THE INFLUENCE OF CHEMICAL FERTILIZERS ON THE GLIADIN - GLUTENIN RATIO TO THE WINTER WHEAT

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Abstract

The gliadin-glutenin ratio is considered one of the quality factors of the flour. This paper proposes an analysis of the influence of chemical fertilizers with nitrogen, phosphorus and potassium, applied both singly and in interaction on this quality element. The research was carried out at Agricultural Research and Developement Statin Lovrin, on the wheat variety Ciprian. The results of the research highlight the major role of fertilizer combinations on the gliadin-glutenin ratio. If, in the case of unilateral application of the three types of fertilizer, both the gliadin / glutenin ratio and the proportion of the two gluten protein components indicated a poor or good gluten quality, by combined application of fertilizers with nitrogen and phosphorus and, especially, those with nitrogen, phosphorus and potassium, the gluten quality is very good. There is a significant negative correlation between unilaterally applied nitrogen fertilizers and gliadin glutenin ratio (r = -0.84 *). The correlation between nitrogen, phosphorus, potassium and the gliadin glutenin ratio is significantly negative, statistically assured at $\alpha = 1\%$ (r = -0.99 **).

Key words: nitrogen, phosphorus, potassium, gluten, the gliadin-glutenin ratio.

The gliadin/glutenin ratio is an important parameter for determining the gluten quality. A low value of this ratio is considered to have high-quality gluten for breadmaking, as increased amounts of gliadin produce a decrease in the bread volume (Fido et al., 1997; Uthayakumaran et al., 2001).

Environment generally has a significant influence on flour quality by its effects on relative quantity of specific proteins, protein subunits and protein groups, proportions of composition, concentration, polymerization and amount and size distribution of polymeric proteins. Temperature, water access and fertilizer are the most crucial environmental conditions (Johansson et al., 2001, 2002; Dupont and Altenbach, 2003). Environmental interactions during grain filling alter the time course for grain development and influence final grain weight, protein and starch contents (Altenbach et al., 2003). The protein quality in the grain is also affected by the entanglements of the glutenin subunits into protein macromolecules which are influenced by the environment in which the wheat plant is cultivated (Jia et al., 1996).

Variation in N application not only influences protein components i.e. glutenin and gliadins but also influences gluten strength among different cultivars (Johansson et al., 2001). Increase in protein content and gliadins to glutenin subunits ratios were found with the increase in the amount of N fertilizer (Gupta et al., 1992; Johansson et al., 2001).

Increased N availability favors the production of storage proteins such as gliadins and glutenins, where gliadins most closely correlated with total protein increase in the grain (Dupont et al., 2006a).

Gliadins increase preferentially over glutenins as N accumulation increases in the grain (Triboi et al., 2000; Wieser and Seilmeier, 1998).

The ratio of gliadins to glutenins was positively correlated with grain N content, even when both gliadins and glutenins increased (Wieser and Seilmeier, 1998).

MATHERIAL AND METHOD

The research was conducted at ARDS Lovrin, under a long-term experience (founded in 1967), on a weakly-gleizated and weakly-alkalinised semicarbonatic chernozem (pH in $H_2O = 6.90$) with a mobile P content of 75.7 ppm, mobile K of 205 ppm and a humus content of

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3.47%. The average yearly rainfall is about 500 mm, and the average temperature of 10.8 ° C.

The research was conducted on wheat variety Ciprian, created at ARDS Lovrin.

The chemical nitrogen fertilizers applied had the following graduations: N_0, N_{30}, N_{60}, N_{90} and N_{120}. They were applied fractionally: ½ spring, at restoration of the vegetation and ½ at elongation of the stem. The precursor culture was soyabean and this is why the nitrogen was not applied to the seed.

The phosphorus was applied autumn under the base layer and applied unilaterally had graduations: : P_0, P_{40}, P_{80}, P_{120} si P_{160}.

The potassium was also applied autumn under the base and the following doses were administered: K_o, K_{40}, K_{80} si K_{120}.

The nitrogen-phosphorus combinations are N_0P_o, N_0P_{80}, $N_{30}P_{80}$, $N_{60}P_{80}$, $N_{90}P_{80}$,, $N_{120}P_{80}$, and nitrogen-phosphorus-potassium are $N_0P_0K_0$, $N_{60}P_{80}K_{80}$, $N_{120}P_{80}K_{40}$ si $N_{120}P_{80}K_{80}$.

Wheat samples were milled and the obtained flour was used for further analysis.

