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Effect of transgenic triticale on grain aphid (*Sitobion avenae* (Fabricius, 1775)) /Hemiptera, Aphidoidea/

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Introduction

The largest single components of the world's economy is its aggregate agricultural output. Environmental stress, pests and pathogens provide a continuous and evolving challenge to agriculture. These factors are the primary elements limiting crop yield and quality. For this reason, there has always been a robust effort in cultural and breeding practices to combat these stresses. More recently, we have begun to understand and exploit the biochemical and genetic basis of plant resistance to and avoidance of these factors (Hein 1998).

Today, plant biotechnology is being used as a tool to give plants new traits that benefit agricultural production, the environment, human nutrition and health. The modification of metabolic pathways, the improvement of plant tolerance to biotic and abiotic stress and the engineering of herbicide resistance into crop plants have been important goals of plant biotechnology in recent years (Mouradov, 2006).

There is a strong tendency to increase cultivation of transgenic plants that offer higher crops of better quality and reduce pathogens and insect pests. However, the introduction of such cultivars to agriculture require a number of tests, including their effect on biology of major pests. The improvement of pest resistance in crop plants by genetic engineering requires knowledge of

more precise details of herbivore feeding behavior. A clear understanding of herbivorous insect-cereal relationships also requires fairly detailed knowledge of plant structure and chemicals involved in the resistance.

In the present paper we report on effect of transgenic triticale on growth, development and feeding behavior of the grain aphid, *Sitobion avenae* (Fabricius, 1775).

Material and methods

Plant material

Experiments were carried out on the previously selected transgenic triticale (MS x 325) and regular cultivar (Bogo) obtained from Institute of Plant Breeding and Acclimation at Radzików/Błonie near Warsaw (Poland). Seeds of the studied triticale were germinated in a climatic chamber, kept at 21°C ± 1°C under 16h daylight and 8h of darkness, and 70 humidity. The seedlings were grown in a medium nutrient fine structure compost with sand, in 7 cm 7 cm 9 cm plastic pots, one plant per pot. The plants were regularly watered and no extra fertilizer was added.

Aphids

The grain aphid (*S. avenae*) came from a stock culture kept at the University of Podlasie at Siedlce. A parthenogenetic clones of *S. avenae* were reared on winter wheat seedlings (cv. 'Tonacja') in an environmental chamber (21°C ± 1°C, 70 relative humidity, 16h:8h L:D photoperiod) and transferred to winter triticale for one generation. Adults *apterae* of the grain aphid were used in the experiments.

Field observations

The field observations were performed at IHAR Radzików experimental plots. The grain aphid abundance on the studied triticale was evaluated according to Wratten *et al.* (1979) and Lykouressis (1984). Observations were carried out from the beginning of the triticale colonization by the grain aphid until the cereal maturity (G.S. 47-80; Tottman, Broad scale, 1987). The number of aphids was counted weekly on 50 plants chosen at random. The all observations were done in three independent replicates, for each triticale. The results of the observations were used to calculate, the cumulative aphid idex/stem and an average percentage of infested stems, of the studied triticale.

Entomological tests

The population tests were conducted in an environmental chamber, at 16h:8h (light: :dark) photoperiod, temperature 21 ± 1°C, 70 relative humidity, in plexiglass cages with a cheese cloth cover. The adult apterous females were caged individually on 5 days seedlings and allowed to deposit nymphs. After 24h, only one nymph remained on each single plant, other offspring and the adult were removed. Development time (from birth until maturity) and reproduction of the individual apterous females were observed daily until death. Pre-reproductive period (time from birth until maturity), total and daily fecundity, intrinsic rate of natural increase (r_m), net reproduction (R_o), multiplication of the population growth (λ) and an average generation development time (T) were determined (Leszczyński, 1996). The experiments were run in 10 independent replicates for each hybride of the studied winter triticale.

EPG recordings

The aphid feeding behavior on the studied triticale was studied with the help of electrical penetration graphs (EPG) method according to Tjallingii (1990). Apterous adults were connected to 2 cm gold electrode, (20 μm wire in diameter), using conductive silver paint (Demetron, L2027, Darmstadt, Germany). The second electrode was inserted into the pot containing transgenic triticale plants. The EPG recordings were performed for 8h in ten independent replicates (ten different adult *apterous* females on ten different plants paced in the Faraday cage). The analysis of the EPG was performed using the computer program Stylet (DOS PCs).

