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PROTEIN COMPOSITION OF SPRING TRITICALE GRAIN IN THE CONDITIONS OF DIVERSIFIED FERTILIZATION SYSTEMS

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Abstract. The aim of the study was the evaluation of the effect of diversified nitrogen fertilization: in-soil, foliar, and foliar in combination with multi-component fertilizer Ekolist, on the content and composition of protein of spring triticale grain cultivar Milewo. Field experiment was carried out at the Didactic-Experimental Centre in Tomaszkowo, which is part of the University of Warmia and Mazury in Olsztyn. Total protein content, as well as the contents of the particular protein fractions in spring triticale grain cultivar Milewo were affected by both the application of foliar additional feeding with Ekolist and mineral nitrogen dose. Nitrogen application at the dose of 80 kg·ha^{-1} with Ekolist, in comparison with the same dose but without the multi-component fertilizer, attributed to the increase in protein amount by 0.72 g per 100 g of grain. The highest proportion was made of storage proteins with the advantage of gliadins over glutenins. Fertilization variants applied in the experiment resulted in a high ratio of gliadins to glutenins. Moreover, significant effect of the total nitrogen dose 120 kg·ha^{-1} was confirmed in comparison with 80 kg·ha^{-1} on the increase in the concentration of ω and α/β gliadins . Cultivation year affected the proportion of γ gliadins and HMW and LMW glutenins in the grain. Increase in nitrogen fertilization by 40 kg·ha^{-1} before sowing as ammonium nitrate contributed to the increase in ω gliadins and HMW glutenins. Additional application of Ekolist fertilization (variant with the dose of 80 kg·ha^{-1}) caused an increase in γ gliadins and LMW glutenins.

Key words: Ekolist fertilizer, foliar fertilization, gliadins, glutenins, protein fractions, storage proteins

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INTRODUCTION

Cereal grain is one of the most nutritious plant products and covers people's demand for protein in over 60%. As global demand for food increases, we face the necessity to use non-bread cereal species. Triticale may be an alternative for other cereals, in particular for wheat because of its poorer response to unfavourable growth conditions and tolerance to a low-cost production system [Erukol and Köhn 2006].

At present, triticale grain is used first of all as fodder for animals. Little use of this species in bakery results from its low baking value determined by the content and functional characteristics of storage proteins. Triticale grain may, however, be applied in bakery as a component of mixtures with high-gluten wheat flour [Mc Goverin *et al.* 2011]. For many years, breeding studies have been carried out in order to create triticale cultivar which would possess high quality standards similar to the ones of winter wheat. According to Gulmezoglu and Aytac [2010], protein content in the grain oscillates between 10% and 18%. Although protein content in triticale grain is higher by 1-2% in comparison with wheat grain and by 3-5% in comparison with rye, gluten efficiency and its functional characteristics (especially elasticity) are definitely poorer than the ones of wheat grain [Zečević *et al.* 2010].

In intensive plant production, fertilization level and application time is a decisive factor for reaching high yield of plants with favourable qualitative properties [Kara and Uysal 2009, Knapowski *et al*. 2009, Zečević *et al*. 2010, Brzozowska and Brzozowski 2011, Ellmann 2011, Malik *et al*. 2011, Piekarczyk *et al*. 2011, Bobrecka-Jamro *et al*. 2013, Janušauskaitė 2013, Stępień and Wojtkowiak 2013]. Positive phenomenon is slow, systematic increase in supplementing basic NPK fertilization with fertilizers that contain a set of adequately chosen microelements [Nadim *et al*. 2012, Nogalska *et al*. 2012, Wojtkowiak *et al*. 2014]. In agricultural practice, more and more often foliar additional feeding of cultivated plants with nitrogen (urea) or liquid multi--component fertilizers is applied. Foliar fertilizer application is recommended at the straw-shooting stage, when intensive cell divisions occur. According to Malik *et al*. [2011], technological value of grain may be influenced through cultivar choice and nitrogen application systems at proper developmental stages of plants.

