JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 42, No. 1 (2002)

CHANGES IN CONTENT AND AMINO ACID COMPOSITION OF SOLUBLE PROTEIN IN WINTER TRITICALE CULTIVARS CAUSED BY GRAIN APHID FEEDING

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Accepted: Decembre 28, 2001

Abstract: The obtained results showed that grain aphid (*Sitobion avenae* Fabr.) feeding caused an increase of the total protein content and a decrease of soluble protein level in the ears of both studied winter triticale cultivars (i.e. susceptible cultivar Grado and relatively resistant – Lasko). Moreover, the content of amino acids in soluble protein increased in Grado plants infested with the aphid, and reduced – in Lasko.

Key words: cereal resistance, *Sitobion avenae*, total protein, soluble protein, protein amino acids

INTRODUCTION

The protein content in plants amounts from 4 to 21% what not always satisfies aphids' food requirements, thereby limiting their development. These substances' concentration in the feeder plants' tissues presents one of the biochemical indicator of their nutritive value for *Lipaphis erysimi* (Kalt.), *Aphis craccivora* (Koch.) and *S. avenae* (Malik 1981; Macfoy and Dąbrowski 1984; Ciepiela 1990). Moreover, in House's (1974) opinion in spite of the fact of not high content of some essential amino acids (e.g. methionine and tryptophan) the plant proteins are their basic source for insects. Therefore the differences in the amino acid composition of protein can be the important determinant of winter triticale resistance to the grain aphid.

So far research point to the fact that *S.avenae* inserts to the attacked feeders tissues the proteolitic enzymes (Urbańska and Niraz 1990) along with the saliva and affects the activity of proteases of plant origin (Ciepiela et al. 1995; 1998a). But there are no studies, which combine these interactions with different fraction of the plant protein content. Moreover, in the subject literature there is no data of how

this aphid species feeding influences on the composition of the amino acid soluble protein isolated from cereal plants in the initial period of the pest feeding (24, 48, 96 and 168 hours). Finding out these relationships is particularly interesting especially in case of winter triticale, which participation in the cereal cultivation structure on the territory of the Poland successively rises.

This paper presents the attempt to estimate the relationship between the grain aphid feeding on the selected cultivars of winter triticale and the total and soluble protein content as well as the amino acid composition of soluble protein, which was obtained from the ears of analyzed cultivars.

MATERIAL AND METHODS

The influence of the grain aphid feeding on the content of the total and soluble protein as well as on the amino acid composition of the soluble protein of winter triticale was tested in the field conditions within three vegetation seasons (1997–2000). The artificially infested and the control ears of two winter triticale cultivars with different resistance to *S. avenae*: Grado – susceptible cultivar and Lasko – relatively resistant – presented the plant materials. The changes in the content of analyzed protein fraction and in the amino acid composition of the soluble protein of the triticale were analyzed after 24, 48, 96 and 168 hours from the moment of its infestation by 5 wingless females of the grain aphid. Entomological experiment was conducted in the period when studied winter triticale cultivars were at the early milk stage (G.S.72 in Tottman and Broad (1987) scale). Number of aphids on isolated ears was controlled and maintained at the constant level of 5 individuals/ear during experiment.

The content of the total and soluble protein was estimated on the basis of the total and protein nitrogen concentration using the method, which was described by Ciepiela (1989).

The soluble protein was isolated from freeze-dried ears of analyzed triticale cultivars by means of 0.2 M NaOH according to the method provided by Ciepiela (1991). Acidic hydrolysis of protein was conducted by 6 M HCl at the temperature of 105–110°C during 20 hours. After hydrolysis the content of flasks was evaporated to dry on the vacuum rotatory evaporator and then the mixture of the protein amino acids was dissolved in 0.2 M citrate buffer pH 2.2 and was analyzed on the automatic amino acids analyzer of T-339 type, plotting each time by 100 mm^3 per column.

The presented results are arithmetical averages calculated on the basis of three years' research. The changes in the content of analyzed protein fractions (the total and soluble protein) and protein amino acids caused by the grain aphid feeding in the ears of tested winter triticale cultivars were expressed as percent (%) in relation to control.

RESULTS AND DISSCUSION

The obtained results showed that 24 hours feeding of the wingless grain aphid females in ears of tested winter triticale cultivars have caused the decrease of the total protein content (Fig. 1). However, in the next considered times (48, 96 and 168

hours) in the ears of Lasko cultivar infested with pest the increase of this protein fraction was recorded. Similar results were obtained for Grado cultivar. There was the increase of total protein amount after 48 and 168 hours and the decline after 96 hours. On average in the time of the experiment duration, *S. avenae* feeding induced the increase of the total protein content in the ears of tested triticale cultivars.

