

Full Length Research Paper

## Impact of nitrogen fertilization on the yield and content of protein fractions in spring triticale grain

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Accepted 16 July, 2013

Spring triticale cv. Andrus was cultivated in 2010 and 2011 with the application of different combinations of nitrogen fertilization with a total dose of 80 or 120 kg ha<sup>-1</sup>. The nitrogen fertilizer was applied into soil or both into soil and on leaves (foliar application) with and without microelements. The average grain yield was 6.21 t ha<sup>-1</sup> with a range from 5.89 to 6.64 t ha<sup>-1</sup> depending on the variant of fertilization. The average protein content at the dose of 80 and 120 kg N ha<sup>-1</sup> was 14.43 and 14.74 g per 100 g of grain, respectively. A higher dose of nitrogen resulted in an increase of albumins with globulins and  $\omega$  and  $\alpha/\beta$  prolamins in grain. This indicates that, even though some variants of fertilization with nitrogen favored the accumulation of protein in the grain, it was mainly the content of monomeric proteins that increased. The increase in their mass, due to a significant predominance in triticale grain, is undesired for potential use in baking. It suggests a lack of possibility for improvement of baking properties in triticale grain as a result of tested variants of fertilization.

**Key words:** Triticale, nitrogen fertilization, glutelins, prolamins.

### INTRODUCTION

Triticale is a hybrid cereal plant derived from wheat and rye genomes. In 2011, the sown area of this species (both winter and spring cultivars) in the world amounted to 3853,000 ha (FAO, 2013; <http://faostat.fao.org>). The advantages of triticale include high grain yield, resistance to biotic and abiotic stresses and valuable grain composition (Zecevic et al., 2010). Despite these features, triticale grain is mainly used as feedstuff material (McGoverin et al., 2011). The low level of use of this species in the baking industry results from low baking properties, which are determined by the content and functional parameters of storage proteins. Although, the average content of protein in triticale grain ranges from 18 to 20% (Gulmezoglu and Aytac, 2010; Zecevic et al.,

2010), the yield of gluten and its parameters are worse than those of wheat grain. For many years, breeders have been allowed to select species for improved triticale gluten quality. Igrejas et al. (1999) have shown that, triticale exhibit great genetic diversity among the group of storage proteins. Triticale proteome is cultivar-dependent and similar to wheat and rye proteomes. However, as with other cereals, it can be modified by the environment. The main agrotechnical factor that influences grain yield is mineral fertilization (Nefir and Tabără, 2011). The availability of nitrogen for plants depends on its form. Urea is the most common form of nitrogen fertilizer, yet the efficacy of nitrogen utilization from urea is conditioned by plant species and the method of fertilization. Results

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**Table 1.** Scheme of field nitrogen fertilization.

Object	Available nitrogen (kg ha <sup>-1</sup> )	Fertilizer type and applying time (rate kg ha <sup>-1</sup> )		
		Before sowing	(BBCH 23-29)	(BBCH 31-32)
K1	80	-	CO(NH <sub>2</sub> ) <sub>2</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> (40)
K2	80	-	CO(NH <sub>2</sub> ) <sub>2</sub> (20) + azofoska <sup>#</sup> (20)	CO(NH <sub>2</sub> ) <sub>2</sub> (40)
K3	80	-	CO(NH <sub>2</sub> ) <sub>2</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> (40)*
K4	80	-	CO(NH <sub>2</sub> ) <sub>2</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> (32) + ekolist <sup>#*</sup> (8)
K5	120	NH <sub>4</sub> NO <sub>3</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> 40
K6	120	NH <sub>4</sub> NO <sub>3</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> (20) + azofoska <sup>#</sup> (20)	CO(NH <sub>2</sub> ) <sub>2</sub> (40)
K7	120	NH <sub>4</sub> NO <sub>3</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> 40*
K8	120	NH <sub>4</sub> NO <sub>3</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> (40)	CO(NH <sub>2</sub> ) <sub>2</sub> (32) + ekolist <sup>#</sup> (8)

CO(NH<sub>2</sub>)<sub>2</sub> - urea; NH<sub>4</sub>NO<sub>3</sub> – ammonium nitrate; <sup>#</sup> multifertilizers, \*foliar fertilization.