For the extraction of gliadins and glutenins, Lab-on-a-Chip (LoaC) technique was used.

This system has the potential for a fast, reliable, and automatable analysis in the field of proteins' separation and quantification (H. Goetz et al, 2004, J. S. Hey, 2007, Živančev, 2015).

The percentage of gliadin and glutenin subunits was determined from 30 mg of flour after removal of albumins and globulins. The gliadins were subsequently extracted with 300 μL of 70% ethanol and 200 μL was transferred into test tube (1.5 mL), whereas the rest of the solution was removed for glutenin extraction. After evaporation of ethanol, gliadins were treated with 350 μL of 2% SDS solution containing 5% β-mercaptoethanol and afterwards heated for 5 minutes to 100◦C. For extraction of full range of the glutenin subunits the same volume of treatment solution (2% SDS solution containing 5% β-mercaptoethanol and 0.0625M Tris-base) and temperature conditions was used.

Final solutions of glutenins were prepared by mixing of 4 μL of the clarified sample extract with 2 μL of Agilent sample buffer and 84 μL of deionized water. Separation of proteins was performed using chip electrophoresis techniqueon Agilent 2100 Bioanalyzer with Protein 230 Plus Lab-on-a-Chip kit, which determined molecular weights of proteins in range from 12.5 to 230 kDa. After analysis, every subunit was manually integrated and their percentage was calculated from the time-corrected area.

The statistical interpretation of the results was made using the variant analysis method (ANOVA).

RESULTS AND DISCUSSIONS

The gliadin/ glutenin ratio has been considered since the beginning of the century as a quality factor for flour.

Table 1

The influence of nitrogen fertilizers on the gluten quality

Variant	Glutenin %	Gliadin %	Gliadin/glutenin	%	Diff.	Semnif.
V1 – control (unfertilized)	**14.67**	**85.33**	**5.8**	100	**mt**	
V2 – fertilized with N_{30}	22.21	77.79	**3.5**	60.3	-2.3	
V3 – fertilized with N_{60}	38.04	61.96	**1.62**	27.9	-4.18	*
V4 – fertilized with N_{90}	31.49	68.51	**2.17**	37.4	-3.63	*
V5 – fertilized with N_{120}	35.10	64.89	**1.84**	31.7	-3.96	*

The gliadin/glutenin ratio **DL 5% =**3.46**; DL 1% =**5.73**; DL 0,1% =** 10.72

In 1980, MacRichie separated the gluten proteins from strong and weak flours into two fractions based on the solubility difference in dilute acetic acid solution, the supernatant containing gliadin in particular, and the precipitated glutenin. By changing the ratio of these fractions and keeping the total protein content constant, he found in the reconstructed flour that the dough development time, determined with the mixograph, correlates directly with the glutenin content.

Later, Kim et al. (1988) have obtained similar results using 70% ethanol to separate gliadin to glutenin from gluten. They have shown that it is possible to greatly change the quality of gluten by changing the proportion of glutenin / gliadin.

Table 1 shows the influence of nitrogen, applied unilaterally, on gliadin glutenin ratio. After 50 years of non-fertilizer application, the gliadin glutenin ratio is 5.8 and the proportion of the two types of gluten proteins is: 14.67% glutenin and 85.33% gliadin. According to the literature, the ratio of 80% gliadin and 20% glutenin indicates a very poor quality gluten (Popescu, 2007). The same aspect is also found in the fertlized variant with only 30 kg nitrogen active substance / ha. With the increase in nitrogen doses, the ratio drops to 1.62 in N60 and 1.84 in N120. The proportion of 68% gliadin and 32% glutenin indicates a good gluten quality. We can say that there is a significant negative correlation between the doses of nitrogen used and the gliadin glutenin ratio (r = -0.84 *).

Table 2

The influence of phosphorus fertilizers on the gluten quality

Variant	Glutenin %	Gliadin %	Gliadin/glutenin	%	Diff.	Semnif.
V1 – control (unfertilized)	14.67	85.33	**5.8**	100	**mt**	
V2 – fertilized with P_{40}	22.21	77.79	**3.5**	60.3	-2.3	
V3 – fertilized with P_{80}	30.30	69.70	**2.29**	39.5	-3.51	*
V4 – fertilized with P_{120}	28.5	71.49	**2.5**	43.1	-3.3	*
V5 – fertilized with P_{160}	26.48	73.51	**2.77**	47.8	-3.03	