Moreover, in the ears of Grado cultivar infested with the grain aphid the increase of the soluble protein content was taken up both in all considered periods (24, 48, 96 and 168 hours) and on average during carried out experiment (Fig. 2). However in case of Lasko cultivar the distinct decrease of this protein fraction concentration after 24 hours and not high after 96 hours of the pest feeding as well as its increase after 48 and 168 hours was observed. On average *S. avenae* feeding on the ears of Lasko triticale caused the decrease of the soluble protein level.

Presented results only partly confirm the results of the prior research of Ciepiela and Niraz (1987; 1988) on basis of which it was affirmed that the grain aphid feeding in the ears of both susceptible and resistant cereal and winter wheat cultivars causes considerable drop of the soluble protein content. Also in Sempruch and Ciepiela's (1998) opinion the result of *S. avenae* feeding on the ears of winter wheat is the drop of the total protein content regardless of the extent of its cultivar resistance. Moreover, the authors observed among cultivars the differences in the soluble protein quantity, which content increased in the infested with pest ears of relatively resistant Saga cultivar and decreased in the susceptible Liwilla cultivar. The decrease of the soluble protein concentration in the ears of Lasko cultivar can be the result of its partial decomposition on the amino acids by the proteolitic en-

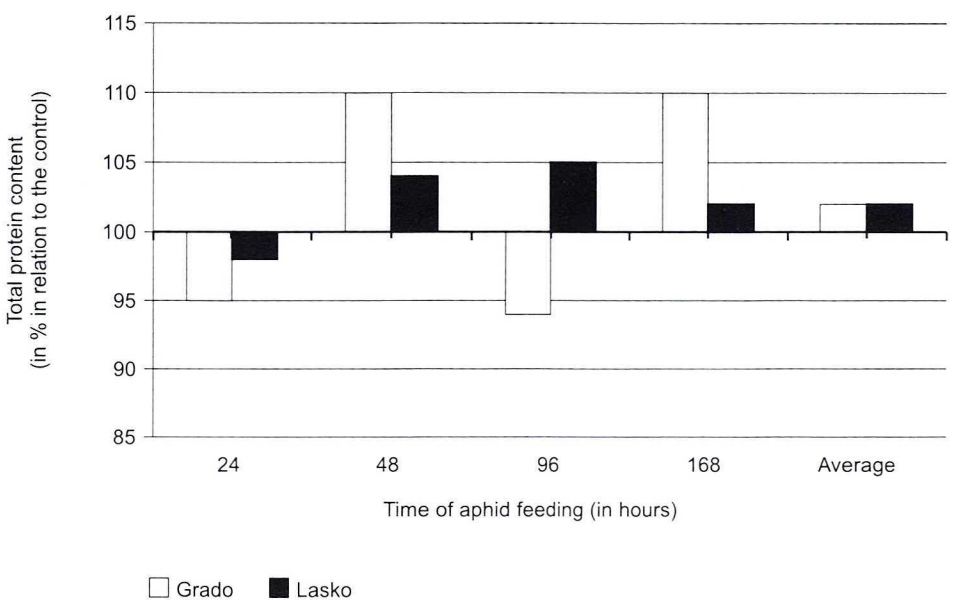

Fig. 1. Changes in the total protein content in the ears of studied winter triticale cultivars caused by the grain aphid feeding