The aim of the work was the evaluation of the effect of diversified nitrogen fertilization: in-soil, foliar, and foliar in combination with multi-component fertilizer Ekolist on the content and composition of protein of spring triticale grain cultivar Milewo. In the context of the assumed study aims, zero hypothesis was verified which assumed the lack of significant differences between the levels of the studied factor. In the case of rejection of the zero hypothesis, an alternative hypothesis was assumed which claimed that there were significant differences between fertilization methods in the formation of total protein content, as well as its particular fractions in the grain of spring triticale.

MATERIAL AND METHODS

Grain of spring triticale cultivar Milewo originated from the field experiment carried out in research years 2010-2011 at the Didactic-Experimental Centre in Tomaszkowo, which is part of the University of Warmia and Mazury in Olsztyn (53°72 N; 20°42 E).

Experiment was set up at split-block design in three replications on proper brown soil, granulometric composition of light clay classified as Haplic Cambisol according to FAO [2006]. Description of the chemical properties of the soil before the set-up of the experiment is presented in Table 1.

Table 1. Soil properties (average values 2010-2011)
Tabela 1. Właściwości gleby (średnia 2010-2011)

Parameter, unit – Parametr, jednostka		Values – Wartości
Soil type (FAO 2006) – Rodzaj gleby		Haplic Cambisol
pH in KCl		5.05
$C_{org.}$, $g \cdot kg^{-1}$		7.71
$N_{og.}$, $g \cdot kg^{-1}$		0.97
Available minerals Dostępne składniki $mg \cdot kg^{-1}$	P	24.90
	K	25.00
	Mg	5.00

Multi-component fertilizer Ekolist was introduced into the experiment with specially processed chelating complex, chelacid. With Ekolist at the dose of 2.0 $dm^{-3} \cdot ha^{-1}$, the following elements were introduced ($g \cdot dm^{-3}$): N – 240.0; K – 130.0; Mg – 40.0; S –10.0; Cu – 10.0; Zn – 5.0; Mn – 1.0; Fe – 2.0; Mo – 0.04, and B – 10.0. Scheme of fertilization with nitrogen and multi-component fertilizer Ekolist is presented in Table 2. In triticale fertilization, triple superphosphate was applied in-soil at the dose equivalent to 30.2 kg $P \cdot ha^{-1}$ and potassium salt at the dose of 83.1 kg $K \cdot ha^{-1}$.

Table 2. Fertilization diagram
Tabela 2. Schemat nawożenia

Treatment Wariant	Sum of N Suma N $kg \cdot ha^{-1}$	Application time and nitrogen fertilization type, dose N $kg \cdot ha^{-1}$ Termin zastosowania oraz forma nawozu azotowego, dawka N $kg \cdot ha^{-1}$		
		before sowing* przed siewem	BBCH 23-29*	BBCH 31-32**
K1	80	–	(40) $CO(NH_2)_2$	(40) $CO(NH_2)_2$
K2	80	–	(40) $CO(NH_2)_2$	(39.76) $CO(NH_2)_2$ + (0.24) Ekolist#
K3	120	(40) NH_4NO_3	(40) $CO(NH_2)_2$	(40) $CO(NH_2)_2$
K4	120	(40) NH_4NO_3	(40) $CO(NH_2)_2$	(39.76) $CO(NH_2)_2$ + (0.24) Ekolist#

$CO(NH_2)_2$ – urea – mocznik
NH_4NO_3 – ammonium nitrate – saletra amonowa
* in-soil fertilization – nawożenie doglebowe
** foliar fertilization – nawożenie dolistne
# multi-component fertilizer – nawóz wieloskładnikowy

During the experiment, temperature and precipitation monitoring was carried out (Table 3). Average temperatures in both research years were similar and monthly temperature distribution did not differ from the many-years' average. Precipitation amount during triticale growth period was diversified. Small precipitation amount in April deserves special attention. In May 2010, precipitation amount was similar to the many-years' average (51.1 mm), whereas in 2011 it exceeded over two times the many--years' average (131.9 mm). From July to August, higher precipitation sums were noted in comparison with the many-years' average. July 2011 deserves special attention, since the precipitation sum was very high (202.0 mm).

