 μm in diameter and approximately 2-3 cm long, and attached to the aphids with silver conductive paint (Demetron L 2027, Darmstadt, Germany), and the second electrode was placed into the soil. Aphids were starved for 2 h before the recording and then were placed on leaves of the tested plants, and feeding behaviour of the insects was analyzed using STYLET 2.2 software. Duration of the following EPG patterns was determined:

Np – non-probing,
ABC – total pathway,
E1 – sieve element salivation,
E2 – ingestion of phloem sap,
G – xylem sap ingestion.

Experiments were run for 8 h for ten aphids on ten different plants, placed in Faraday's cage.

Influence of Grain Aphid Feeding on Tyrosine Decarboxylase Activity

The seven-day-old seedlings of the studied cultivars were artificially infested with five wingless females of *S. avenae* and isolated with Plexiglas cages with a cheese cloth cover. Control plants (without aphids) were similarly isolated. Infested and control seedlings were collected after 24 h, one week and two weeks from the beginning of the experiment. Aphid number was determined on five randomly selected shoots during the plant material collection.

Obtained results were calculated as an average aphid number per seedling and the studied seedlings were divided into aerial parts and roots and used immediately for the enzyme assay.

Tyrosine Decarboxylase Assay

Extraction and determination of the TYDC activity was conducted according to the method described by Phan et al. [21].

Fresh plant material (2 g) was homogenized in 10 cm^3 of 0.5 M acetate buffer pH 5.6, and obtained suspension was filtered through cheese cloth and centrifuged at 18,000 × g for 20 min at 5ºC. Supernatant was used for determination of the tyrosine decarboxylase activity.

0.5 cm^3 of the enzyme extract was mixed with 0.8 cm^3 of 8 mM tyrosine and 0.2 cm^3 of 0.1 mM of pyridoxal 5'-phosphate (PLP). Then the mixture was incubated at 30ºC for 30 min and enzymatic reaction was stopped by the addition of 1 cm^3 of 1 M potassium carbonate and 1 cm^3 of 10.2 mM trinitrobenzenesulfonic acid (TNBS). The obtained mixture was kept at 40ºC for 5 min, and 2 cm^3 of toluene was added and after mixing centrifuged at 2,000 × g for 5 min. Absorbance of the toluene layer was measured on a Hewlett Packard UV-VIS Spectrophotometer type 8453 at 340 nm. Tyramine content was quantified on the basis of standard curve (Sigma), and the TYDC activity was expressed in μM of tyramine generated during 1 h of the enzymatic reaction per 1 mg of protein. Protein quantity within the enzymatic extracts was measured according to Lowry et al. [22]. All chemical analyses were done in three independent replications.

Statistics

Dynamics of the grain aphid population on the triticale cultivars were analyzed with Tukey's test. The effect of the cultivars on *S. avenae* population density, feeding behaviour and performance as well as differences in the TYDC activity within plant tissues were subjected to Mann's and Whitney's U-test.

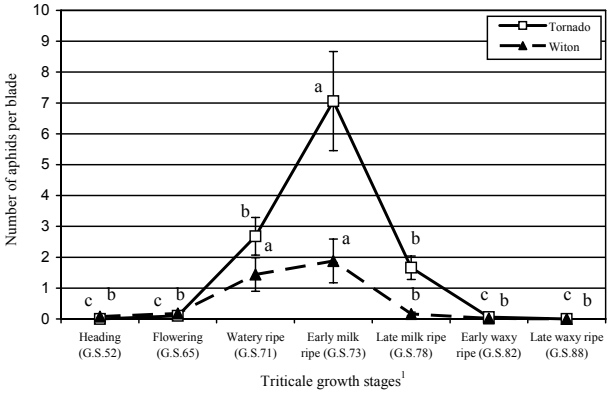

Fig. 1. Population dynamics of the grain aphid on the studied winter triticale cultivars:
[1] Developmental stages of triticale according to Tottman and Broad [18] scale;
Values signed by different letters are statistically different at P≤0.05 (Tukey's test).

Table 1. Acceptability of the studied winter triticale cultivars by the grain aphid.