Statistics

Differences in the aphid growth and development and feeding behavior in the conducted experiments were subjected to one-way ANOVA, followed by Duncan's test.

Results

The obtained results suggest that the transgenic triticale (MSx325) was more resistant to the grain aphid than the regular one. Field observations showed that the transgenic plants were less accepted by the grain aphid in comparison to plants of the 'Bogo' cultivar. The transgenic triticale also reduced the aphid number in the field. *S. avenae* formed a smaller colonies and developed worse on the transgenic hybrid of the triticale. As a consequence,

a constantly lower value of the cumulative aphid index and lower percentage of infested stems of the transgenic triticale were found (Tab. 1).

The population tests showed that transgenic triticale prolonged the grain aphid maturity, decreased fecundity and reduced values of the intrinsic rate of population increase (r_m), net reproduction (R_O) and multiplication rate of population increase (λ). In addition, T value (mean time of generation development) was prolonged (Tab. 2 and 3).

The transgenic triticale also changed the grain aphid feeding behavior. Adult *apterae* of *S. avenae* which fed on the hybrid, showed an increase in the number of short probes into the peripheral tissues and lower ingestion of the phloem sap in comparison to regular triticale (Tab. 4). EPG recordings also showed that transgenic triticale increased the duration of non-penetration and probing into peripheral tissues. Moreover, such triticale hybrid reduced ingestion of phloem sap by the grain aphid (Tab. 4).

Table 1. Occurrence of the grain aphid on the studied triticale in the field

Cultivar/Hybrid	Cumulative aphid index	Percentage of infested stems
BOGO	10.33 a	23.89 a
MS x 325	3.05 b	8.89 b

Values in columns followed by various letters are significantly different at $p \leq 0{,}01$ (Duncan's test).

Table 2. Comparison of the grain aphid development and fecundity on the studied triticale

Cultivar/Hybrid	Pre-reproductive period (days)	Daily fecundity (per female)	Total fecundity (per female)
BOGO	6.6 a	2.2 a	46.2 a
MS x 325	7.5 b	1.1 b	23.5 b

Values in columns followed by various letters are significantly different at $p \leq 0{,}01$ (Duncan's test).

Table 3. The grain aphid performance on the triticale in laboratory conditions

Cultivar/Hybrid	r_m	λ	R_o	T
BOGO	0.40	1.45	26.4	8.10
MS x 325	0.22	1.25	8.20	9.30

Values in columns followed by various letters are significantly different at $p \leq 0{,}01$ (Duncan's test).

Table 4. The grain aphid feeding behavior on the studied triticale

Cultivar/Hybrid	Number of peripheral tissues probing	Time to the firs phloem sap ingestion (s)	Duration of the phloem sap ingestion (s)
BOGO	8.4 b	2156 b	12546 a
MS x 325	10.2 a	5823 a	9862 b

Values in columns followed by various letters are significantly different at $p \leq 0{,}01$ (Duncan's test).

Discussion

Cereal aphids are major agricultural pests because of their unparalleled reproductive capacity and their ability to manipulate host plant physiology (Goggin, 2007). *S. avenae* is an important pest of cereals worldwide (Sanderson & Mulholland, 1969; George, 1974; Kolbe & Linke, 1974; Wratten, 1975; George & Gair, 1979; Vickerman & Wratten, 1979; Carter *et al.*, 1989; Legrand *et al.*, 2004; Cardoso *et al.*, 2006). The integrated pest management of the cereals has been implemented to maintain environmental quality and reduce unnecessary use of pesticides since the *S. avenae* cause an economic injury. They are based on cereal resistance to aphids, however, high level of antixenosis and/or antibiosis mechanisms are extreme rare in nature. Thus the transgenic plants fill out the gap in the integrated pest management of cereal control (Vickerman & Wratten, 1979; Higley & Pedigo, 1993; Leszczyński, 1999; Larsson, 2005).