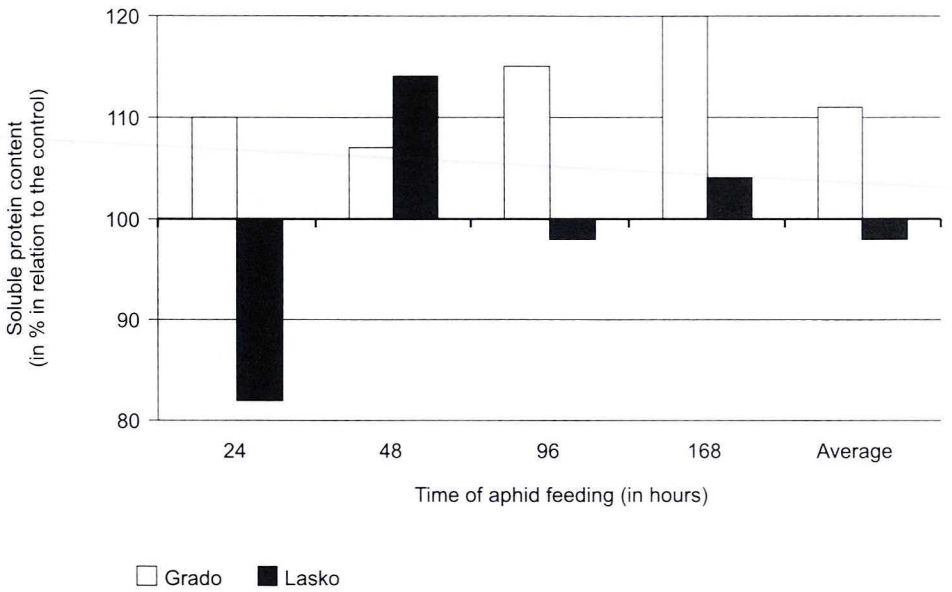

Fig. 2. Changes in the soluble protein content in the ears of studied winter triticale cultivars caused by the grain aphid feeding

zymes secreted by aphids with saliva to the host tissues (Boczek 1977). However, the increase of the amount of this protein fraction in Grado triticale as well as the total protein in both tested triticale cultivars seems to indicate the existence of the defensive mechanisms in those plants. These mechanisms' activities apply a displacement of balance in the protein metabolism in the direction of its synthesis or an inhibition of its decomposition by raising the proteolitic enzyme inhibitors' concentration (Ciepiela 1984). The presented results and quoted literature allow to suppose that the direction of changes in the content of analyzed protein fractions caused by *S. avenae* feeding is dependent both on the species and the cultivar. The time of aphid feeding as well as the developmental phase of winter triticale is of great importance for the considered problem.

The carried out research proved also that after 24 hours of the grain aphid feeding in susceptible Grado cultivar ears there was recorded the increase of majority analyzed amino acids content except of threonine, valine, isoleucine and arginine. Their content decreased in comparison to plants without aphids. Moreover, glycine and histidine concentration in this protein remained on the level of the control plants (Tab. 1). Similar increase of protein amino acids content was shown after 48 hours of the pest feeding on this cultivar. But then the decrease of asparatic acid, threonine and glycine content was also recorded as well as no changes in alanine and tyrosine concentration with relation to the control plants. However, *S. avenae* feeding in the ears of Grado cultivar over 96 hours caused the increase of threonine and methionine content as well as the decrease of the other protein amino acids (exception histidine). After 168 hours the lower content of phenylalanine and histidine and the increase of amount of the other investigated amino acids in the

Table 1. Changes in the amino acids content (in % in relation to the control) in the ears of winter triticale cultivar Grado caused by the grain aphid feeding

Amino acid	Time of aphid feeding (in hours)				Mean
	24	48	96	168	
Asparatic acid	130±2a	89±1ab	95±2abcd	110±6ab	106±2ab
Threonine*	94±4bc	98±5ab	102±1a	109±12ab	101±3ab
Serine	108±4abc	102±4ab	90±5cde	122±5a	105±2ab
Glutamic acid	109±2abc	105±5ab	84±4e	108±3ab	102±2ab
Proline	112±6abc	112±5a	94±1bcde	107±4ab	106±3ab
Glycine	100±1abc	99±6ab	92±3bcde	117±6ab	102±2ab
Alanine	110±1abc	100±7ab	92±3bcde	109±7ab	103±7ab
Valine*	92±2bc	110±3a	98±2abc	112±6ab	103±7ab
Methionine*	114±2ab	107±6ab	102±4a	113±4ab	109±2a
Isoleucine*	93±8bc	107±2ab	97±2abcd	108±3ab	101±3ab
Leucine*	101±3abc	110±4a	94±2abcd	108±6ab	103±3ab
Tyrosine	117±5a	100±1ab	88±3de	101±2ab	102±1ab
Phenylalanine*	112±1abc	111±10a	91±2cde	98±9ab	103±3ab
Histidyne*	100±3abc	102±3ab	100±1ab	92±14b	99±2b
Lysine*	105±5abc	104±1ab	98±1abc	104±1ab	103±1ab
Arginine*	91±7c	104±5ab	94±3abcd	102±1ab	98±3b

*Essential amino acids

Values in the same columns followed by different letters are significantly different at P≤0.05 (Duncan's test)

soluble protein composition extracted from the ears of susceptible triticale attacked by aphids was revealed. On average the grain aphid feeding caused a decline of histidine and arginine content in the soluble protein of Grado cultivar, with simultaneous growth of level of the other tested amino acids in comparison to control. Moreover, it was proved that in the protein obtained from the ears of Grado cultivar infested with *S. avenae* there was an increase of sum of essential and nonessential amino acids as well as their total content after 24, 48 and 168 hours and on average in considered period (Fig. 3). Exceptionally, 96 hours of pest feeding on Grado cultivar induced the reduction of all analyzed groups of amino acids in the soluble protein.