Parameters	Cultivar		U_3	P
	Tornado $\bar{x} \pm SE$	Witon $\bar{x} \pm SE$		
An average in vegetation season				
Number of individuals/blade	1.65 ± 0.16	0.54 ± 0.08	0.00	0.05
Percentage of infested plants	22.29 ± 2.40	15.10 ± 2.07	1.00	0.126
Peak density				
Number of individuals/blade	7.06 ± 0.29	1.88 ± 0.29	0.00	0.05
Percentage of infested plants	72.29 ± 6.93	48.00 ± 4.629	0.00	0.05

Mann-Whitney's U-test; comparing of density grain aphid population on studied winter triticale cultivars.

Results

Abundance of the Grain Aphid on the Studied Triticale Cultivars

The first appearance of the grain aphid on the tested cultivars occurred during triticale heading (G.S. 52; [18]) (Fig. 1). The number of the aphids increased later on, and peak density of *S. avenae* population was achieved at early milk ripe stage (G.S. 73). The aphid population was strongly reduced during late milk ripe stage (G.S. 78) and at early waxy ripe stage (G.S. 82), and finally disappeared at late waxy ripe stage (G.S.88).

A higher level of the grain aphid population was observed on Tornado cv at peak density and during the all vegetation season as well (Table 1). A percentage of the infested plants were significantly higher on more accepted Tornado cv during most of the growing stages and especially at population peak density.

Grain Aphid Performance on Seedlings of Studied Triticale Cultivars

Obtained results showed that individuals of *S. avenae* occurring on Witon cv were characterized by significantly lower values of daily fecundity and intrinsic rate of natural increase (r_m) than aphids from Tornado cv (Table 2). Moreover, aphids settled seedlings of Witon cv proved to be a longer prereproductive period and mean time of population development (*T*). Differences in duration of prereproductive and population development period were not significant (Table 2).

Table 2. Values of the grain aphid population parameters on the studied winter triticale cultivars.

Cultivar	Population parameters			
	Prereproductive period (days) $\bar{x} \pm SE$	Daily fecundity per female $\bar{x} \pm SE$	Mean time of generation development (T) (days) $\bar{x} \pm SE$	Intrinsic rate of natural increase (r_m) $\bar{x} \pm SE$
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
U_{25}	280.5	148.0	276.5	4. 0
P	0.5352	0.0005	0.484	$0.0005 \cdot 10^{-3}$

Mann-Whitney's U-test; comparing population parameters for grain aphid on the studied winter triticale cultivars.

Grain Aphid Feeding Behavior on the Studied Triticale Cultivars

EPG recordings indicated that the grain aphid individuals that fed on seedlings of Witon cv showed a longer duration of non-probing (Np activity) and penetration of peripheral tissues (ABC) in comparison to those fed on Tornado one (Table 3). Such insects also spent more time on salivation into sieve elements (E1) and on ingestion of the xylem sap (G). Moreover, aphids on less accepted Witon cv were characterized by significantly shorter total penetration time (ABC + E1 + E2 + G) and shorter duration of phloem sap ingestion and total phloem phase (E_2, $E_1 + E_2$) (Table 3).

Influence of Grain Aphid Feeding on Activity of Tyrosine Decarboxylase within Tissues of the Triticale

S. avenae feeding-induced activity of the TYDC within the aerial parts of the Tornado cv seedlings and roots after 24 h and after two weeks and decreased its activity after one week (Fig. 2). Such fluctuations in the TYDC activity within less accepted seedlings of Witon cv were not observed, since clear induction of the activity was proved. In addition, a clear systemic effect in changes of the enzyme activity within root tissues of the studied triticale cultivars was observed as well (Fig. 2).