Table 3. Weather conditions during the study period
Table 3. Warunki meteorologiczne w okresie prowadzenia badań

Year – Rok	March marzec	April kwiecień	May maj	June czerwiec	July lipiec	August sierpień	Mean średnia
	Temperature – Temperatura, °C						
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 – Opady, 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	75.9
1961-2005	27.6	35.7	51.9	78.5	75.1	66.1	55.8

Quantitative and qualitative characteristics of protein were determined by the RP--HPLC technique described by Konopka *et al.* [2007]. Contents of albumin and globulin, gliadin and glutenin fractions were analyzed. Analyses were performed using Hewlett Packard chromatograph series 1050. Protein fraction detection was carried out at the wavelength of 210 nm, and their identification was based on the analyses of spectra and retention times of model proteins (division into classes was assumed on the basis of the retention time of typical wheat grain proteins) Protein content was expressed in g per 100 g of grain, using the calibration curves for bovine serum albumin (BSA) and gliadins and glutenins of wheat cultivar Tonacja (Table 4).

Table 4. Protein fractions in spring triticale grain cultivar Milewo, $g \cdot 100\ g^{-1}$
Tabela 4. Frakcje białek w ziarnie pszenżyta jarego odmiany Milewo, $g \cdot 100\ g^{-1}$

Fertilization method Sposób nawożenia		Albumins + globulins Albuminy + globuliny	Gliadins Gliadyny	Glutenins Gluteniny	Gliadins/ Glutenins Gliadyny/ Gluteniny	Sum of protein fractions Suma frakcji białek
Study years Lata badań	2010	2.47[a] ±0.08	9.34[a] ±0.52	5.40[a] ±0.43	1.73[b] ±0.07	17.21[a] ±0.74
	2011	2.39[a] ±0.08	9.52[a] ±0.22	5.18[b] ±0.18	1.84[a] ±0.07	17.09[a] ±0.33
Treatment Wariant	K1	2.41[a] ±0.02	9.01[b] ±0.46	5.14[b] ±0.17	1.76[a] ±0.11	16.56[b] ±0.47
	K2	2.45[a] ±0.14	9.46[a] ±0.39	5.38[a] ±0.04	1.76[a] ±0.08	17.28[a] ±0.38
	K3	2.45[a] ±0.10	9.51[a] ±0.21	5.36[a] ±0.12	1.77[a] ±0.02	17.33[a] ±0.36
	K4	2.41[a] ±0.09	9.74[a] ±0.17	5.28[ab] ±0.41	1.85[a] ±0.12	17.44[a] ±0.64
Mean for nitrogen doses Średnia dla dawek azotu	80	2.42[a] ±0.09	9.24[b] ±0.46	5.26[a] ±0.17	1.76[a] ±0.09	16.92[a] ±0.55
	120	2.43[a] ±0.09	9.63[a] ±0.21	5.32[a] ±0.28	1.81[a] ±0.09	17.38[a] ±0.49

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mean values in columns (separately for years, treatments and nitrogen doses) marked with the same letter are insignificant ($P \leq 0.05$) – średnie w kolumnach (osobno dla lat, obiektów i dawek azotu) oznaczone tą samą literą są nieistotne ($P \leq 0,05$)
± standard deviation – odchylenie standardowe

Results were processed statistically with the use of the program STATISTICA 9.0 by Statsoft. One-factor analysis of variance was used for statistical calculations. In addition to the basic statistical parameters, also standard deviation and homogenous groups were set with the Duncan's test, at the significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Spring triticale cultivar Milewo was bred at the Plant Breeding and Acclimatization Institute in Strzelce Opolskie and registered in 2008. According to the data by the Research Centre for Cultivar Testing [2013], it was the spring triticale cultivar that gave the highest yield in the years 2011 and 2012 and was characterized by rather high mass of 1000 grains and protein content.