However, *S. avenae* feeding in the ears of relatively resistant Lasko cultivar caused decisive decrease of content of overwhelming majority of amino acids in the soluble protein as well as their groups both in each of tested periods and on average in the time of carried out experiment (Tab. 2, Fig. 4). Only in case of methionine (24 hours) and lysine (96 hours) the increase of concentration in the composition of soluble protein isolated from the ears of Lasko cultivar infested by aphids was taken down.

Presented results are similar to the results of prior experiments, which have showed that the grain aphid feeding in the ears of rye and winter wheat does not cause the qualitative changes in the amino acid soluble protein composition (Ciepiela and Niraz 1987; 1988). However, it was revealed that in the plants of susceptible and resistant cultivars of both species attacked by this species of aphid there is observed the drop of protein amino acids content, especially essential such as methionine and histidine ensues. Though Sempruch and Ciepiela (1998b)

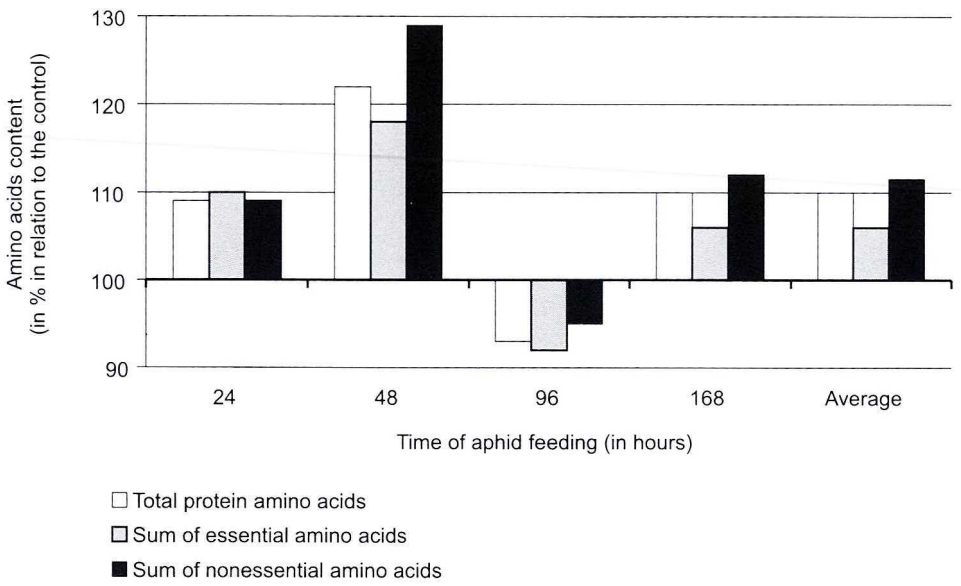

Fig. 3. Changes in the amino acids content in the ears of winter triticale cultivar Grado caused by the grain aphid feeding

proved that as a result of *S. avenae* feeding in the ears of Liwilla susceptible winter wheat and relatively resistant – Saga, the content of amino acids in the soluble protein obtained from these cultivars increase. Moreover, in Ciepiela et al. (1998b) opinion the grain aphid feeding contributes to the increase of nutritive value of protein of the ears of susceptible triticale Grado cultivar and the decrease in partly re-

Table 2. Changes in the amino acids content (in % in relation to the control) in the ears of winter triticale cultivar Lasko caused by the grain aphid feeding

Amino acid	Time of aphid feeding (in hours)				Mean
	24	48	96	168	
Asparatic acid	89±3ab	86±2a	92±4ab	75±7a	86±1a
Threonine*	88±3ab	81±6a	90±3ab	75±7a	84±2a
Serine	89±4ab	87±2a	93±5ab	75±1a	86±3a
Glutamic acid	91±2ab	89±2a	93±2ab	84±3a	89±1a
Proline	99±1a	78±9a	89±1ab	74±1a	85±7a
Glycine	98±2ab	85±7a	95±1ab	76±8a	87±3a
Alanine	94±2ab	79±7a	90±7ab	79±9a	85±5a
Valine*	94±5ab	88±6a	93±3ab	81±7a	89±5a
Methionine*	111±6a	85±1a	83±6b	88±8a	92±6a
Isoleucine*	93±1ab	84±5a	85±6b	73±7a	84±5a
Leucine*	91±6ab	83±6a	92±3ab	78±1a	86±5a
Tyrosine	89±3ab	94±1a	93±3ab	79±9a	89±5a
Phenylalanine*	91±2ab	90±4a	88±2ab	95±1a	91±4a
Histidyne*	97±2a	87±1a	84±7b	86±7a	89±5a
Lysine*	91±3ab	98±3a	103±4abc	81±1a	93±3a
Arginine*	87±7ab	93±1a	90±5ab	92±7a	91±2a