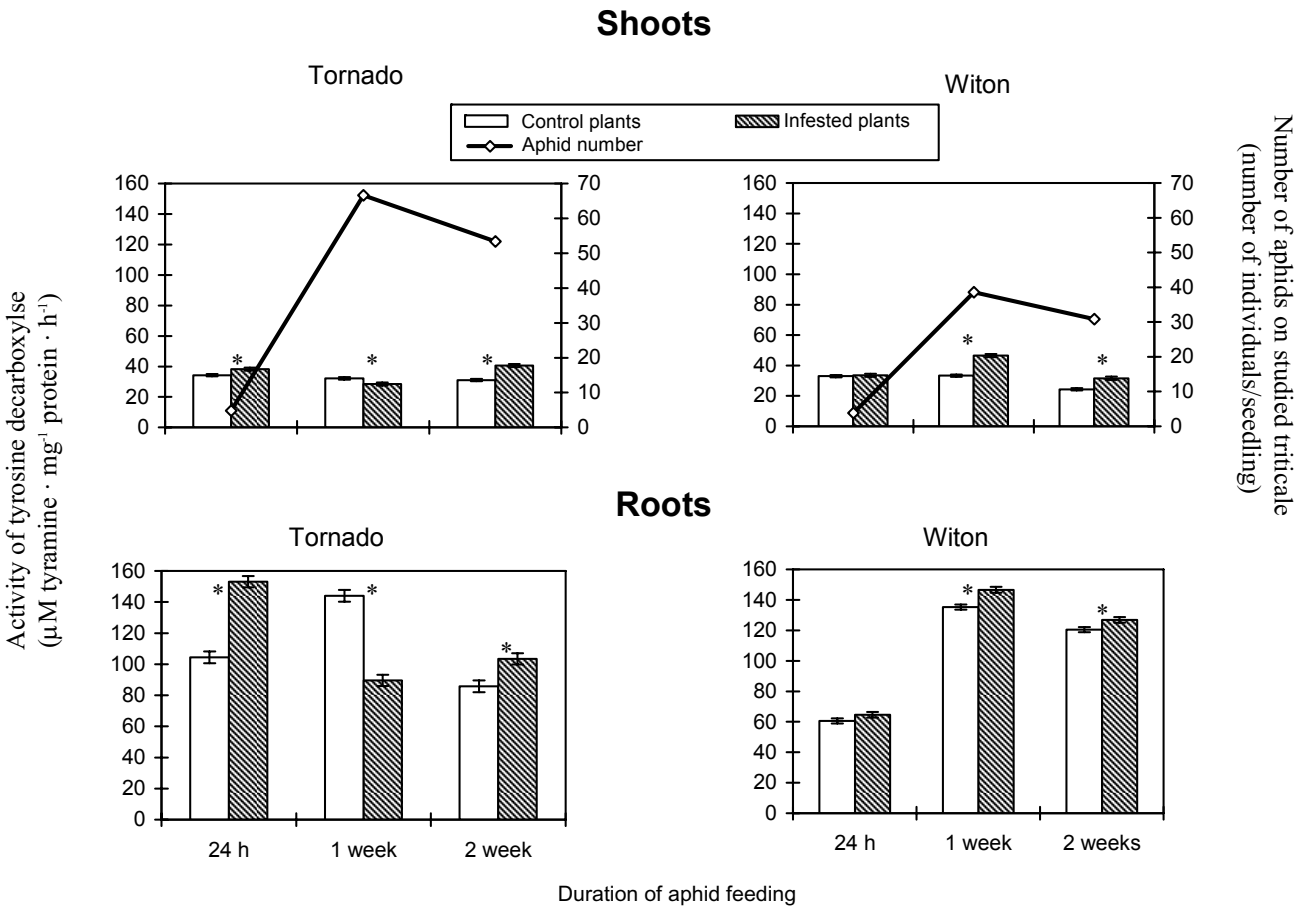

Fig. 2. Influence of the grain aphid feeding on activity of the tyrosine decarboxylase within tissues of the studied triticale cultivars: * differences between control and infested plants significant at P≤0.05 (Mann-Whitney's U-test).

Table 3. Grain aphid feeding behaviour on the studied winter triticale cultivars during 10 h of EPG recordings.

EPG events		Cultivar		U_{10}	P
		Tornado $\bar{x} \pm SE$	Witon $\bar{x} \pm SE$		
Np	Average time (min)	3.80 ± 1.37	6.77 ± 1.47	20.0	0.0075
	Number	5.90 ± 1.21	7.60 ± 1.49	39.5	0.4296
ABC	Average time (min)	10.79 ± 3.04	11.02 ± 1.72	41.0	0.4964
	Number	7.30 ± 1.24	8.70 ± 1.45	39.0	0.4066
E1	Average time (min)	4.28 ± 1.17	6.59 ± 2.16	36.5	0.3078
	Number	2.10 ± 0.41	1.30 ± 0.26	31.0	0.1498
E2	Average time (min)	83.38 ± 14.79	59.74 ± 8.20	24.0	0.0484
	Number	2.00 ± 0.37	1.20 ± 0.25	35.0	0.2564
G	Average time (min)	13.14 ± 5.80	22.30 ± 11.88	41.0	0.4964
	Number	0.70 ± 0.26	0.80 ± 0.33	53.0	0.7040
E1+E2	Average time (min)	87.56 ± 15.57	66.34 ± 7.96	23.0	0.0414
ABC+E1+ E2+G	Average time (min)	115.79 ± 15.57	99.66 ± 12.60	24.0	0.0484