Protein content in spring triticale grain was significantly diversified by plant fertilization method. Significantly lower protein fraction sum was found in the grain of triticale fertilized with the dose of N 80 $kg \cdot ha^{-1}$ exclusively in the foliar form (variant K1). The remaining fertilization methods had no significant effect on the formation of protein content in the grain. Moreover, a tendency was observed (but not statistically proven) for an increase in the protein content under the effect of the increase in the nitrogen dose from 80 to 120 $kg \cdot ha^{-1}$.

Positive effect of foliar fertilization with nitrogen on protein accumulation in cereal grain is confirmed in literature. According to Malik *et al*. [2011], main environmental factors that affect protein accumulation in grain are nitrogen availability and temperature during growth (especially period from flowering to grain maturation). Garrido-Lestache *et al*. [2004] confirmed better use of nitrogen from urea applied in a half or one-third of the total dose, foliar rather than in-soil. Kara and Uysal [2009] indicated that late-growth foliar nitrogen application increased protein content by 9.0%. According to Mut *et al*. [2005], increase in fertilization level with this element from 60 to 120 $kg \cdot ha^{-1}$ increased protein content by 1.5%, and further 60 $kg \cdot ha^{-1}$ N only by 0.3%. Tababtabaei and Ranjbar [2012] pointed out to a clear increase in protein proportion in grain with the increase in nitrogen fertilization dose to the level of 160 kg $N \cdot ha^{-1}$.

In the conducted research, year and fertilization variant did not have a significant effect on the contents of albumins and globulins in triticale grain. From the analyzed proteins, storage proteins had the highest proportion with the prevalance of gliadins (54.41%-55.85%) over glutenins (30.28%-31.04%). With the increase in participation in the total protein of storage proteins, structural protein (albumin and globulin) contents decreased. Also Johansson *et al*. [2004], Barczak and Knapowski [2008] and Hurkman *et al*. [2013] found a relation between nitrogen fertilization and storage protein accumulation in grains. In the conducted research, the highest amount of gliadins was found after nitrogen application at the dose of 120 $kg \cdot ha^{-1}$ in-soil and foliar, in the form of urea and with Ekolist. Increased nitrogen doses from 80 to 120 $kg \cdot ha^{-1}$ (in variants without Ekolist) through in-soil application of ammonium nitrate, significantly increased gliadin content by 5.5%. Also glutenin content increased by 4.1% but the relation was not statistically significant.

Baking value of bread grain is determined first of all by gluten protein content and the ratio of gliadins to glutenins. Konopka *et al*. [2007] and Altenbach [2012] showed that the highest proportion of gliadins may indicate a potential predominance of viscous features over elasticity of this flour proteins. Konopka *et al*. [2007], Shewry *et al*. [2002] and Singh and MacRitchie [2001] indicated that some wheat cultivars have even proportions of gliadins to glutenins. Studies of different wheat genotypes showed preferences for gliadin accumulation in the grain [Gil-Humanesi *et al*. 2012]. It is generally assumed that the proportions depend on genetic and environmental factors [Malik 2011, Erekul and Köhn 2006]. In the grain of the studied spring triticale cultivar, ratio of gliadins to glutenins was from 1.75 to 1.84 and varied in the study years but did not depend on the fertilization method.

Sample chromatograms of the particular protein fractions of triticale grain are presented in Figure 1. It was found that the most diversified fractions were albumins and globulins, in which circa 27 peaks were observed with the retention times from 6 to 15 minutes. Comparison of two extreme samples for this protein fraction (Fig. 1A) demonstrated only quantitative differences, with small advantage of the grain in variant K4 over K2 (both samples from the year 2010). Also differences in gliadin contents between the extreme samples had quantitative character (Fig. 1B). Grain from 2011 was the richest in gluten proteins (variant K1), whereas the lowest amount of those proteins was found in variant K3 in 2010. Analysis of Figure 3C showed the presence of 15 main chromatographic peaks. Extreme differences in this case were noted for samples from 2010 (variants K1 and K4), with noticeable tendency for glutenin increase under the effect of an increasing nitrogen dose.