**Table 2.** Climate conditions during triticale vegetation.

Year	Temperature (°C)						Average
	March	April	May	June	July	August	
2010	2.1	8.1	12.0	16.4	21.1	19.3	13.2
2011	1.6	9.1	13.1	17.1	17.9	17.6	12.7
1961-2005	1.2	6.9	12.8	15.9	17.8	17.7	12.1
Precipitation (mm)							
2010	36.7	18.2	131.9	84.8	80.4	95.3	74.6
2011	16.3	22.5	51.1	81.7	202.0	82.1	76.0
1961-2005	27.6	35.7	51.9	78.5	75.1	66.1	55.8

of numerous studies have indicated better utilization of nitrogen from foliarly-applied urea than from the soil. It has been shown that, the foliar application of nitrogen in a late phase of vegetation generates an increase in yielding and protein content by approximately 7 and 9%, respectively (Kinaci and Gulmezoglu, 2007). Mut et al. (2005) and Nefir and Tabără (2011) recorded an increase in triticale grain yield together with an increase in the dose of nitrogen, including the applied foliarly and supplemented with multi-component fertilizers. Supplementation of basic fertilization with copper, zinc, manganese and iron is particularly important (Nadim et al., 2012). The use of mineral fertilization combined with microelements increases grain yield and simultaneously improves the nutritional value of cereal grain (Malakouti, 2008).

This paper discusses the impact of nitrogen fertilization applied at doses of 80 and 120 kg ha<sup>-1</sup> into soil and into both soil and foliarly, with and without multi-component fertilizers as well as the impact of the year of harvesting on the yield of grain and protein and its composition in spring triticale grain.

## MATERIALS AND METHODS

Spring triticale cv. Andrus was cultivated in 2010 to 2011 in the

Education and Research Station of University of Warmia and Mazury in Tomaszkowo (53°72' N; 20°42' E), Poland, on typical brown soil with a texture of light loam. The soil was characterised by an acidic reaction, a low content of organic carbon (7.7 g kg<sup>-1</sup>), a high content of phosphorus (10.9 mg kg<sup>-1</sup> in d.m and potassium (20.7 mg kg<sup>-1</sup> in d.m), a medium content of magnesium (5.0 mg kg<sup>-1</sup> in d.m), an average content of available zinc (14.5 mg kg<sup>-1</sup> in d.m), manganese (182 mg kg<sup>-1</sup> in d.m) and iron (1100 mg kg<sup>-1</sup> in d.m) and a low copper content (2.1 mg kg<sup>-1</sup> in d.m). The experiment was set in a randomized block model in 3 replications. The doses of 30.2 kg P ha<sup>-1</sup> as triple superphosphate and 83.1 kg K ha<sup>-1</sup> as potassium salt were applied on all experimental objects. The fertilization with nitrogen was only a differentiating factor (Table 1).

The average temperature and precipitations during vegetation of triticale in the month of March to August period are presented in Table 2. The temperatures and their monthly distributions did not differ from the multi-annual mean (12.1°C). More diversified were precipitations. The average monthly volume of precipitation in the month of March to August period was 74.6 mm in 2010 and 75.9 mm in 2011 and was higher by approximately 35% than the multi-annual mean.

## Protein content and yield

Triticale grain samples were collected at harvest. The grain was then dried to approximately 14% and cleaned from dust and tailings in a laboratory sieve-air separator. Finally, the grain was milled to particles below 300 nm. Ground samples were stored in a refrigerator before the analyses. The content of nitrogen in the milled grain samples was determined with the Kjeldahl method and

**Table 3.** Impact of fertilization variant, year of harvesting and nitrogen dose on the grain ( $\text{t ha}^{-1}$ ) and protein yield ( $\text{kg ha}^{-1}$ ) and the composition of proteins in grain (g per 100 g of grain).