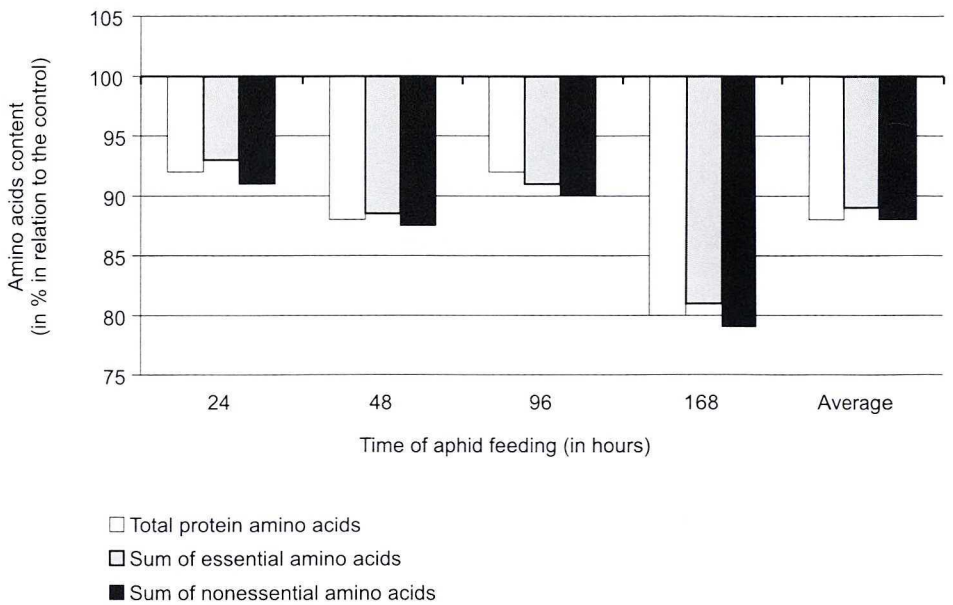

Fig. 4. Changes in the amino acids content in the ears of winter triticale cultivar Lasko caused by the grain aphid feeding

sistant Lasko cultivar. The results presented in this paper proved that changes in the amino acid composition of soluble protein might be one of more important factors forming the chemical basis of interactions between winter triticale and the grain aphid as regards food demands of the pest. The increase of amino acids content which rises the nutritive value of soluble protein of the Grado cultivar ears can determine high level of susceptibility of this triticale to *S. avenae* while the decrease of these substances quantity in protein of Lasko cultivar probably determines the functioning of well developed mechanisms of inducted antibiosis in this triticale.

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POLISH SUMMARY

ZMIANY W ZAWARTOŚCI I SKŁADZIE AMINOKWASOWYM BIAŁKA ROZPUSZCZALNEGO W ODMIANACH PSZENŻYTA OZIMEGO WYWOŁANE ŻEROWANIEM MSZYCY ZBOŻOWEJ

Celem podjętych badań było określenie wpływu żerowania mszycy zbożowej na zawartość i skład aminokwasowy białka rozpuszczalnego wyizolowanego z kłosów dwóch odmian pszenżyta ozimego o zróżnicowanym stopniu odporności na tego szkodnika.

W oparciu o uzyskane rezultaty stwierdzono, że żerowanie bezskrzydłych samic *S. avenae* wywołuje wzrost zawartości zarówno białka ogólnego jak i rozpuszczalnego w podatnej odmianie Grado. Natomiast w zasiedlonych przez mszyce roślinach względnie odpornej odmiany Lasko wzrastało stężenie białka ogólnego przy równoczesnym spadku poziomu białka rozpuszczalnego.

Analiza składu aminokwasowego białka rozpuszczalnego wykazała, że pod wpływem żerowania *S. avenae* w pszenżycie Grado wzrasta zawartość aminokwasów egzogennych i endogennych, a w odmianie Lasko – obniża się.

Otrzymane rezultaty dowiodły ponadto, że zmiany w zawartości i składzie aminokwasowym analizowanych frakcji białka uzależnione są w dużej mierze od czasu żerowania szkodnika.