The gliadin/glutenin ratio DL 5% = 3.14; DL 1% = 5.21; DL 0,1% = 9.74

Table 3

The influence of potassium fertilizers on the gluten quality

Variant	Glutenin %	Gliadin %	Gliadin/glutenin	%	Diff.	Semnif.
V1 – control (unfertilized)	14.67	85.33	**5.8**	**100**	**mt**	
V2 – fertilized with K_{40}	49.61	50.39	**1.01**	17.4	-4.79	**
V3 – fertilized with K_{80}	48.79	51.21	**1.04**	17.9	-4.76	**
V4 – fertilized with K_{120}	43.48	56.52	**1.29**	22.2	-4.51	**

The gliadin/glutenin ratio DL 5% = 4.87 ; DL 1% =8.38 ; DL 0,1% = 19.80

Table 2 shows the influence of phosphorus on gliadin glutenin ratio. The difference from the non-fertilized control is significant in the fact that the doses of nitrogen 80 and 120 kg of active substance / ha are used. The proportion of 30% glutenin and 70% gliadin indicates a good gluten quality.

The unilateral application of chemical fertilizers with potassium indicates distinctly significant differences compared to the unfertilized control variant.

The potassium doses of 40, 80 and 120 kg of active substance / ha determine a gliadin glutenin ratio between 1.01 and 1.29 and a proportion of the two gluten proteins ranging from 68% gliadin and 32% glutenin, corresponding to a very good gluten qualit (Table 3).

Table 4

The influence of nitrogen-phosphorus-potassium combinations on the gluten quality

Variant	Glutenin %	Gliadin %	Gliadin/glutenin	%	Diff.	Semnif.
V1 – control (unfertilized)	14.67	85.33	**5.8**	**100**	**mt**	
V2 – fertilized with $N_{60}P_{80}K_{80}$	42.90	57.10	**1.33**	22.9	-4.47	**
V3 – fertilized with $N_{120}P_{80}K_{40}$	47.64	52.36	**1.09**	18.8	-4.71	**
V4 – fertilized with $N_{120}P_{80}K_{80}$	49.20	50.80	**1.03**	17.8	-4.77	**

The gliadin/glutenin ratio DL 5% =1.67 ; DL 1% =2.77 ; DL 0,1% = 5.1

Table 5

The influence of nitrogen-phosphorus combinations on the gluten quality

Variant	Glutenin %	Gliadin %	Gliadin/glutenin	%	Diff.	Semnif.
V1 – control (unfertilized)	14.67	85.33	**5.8**	**100**	**mt**	
V2 – fertilized with N_0P_{80}	19.12	80.88	**4.22**	72.8	-1.58	
V3 – fertilized with $N_{30}P_{80}$	43.69	56.31	**1.28**	22.1	-4.52	*
V4 – fertilized with $N_{60}P_{80}$	43.31	56.69	**1.3**	22.4	-4.5	*
V5 – fertilized with $N_{90}P_{80}$	47.93	52.07	**1.1**	19.0	-4.7	*
V6 – fertilized with $N_{120}P_{80}$	43.81	56.19	**1.28**	22.1	-4.52	*

The gliadin/glutenin ratio **DL 5% =** 3.29 **; DL 1% =**5.17**; DL 0,1% =** 8.79

By using fertilizer mixtures with nitrogen and phosphorus and with nitrogen, phosphorus and potassium, there is considerable improvement in the quality of gluten, rather than the unilateral application of gluten.

Table 5 presents the influence of nitrogen - phosphorus combinations. The combinations of the two types of fertilizer are positively correlated with the glutenin content of the gluten (r = 0.84 *). Between the glutenin ratio and gliadin glutenin ratio there is a distinctly significant negative correlation (r = -0.93 **), statistically assured at α = 1%.

The value of the gliadin / glutenin ratio and the the proportion of gliadin and glutenin of the gluten structure indicate a very good quality. By the combined application of the three types of fertilizer (Table 4), in all three experimental variants the proportion of gluten structural proteins and the value of their ratio indicated a very good gluten quality.

The correlation between nitrogen, phosphorus, potassium and the gliadin glutenin ratio is distinctly significant negative , statistically assured at α = 1% (r = -0.99 **).

CONCLUSIONS

This study highlights the need for combined application of chemical fertilizers for a good quality gluten. . If, in the case of unilateral application of the three types of fertilizer, both the gliadin / glutenin ratio and the proportion of the two gluten protein components indicated a poor or good gluten quality, by combined application of fertilizers with nitrogen and phosphorus and, especially, those with nitrogen, phosphorus and potassium, the gluten quality is very good.

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