Fertilization variant		Grain yield	Protein yield	Albumins + globulins	Prolamins	Glutelins	Sum of protein fractions
<b>Average</b>		<b>6.21</b>	<b>786.4</b>	<b>2.25</b>	<b>8.79</b>	<b>3.65</b>	<b>14.69</b>
Year	2010	6.07 <sup>a</sup>	773.2 <sup>a</sup>	2.31 <sup>a</sup>	8.77 <sup>a</sup>	3.77 <sup>a</sup>	14.85 <sup>a</sup>
	2011	6.35 <sup>a</sup>	799.6 <sup>a</sup>	2.19 <sup>b</sup>	8.81 <sup>a</sup>	3.53 <sup>b</sup>	14.53 <sup>b</sup>
Object	K1	5.89 <sup>a</sup>	754.4 <sup>a</sup>	1.96 <sup>abc</sup>	8.52 <sup>c</sup>	3.18 <sup>d</sup>	13.66 <sup>c</sup>
	K2	6.14 <sup>a</sup>	788.5 <sup>a</sup>	2.29 <sup>abc</sup>	8.73 <sup>bc</sup>	3.62 <sup>a</sup>	14.64 <sup>bc</sup>
	K3	6.04 <sup>a</sup>	778.0 <sup>a</sup>	2.15 <sup>c</sup>	8.43 <sup>c</sup>	3.37 <sup>a</sup>	13.95 <sup>c</sup>
	K4	6.33 <sup>a</sup>	817.3 <sup>a</sup>	2.29 <sup>bc</sup>	8.86 <sup>b</sup>	3.79 <sup>a</sup>	14.94 <sup>ab</sup>
	K5	5.95 <sup>a</sup>	750.3 <sup>a</sup>	2.41 <sup>bc</sup>	9.21 <sup>a</sup>	3.80 <sup>a</sup>	15.42 <sup>a</sup>
	K6	6.64 <sup>a</sup>	813.3 <sup>a</sup>	2.23 <sup>bc</sup>	8.71	3.79 <sup>a</sup>	14.73 <sup>b</sup>
	K7	6.35 <sup>a</sup>	819.1 <sup>a</sup>	2.39 <sup>bc</sup>	9.20 <sup>a</sup>	3.84 <sup>a</sup>	15.43 <sup>a</sup>
	K8	6.36 <sup>a</sup>	770.1 <sup>a</sup>	2.25 <sup>bc</sup>	8.66	3.81 <sup>a</sup>	14.72 <sup>b</sup>
Nitrogen dose	80	6.13 <sup>a</sup>	778.4 <sup>a</sup>	2.18 <sup>a</sup>	8.72 <sup>a</sup>	3.53 <sup>a</sup>	14.43 <sup>b</sup>
	120	6.12 <sup>a</sup>	783.5 <sup>a</sup>	2.29 <sup>a</sup>	8.81 <sup>a</sup>	3.64 <sup>a</sup>	14.74 <sup>a</sup>

Means in the same column (separately for year, object and nitrogen dose) followed by different letters are significantly different ( $\alpha \leq 0.05$ ).

the content of total protein was then calculated using a 5.7 multiplier. The yield of protein was calculated based on the grain yield and content of protein in the grain.

#### Protein extraction and analysis

The quantitative and qualitative protein characteristics were determined with the RP-HPLC technique according to Konopka et al. (2007). The content of albumins and globulins, prolamins and glutelins was analyzed. The assays were performed with a Hewlett Packard 1050 series apparatus. Detection of protein fractions was carried out at the 210 nm wavelength and their identification consisted in an analysis of spectra and standard protein retention times. The content of protein is expressed in g per 100 g of grain using the standard curves for bovine serum albumin (BSA) and gliadins and glutenins of wheat cv. Tonacja.

#### Statistical analysis of data

Data were statistically processed with the ANOVA and a "post-hoc" Duncan test. The comparisons between the average values were performed separately for the year of cultivation, variant of nitrogen fertilization and the total dose of nitrogen fertilizer. The calculations were performed at level of significance  $\alpha = 0.05$  with STATISTICA v.10 software (StatSoft, Inc.).

## RESULTS AND DISCUSSION

### Grain yield

The average grain yield was  $6.21 \text{ t ha}^{-1}$  with the variation ranging from  $5.89$  to  $6.64 \text{ t ha}^{-1}$ , depending on the variant of fertilization with nitrogen (Table 3). The recorded differences were, however, statistically insignificant. The yield volume was significantly higher than the average