Plant defense might be of different origin but is always due to certain plant characters, limiting the insect pest colonization (Strong *et al.*, 1984; Southwood, 1986; Leszczyński, 1999). Morphologically specialized structures such as trichomes, spines, awns, thickness and lignification of cell walls and waxes located in plant surface may serve as physical barriers to prevent herbivore feeding: an array of chemicals plants produce may deter herbivores or retard their growth, and/or compounds (volatile signals) that attract herbivore predators (Niraz *et al.*, 1982; Mittler, 1988; Wagner, 1991; Eigenbrode & Espelie, 1995; Juniper, 1995; De Moraes *et al.*, 2001; Kessler & Baldwin, 2002; Huang *et al.*, 2003; Wagner *et al.*, 2004; Wei *et al.*, 2007).

Secondary metabolites had a marked insecticidal and ecological effect (Kelsay *et al.*, 1984; Barenbaum, 1995; Wei *et al.*, 2007). Many of the secondary compounds including phenolics, especially o-dihydroxyphenols, glucosinolates, alkaloids, cyanogenic glycosides, furanocumarins are known as protection agents towards various species of aphids. They seriously affect aphid behavior, physiology and metabolism and as a result reduce aphid populations on resistant plants (Lowe, 1981; Beck *et al.*, 1983; Leszczyński *et al.*, 1985; Leszczyński

et al., 1989; BENNETT & WALLSGROVE, 1994). Among these compounds are which exhibit a wide range of biological activities, including neurotoxicity, cytotoxicity, and antimitotic (LIN & WAGNER, 1994; WHITE *et al.*, 1998; LAUE *et al.*, 2000). WANG *et al.* (2001) reported that increased levels of membrane derivatives produced in tobacco trichome glands can significantly enhance plant resistance to phloem-feeding aphid. WITTSTOCK & GERSHENZEN (2002) found that these toxins accumulated in glandular trichomes are released in large amounts as soon as these structures are raptured by herbivore feeding, the movement on the plant surface or the growth of pathogens.

The results presented in this paper showed that transgenic manipulations caused a significant increase in the resistance of the regular triticale to the grain aphid. It considered not only the stage of the host plant colonization (antixenosis mechanism) but also feeding behavior and growth and development of the grain aphid (antibiosis mechanism). Similar results were obtained by WEI *et al.* (2007) who showed that the density of secretory glandular trichomes was significantly greater in transgenic than in wild-type plants and the mortality of adult whiteflies fed transgenic tobacco plants was significantly higher than those reared on control plants). WEI *et al.*, (2007) showed that transgenic *Nicotiana tabacum* plants, compared with wild-type controls did not differ significantly in seed germination, plant growth rate, plant height, or flowering time. However, in transgenic plants, seed germination and the beginning of flowering were significantly delayed, and the leaf area and plant fresh weight were significantly reduced. Transgenic plants had a marked insecticidal effect on whiteflies (*Bemisia tabaci*) and on *Diptera* spp. flies. Scanning electron microscopy revealed that dead and shrunk whiteflies on transgenic leaf surface were found physically associated with the veins where the trichomes were densely located.

The transgenic triticale clearly affected bionomy and feeding behavior of the grain aphid, thus genetically modified hybrids might be useful in control of this cereal pest.

Thus the transgenic triticale seems to be pretty effective in controlling the grain aphid population. However, further study needed to explain the detailed mechanism of the resistance and to exclude any harmful effect of such cereals to man, animals and environment.

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Wpływ transgenicznego pszenżyta na mszycę zbożową (*Sitobion avenae* (Fabricius, 1775)) /Hemiptera, Aphidoidea/

Streszczenie

Na podstawie przeprowadzonych badań stwierdzono, że rośliny pszenżyta ozimego MS x 325, był w znacznie mniejszym stopniu akceptowane przez mszycę zbożową. Wyrażało się to w postaci mniejszej liczebności populacji i niższym procencie porażonych roślin, w stosunku do klasycznego pszenżyta odmiana – Bogo. Wyniki uzyskane podczas obserwacji polowych potwierdziły testy laboratoryjne, podczas których obserwowano duże różnice w tempie rozwoju populacji szkodnika. Ponadto, na roślinach pszenżyta transgenicznego *Sitobion avenae* pobierała mniejsze ilości soku floemowego, wykonując krótsze, liczniejsze nakłucia.

Rezultaty przeprowadzonych badań sugerują, że nowoczesna hodowla roślin połączona z najnowszymi osiągnięciami biotechnologii pozwoli uzyskać hybrydy znacznie skuteczniej chroniące zboża przed mszycami.