Contents of high molecular weight glutenins (HMW) and low molecular weight glutenins (LMW) are genetically controlled [Wieser 2007] but may slightly increase under the effect of nitrogen application at latter developmental stages [Luo *et al*. 2000]. High temperatures and water deficit in the spring may shorten grain filling and maturation, thus decreasing grain yield and increasing protein content. This affects changes in protein composition, which may then negatively affect gluten endurance [Garrido-Lestache *et al*. 2004]. Both the increase in nitrogen dose and dose division lead to the increase in high molecular weight glutenins HMW, which make it possible to form disulphide bonds, which leads to the increase in the polymerization degree and, as a result, to the improvement of dough and bread quality [Fuertes-Mendizábal *et al*. 2010]. In the conducted experiment, fertilization variants affected the changes in the content of subunits in storage protein fractions (Table 5). Within glutenins, HMW proteins made up from 1.40 to 1.52, and LMW proteins from 3.74 to 3.91 $g \cdot 100\ g^{-1}$ of grain. Statistically significant effect of the harvest year and fertilization variant on the HMW accumulation was observed. Significantly higher HMW content was characteristic for triticale grain in 2010, and foliar plant fertilization caused a significant increase in its accumulation in the grain in comparison with only in-soil fertilization. At the same time, no significant effect was found of nitrogen dose on HMW content in the grain.

Results of the present research indicate a significant effect of the cultivation years on the proportion of γ gliadins and HMW and LMW glutenins. Weather conditions could have affected nitrogen use by the plants. Many authors point out plant response to changeable weather conditions, in particular hydrological conditions, which may create protein content and composition [Erekul and Köhn 2006, Janušauskaitė 2013].