spring triticale yielding in other EU countries (FAO, 2013; <http://faostat.fao.org>). According to COBORU (2013 <http://coboru.pl>), with a transition from average to intensive fertilization, the grain yield for this cultivar may increase from  $5.79$  to  $6.67 \text{ t ha}^{-1}$ . However, Piekarczyk et al. (2011) found only a minor increase in wheat grain yield as a result of the application of nitrogen at the dose of  $40$  to  $160 \text{ kg ha}^{-1}$ . According to Nefir and Tabără (2011), the increase in the dose of nitrogen from  $80 \text{ kg ha}^{-1}$  to  $160 \text{ kg ha}^{-1}$  generates an increase in yielding as high as approximately 12%. These authors have also shown that, mineral fertilization with dose of  $160 \text{ kg N}$ ,  $60 \text{ kg P}$ , and  $60 \text{ kg K}$  per ha promoted the growth of production yield by 44% comparing to unfertilized plot. The increase in spring triticale yielding is favored by a combination of nitrogen fertilization with the foliar application of zinc (Knapowski et al., 2009).

### Protein grain yield

The protein yield ranged from  $704.9$  to  $861.7 \text{ kg ha}^{-1}$  with the total average value of  $786.4 \text{ kg ha}^{-1}$  (Table 3). Neither the year of cultivation nor any of the fertilization variants generated a statistically significant change in the protein yield. Only a tendency towards an increase in the protein yield in 2011 and under the influence of higher nitrogen dose ( $120 \text{ kg ha}^{-1}$ ) was noted. Alaru et al. (2003) showed that, the main factor influencing the content of protein in triticale grain was the cultivar. Weather conditions in the growth period have a lesser impact, and nitrogen fertilization being the least important. According to these authors, fertilization with nitrogen at tillering caused an average increase in the protein content in triticale grain of

**Table 4.** Impact of fertilization variant, year of harvesting and nitrogen dose on the characteristics of storage proteins in grain (g per 100 g of grain).

Fertilization variant		Prolamins			Glutelins	
		$\omega$	$\alpha/\beta$	$\gamma$	HMW	LMW
Average		0.54	4.90	3.35	1.26	2.39
Year	2010	0.54 <sup>a</sup>	4.86 <sup>a</sup>	3.36 <sup>a</sup>	1.35 <sup>a</sup>	2.42 <sup>a</sup>
	2011	0.54 <sup>a</sup>	4.93 <sup>a</sup>	3.34 <sup>a</sup>	1.17 <sup>b</sup>	2.36 <sup>b</sup>
Object	K1	0.55 <sup>b</sup>	4.73 <sup>bc</sup>	3.25 <sup>a</sup>	1.24 <sup>bc</sup>	1.94 <sup>d</sup>
	K2	0.64 <sup>a</sup>	4.78 <sup>bc</sup>	3.30 <sup>a</sup>	1.23 <sup>bc</sup>	2.38 <sup>b</sup>
	K3	0.51 <sup>bc</sup>	4.66 <sup>c</sup>	3.26 <sup>a</sup>	1.20 <sup>c</sup>	2.17 <sup>c</sup>
	K4	0.55 <sup>b</sup>	4.93 <sup>b</sup>	3.38 <sup>a</sup>	1.29 <sup>bc</sup>	2.50 <sup>a</sup>
	K5	0.62 <sup>ab</sup>	5.14 <sup>a</sup>	3.46 <sup>a</sup>	1.31 <sup>a</sup>	2.48 <sup>a</sup>
	K6	0.48 <sup>c</sup>	4.92 <sup>b</sup>	3.31 <sup>a</sup>	1.23 <sup>bc</sup>	2.56 <sup>a</sup>
	K7	0.51 <sup>bc</sup>	5.20 <sup>a</sup>	3.49 <sup>a</sup>	1.30 <sup>ab</sup>	2.53 <sup>a</sup>
	K8	0.47 <sup>c</sup>	4.80 <sup>bc</sup>	3.38 <sup>a</sup>	1.26 <sup>abc</sup>	2.54 <sup>a</sup>
Nitrogen dose	80	0.54 <sup>b</sup>	4.85 <sup>a</sup>	3.33 <sup>a</sup>	1.25 <sup>a</sup>	2.28 <sup>b</sup>
	120	0.58 <sup>a</sup>	4.88 <sup>a</sup>	3.35 <sup>a</sup>	1.26 <sup>a</sup>	2.39 <sup>a</sup>

Means in the same column (separately for year, object and nitrogen dose) followed by different letters are significantly different ( $\alpha \leq 0.05$ ).