Mann-Whitney's U-test; comparing the number and average time of EPG activities for grain aphid on the studied winter triticale cultivars.
Np – non-probing, ABC – total pathways, E1 – salivation into sieve elements, E2 – phloem sap ingestion, G – xylem sap ingestion, E1 + E2 – duration of total phloem activity, ABC + E1 + E2 + G – duration of total activity within plant tissues.

Discussion

Obtained results showed that tested cultivars differed in acceptability to the grain aphid. The aphid number and percentage of the infested plants on Witon cv, especially during population peak density, suggested that this cultivar was less accepted by *S. avenae* than Tornado one. Laboratory tests confirmed the field observations, because *S. avenae* individuals fed on Witon seedlings were characterized by lower values of daily fecundity and intrinsic rate of natural increase (r_m) than the individuals settled in Tornado cv. Obtained results are in accordance with an earlier data by Ciepiela et al. [23], who claimed that the antibiotic effect of host plants towards the grain aphid is realized as reduction of their growth, development and fecundity. Differences in acceptability of the triticale by *S. avenae* were also connected with various non-probing periods, sieve element activity and total penetration of plant tissues. On the other hand, aphid feeding behaviour was strictly related to chemical composition of their host plants [24, 25].

Under the grain aphid feeding, activity of the TYDC within Tornado tissues increasing at the initial period of infestation, decreasing after one week and again was induced after two weeks. Such fluctuations suggest reaction on biotic stress of this cultivar, dependent on duration of feeding and aphid numbers. Such a phenomenon was not observed for Witon cv, where constant induction of activity of the enzyme occurred. In addition, differences in TYDC activity within the studied triticale had a systemic character, since the enzyme activity varied not only in aerial parts of the seedlings directly attacked by the pest, but also showed a similar picture within their root tissues. Thus we hypothesize that TYDC activity changed as a result of the wounding effect caused by the aphid stylets while puncturing peripheral tissues and could be a part of induced defence mechanisms dependent on jasmonates and/or other signalling compounds proven earlier [5, 7]. It could be related with lower acceptability of Witon cv by the grain aphid, since the higher activity of the enzyme is responsible for the increase of tyramine levels and its HCAAs derivatives. Moreover, it is well known that aphid feeding behaviour, growth, development and fecundity is strictly related to the content of plant allomones that belong to various classes of secondary plant metabolites [5, 26] and/or primary metabolites such as amino acids [27, 28]. Thus an increase in the TYDC activity may be connected with the reduction of nutritive value of triticale tissues for *S. avenae*. According to Abbot et al. [29], changes in nutritive value of host plants are important factors of their lower acceptance by herbivorous insects. Thus higher and faster induction of the enzyme activity by *S. avenae* within Witon tissues might cause a higher level of antibiotic response of this cultivar to the grain aphid. However, further studies are needed to focus on the role of tyramine and its HCAA derivatives in cereal responses to the grain aphid attack.

Conclusions

In conclusion, we can state that faster and more intense conversion of tyrosine to tyramine and/or its derivatives within seedlings of triticale cv is less accepted by aphids and might be part of an induced defensive mechanism toward *S. avenae.* Changes in TYDC activity within the studied triticale caused by the aphids had a systemic character.

Abbreviations

TYDC – tyrosine decarboxylase;
HCAAs– hydroxycinnamic acid amides;
DOPA – 3,4-dihydroxyphenylalanine;
CT – coumaroyltyramine;
FT – feruloyltyramine;
EPG – electrical penetration graphs;
PLP – pyridoxal 5'- phosphate;
TNBS – trinitrobenzenesulfonic acid

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