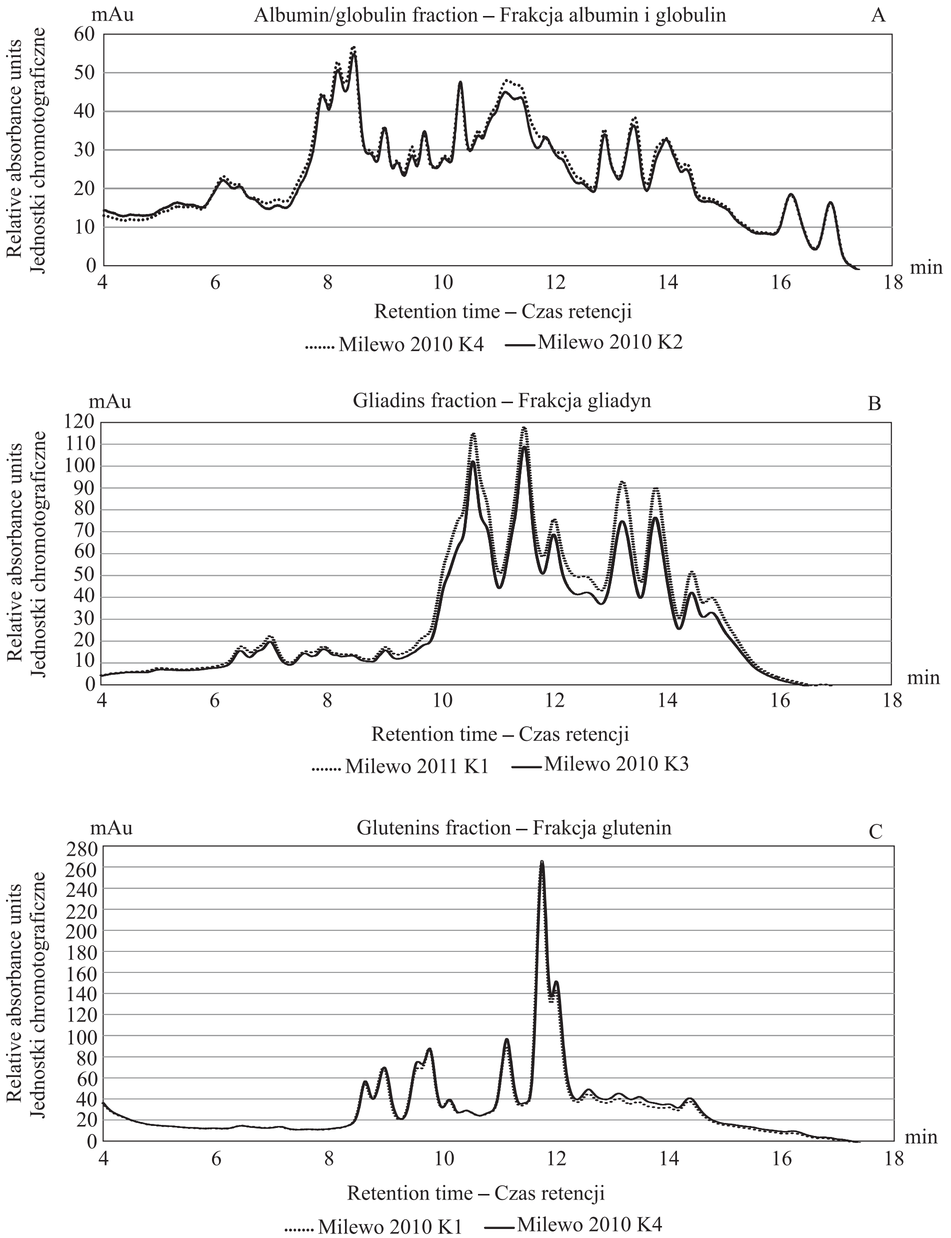

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Fig. 1. Examples of chromatograms of the analyzed protein fractions: A) albumins and globulins, B) gliadins, C) glutenins

Rys. 1. Przykładowe chromatogramy analizowanych frakcji białek: A) albuminy i globuliny, B) gliadyny, C) gluteniny

Total nitrogen dose 120 kg·ha^{-1} in comparison with 80 kg·ha^{-1} contributed to the increase in concentration of gliadins ω and α/β respectively by 9.38% and 6.69%. Increase in nitrogen fertilization by 40 kg before sowing in the form of ammonium nitrate (variants K1 in comparison with K3) contributed to the increase in ω gliadins (by 18.03%) and HMW glutenins (by 8.57%). Additional application of fertilization with Ekolist (variant K2 with the N dose of 80 kg·ha^{-1}) affected the increase in γ gliadins and LMW glutenins, respectively by 5.81% and 4.55% in comparison with in-soil and foliar fertilization with urea (variant K1 with the N dose of 80 kg·ha^{-1}).

Table 5. Gliadin and glutenin fractions in spring triticale grain cultivar Milewo, g·100 g^{-1}
Tabela 5. Frakcje gliadyn i glutenin w ziarnie pszenżyta jarego odmiany Milewo, g·100 g^{-1}

Fertilization method Sposób nawożenia		Gliadins – Gliadyny			Glutenins – Gluteniny	
		ω	α/β	γ	HMW	LMW
Study years Lata badań	2010	0.68^{a} ±0.07	5.39^{a} ±0.43	3.27^{b} ±0.11	1.52^{a} ±0.13	3.89^{a} ±0.11
	2011	0.66^{a} ±0.05	5.42^{a} ±0.13	3.44^{a} ±0.12	1.43^{b} ±0.08	3.75^{b} ±0.13
Treatment Wariant	K1	0.61^{c} ±0.03	5.13^{b} ±0.33	3.27^{b} ±0.20	1.40^{b} ±0.07	3.74^{b} ±.013
	K2	0.66^{b} ±0.04	5.34^{ab} ±0.30	3.46^{a} ±.013	1.47^{ab} ±0.04	3.91^{a} ±0.07
	K3	0.72^{a} ±0.01	5.48a^{b} ±0.20	3.31^{b} ±0.03	1.52^{a} ±0.07	3.85^{ab} ±0.06
	K4	0.68^{ab} ±0.08	5.68^{a} ±0.16	3.37^{ab} ±0.12	1.51^{a} ±0.20	3.77^{ab} ±0.22
Mean for nitrogen doses Średnia dla dawek azotu	80	0.64^{b} ±0.04	5.23^{b} ±0.31	3.37^{a} ±0.19	1.44^{a} ±0.06	3.82^{a} ±0.14
	120	0.70^{a} ±0.06	5.58^{a} ±0.20	3.34^{a} ±0.09	1.51^{a} ±0.14	3.81^{a} ±0.15