1.57%. Lestingi et al. (2010) proved that, the optimal dose of nitrogen for maintaining good quality of triticale grain was approximately 50 kg ha<sup>-1</sup>.

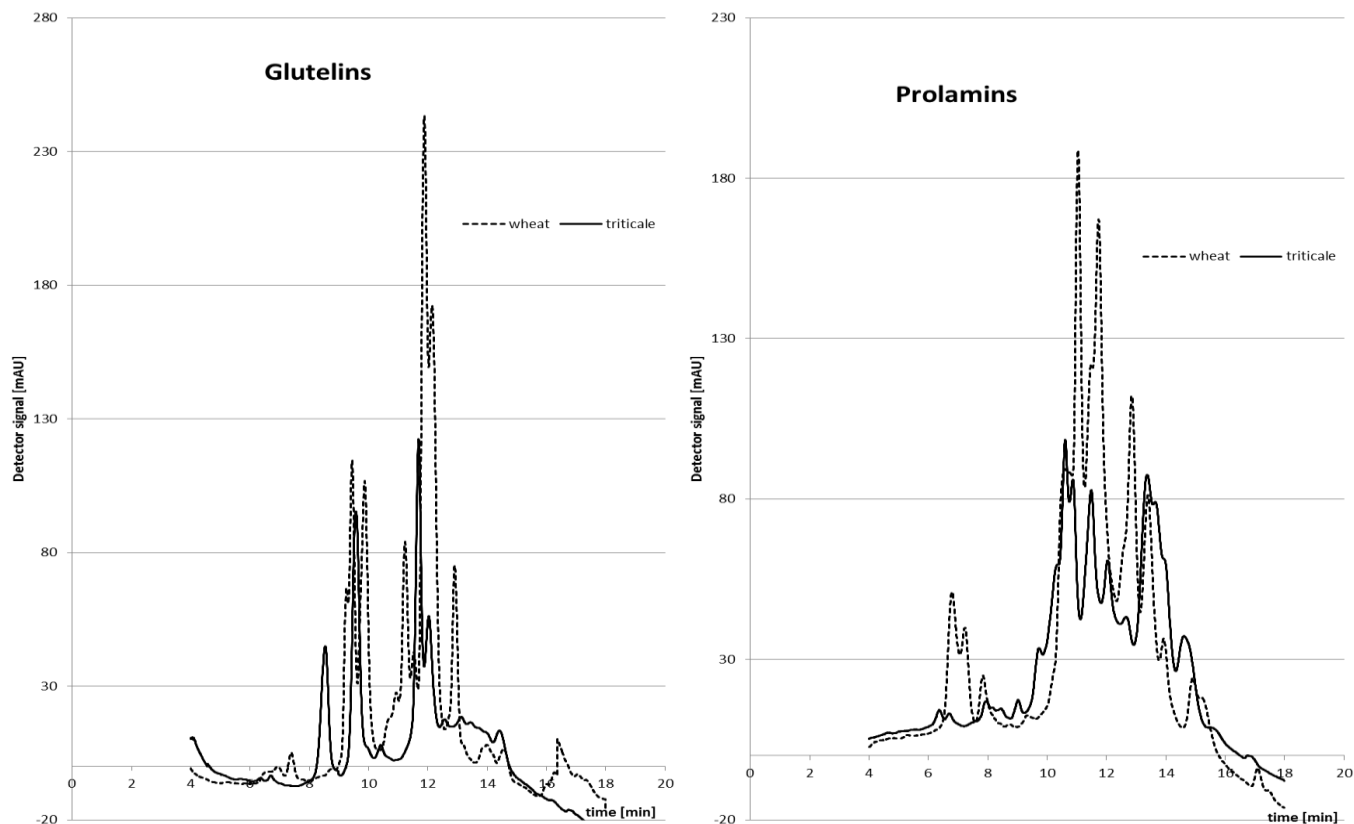
### Protein characteristics

The average protein content in the grain of tested triticale cultivar was 14.69 g per 100 g of grain (Table 3). Of this, prolamins constituted 59.8 and glutelins 24.8%, whilst the amount of albumins and globulins was the lowest, amounting to 15.4% in total. The year of harvesting and the total dose of nitrogen fertilizer impacted the total concentration of protein (higher values were recorded in 2010 and after the application of 120 kg N ha<sup>-1</sup>). Moreover, statistically significant differences were found between individual fertilization variants. The difference between the highest and the lowest total protein content was 1.77 g per 100 g of grain. The comparison between K3 and K7 variants showed that, the application of additional pre-sowing fertilization with nitrogen at the dose of 40 kg ha<sup>-1</sup> generated an increase in the relative protein content in the grain by as much as 10.6%.