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mean values in columns (separately for years, treatments and nitrogen doses) marked with the same letter are insignificant ($P \le 0.05$) – średnie w kolumnach (osobno dla lat, obiektów i dawek azotu) oznaczone tą samą literą są nieistotne ($P \le 0,05$)
± standard deviation – odchylenie standardowe

CONCLUSIONS

1. Regardless of the fertilization method, spring triticale grain cultivar Milewo was characterized by the high storage protein share with significant advantage of gliadins over glutenins.
2. Additional application of fertilization with Ekolist against the nitrogen dose of 80 kg·ha^{-1} caused an increase in the proportion of γ gliadin fractions and LMW glutenins.
3. Increase in the nitrogen dose from 80 to 120 kg·ha^{-1} without Ekolist contributed to the increased accumulation of ω gliadins and HMW glutenins. Additional application of Ekolist at the nitrogen dose of 120 kg·ha^{-1} increased the concentration of α/β gliadins.
4. Triticale growth year diversified significantly the contents of γ gliadins, as well as HMW and LMW glutenins.

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SKŁAD BIAŁKA ZIARNA PSZENŻYTA JAREGO W WARUNKACH ZRÓŻNICOWANYCH SYSTEMÓW NAWOŻENIA

Streszczenie. Przedmiotem badań była ocena oddziaływania zróżnicowanego nawożenia azotem stosowanego doglebowo, dolistnie oraz dolistnego nawożenia azotem w połączeniu z nawozem wieloskładnikowym Ekolist na zawartość i skład białka ziarna pszenżyta jarego odmiany Milewo. Doświadczenie polowe przeprowadzono w Zakładzie Dydaktyczno-Doświadczalnym w Tomaszkowie, należącym do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Na zawartość białka ogółem i poszczególnych jego frakcji w ziarnie pszenżyta jarego odmiany Milewo miały wpływ zarówno zastosowanie dokarmiania dolistnego Ekolistem, jak i dawka azotu mineralnego. Zastosowanie azotu w dawce 80 $kg \cdot ha^{-1}$ łącznie z Ekolistem, w porównaniu z tą samą dawką, ale bez nawozu wieloskładnikowego, przyczyniło się do wzrostu ilości białka o 0,72 g w 100 g ziarna. Największy udział stanowiły białka zapasowe z przewagą gliadyn nad gluteninami. Zastosowane w doświadczeniu warianty nawozowe kształtowały wysoki stosunek gliadyn/glutenin. Wraz ze wzrostem białek zapasowych (gliadyny + gluteniny) zmniejszała się zawartość białek strukturalnych (albumin i globulin). Potwierdzono ponadto istotny wpływ całkowitej dawki azotu 120 $kg \cdot ha^{-1}$ w porównaniu z 80 $kg \cdot ha^{-1}$ na zwiększenie koncentracji białek gliadynowych frakcji ω i α/β. Lata badań wpłynęły na udział gliadyn γ oraz glutenin HMW i LMW w ziarnie. Zwiększenie nawożenia azotem o 40 $kg \cdot ha^{-1}$ przedsiewnie, w postaci saletry amonowej, przyczyniło się do zwiększenia frakcji gliadyn ω i glutenin HMW. Dodatkowe zastosowanie nawożenia Ekolistem (wariant z dawką 80 kg·ha^{-1}) wpłynęło na zwiększenie frakcji gliadyn γ i glutenin LMW.

Słowa kluczowe: białka zapasowe, Ekolist, frakcje białek, gliadyny, gluteniny, nawożenie dolistne

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