Examples of chromatograms depicting the individual protein fractions in the tested triticale grain are presented in Figure 1. Comparison of these data to typical chromatogram of storage proteins in wheat grain (cv. Tonaćja) showed that, these species are distinctly different. Analyzed triticale grain contained less prolamins with retention times up to approximately 12 min, and in glutelins composition, it can be a visible balance between the subunits of high (with retention times up to 10 min)

and low molecular weight. Under the influence of lower nitrogen dose were detected statistically lower contents of glutelins and albumins + globulins in the grain harvested in 2011 (except for high molecular weight (HMW) glutelins). The large difference in the content of albumins and globulins was stated between K1 and K5 variants, and for prolamins between K3 and K7 (Tables 3 to 4). The application of additional pre-sowing nitrogen fertilization generated an increase of 22.9 and 9.1%, respectively. Within the prolamins, the applied fertilization variants only generated a change in  $\omega$  and  $\alpha/\beta$  subunits and statistically significant differences were detected mainly between variants with additional pre-sowing nitrogen fertilization: K1 and K5 ( $\omega$  and  $\alpha/\beta$ ) and K3 and K7 ( $\alpha/\beta$ ). It was also found that, a higher dose of nitrogen favored the accumulation of  $\omega$  gliadins and the increase was 7.4%. The variability in the content of glutelins under different variants of fertilization was also high and ranged from 3.18 to 3.84%. Within this group of proteins, the low-molecular-weight (LMW) glutelins showed more pronounced changes (from 1.94 to 2.56%) than HMW subunits (from 1.20 to 1.31%). The amount of both fractions was statistically lower in 2011, whereas the content of LMW glutelins was significantly higher in grain fertilized with nitrogen at 120 kg ha<sup>-1</sup>.

Many authors have indicated the potential utility of triticale grain in the baking industry (Amiour et al., 2002; Martinek et al., 2008). The baking value of triticale grain depends on the amount and quality of storage proteins, which is influenced by genetic and environmental factors (Ereku and Köhn, 2006; Burešová et al., 2010; McGovern et al., 2011). The hallmark feature of the



**Figure 1.** Comparison of storage proteins in triticale cv. Andrus and wheat cv. Tonacja grain.

Andrus cultivar is a high thousand grain weight with a low protein content (COBORU, 2013). Improvements in grain quality parameters can be achieved with adequate agricultural engineering procedures. According to Luo et al (2001), the content of HMW and LMW fractions, although genetically determined, may increase slightly under the influence of nitrogen application in later growth stages.

High temperatures after anthesis and abiotic stress in the early stages of grain filling exert a negative impact on the accumulation of protein (Knezevic et al., 2007), while drought stress has a positive effect (Fernandez-Figares et al., 2000). The present study confirmed the significant impact of the climate on the characteristics of protein. An analysis of meteorological data (Table 2) has shown that, the average temperature during vegetation in 2011 year was slightly lower than in 2010. The biggest difference (3.2°C) was recorded in July. Furthermore, both years were much abundant in rainfall than the multi-annual mean. This indicates that, the climatic conditions in 2011 were less favorable for the accumulation of storage proteins. The more intensive fertilization with nitrogen contributed to an increase in the  $\omega$  fraction and LMW glutelins. A similar phenomenon was observed by Wieser and Seilmeier (1998) who suggested that, nitrogen fertilization generates a higher increase in the content of

gliadins than of glutenins. This results in an increase of monomeric proteins and a reduction of polymeric proteins. The ratio of prolamins to glutelins in bread wheat grain should approximately be 1:1 (Singh and MacRitchie, 2001; Shewry and Halford, 2002). In the grain of tested triticale cultivar, this value ranged from 2.2 to 2.7:1. Some variants of used fertilization favored the accumulation of prolamins, what can additionally increase viscous properties of protein over its elasticity. This indicates a lack of potential for improvements in the baking properties in triticale grain as a result of proposed fertilization variants.

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