THE INFLUENCE OF NITROGEN CHEMICAL FERTILISERS ON WINTER WHEAT GLUTEN QUALITY

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Abstract

This paper aims to show the influence that nitrogen fertilizer has on the quality of wheat. Research has been carried out at Lovrin Agricultural Development Research Center, introducing a long-lasting fertilizer experience between 2015 and 2016 on Ciprian's wheat variety. The applied nitrogen fertilizers had the following graduations: 0, 30, 60, 90, 120 kg nitrogen active substance/ha. Speaking about the quality of wheat, it is inappropriate to refer only to the percentage of protein and wet gluten. The gluten structure is important for a high quality raw material in the milling and bakery industry. Chemical nitrogen fertilizers do not only influence the amount of wet gluten but also that of gliadins, glutenins, the accumulation of high molecular weight glutenin subunits and gliadin - glutenin ratio. Between the doses of nitrogen applied, the amount of glutenin and the proportion of HMW units of glutenin there is a significant positive correlation, statistically assured at $\alpha = 5\%$ ($r = 0.95$ * for glutenin, $r = 0.90$ * for HMW subunits).

Key words: gliadins, glutenins, chemical fertilizers, wet gluten.

In order to obtain wheat with superior quality indices, besides the genetic characteristics of the varieties and the favorable climatic conditions, there is also a need for an appropriate technology. Chemical nitrogen fertilizers significantly affect both production and quality of winter wheat.

Of the total protein, gluten proteins are the ones that most influence the quality of wheat baking.

It is known that two proteins condition the quality of gluten and bread: gliadin that belongs to prolamins and glutenin which belongs to glutamines. (Ceapoiu, 1984)

Gliadines give the dough extensibility and viscosity, and glutenins are responsible for elasticity and resistance (Tazzini, 2015).

Gliadines seem to have less influence on the technological behavior of the flour than glutenin. If the gliadin fraction is interchanged between different flavors of flour, the effect on bread volume is very small compared to the case of glutenin interchange (MaRichie, 1980).

The influence of glutenin on the bakery attributes of the flour is much higher, being the main component influencing the time of framing and the quality of bread. Its influence is given by the gliadin/glutenin ratio, the molecular weight distribution and the presence of high molecular weight glutenin subunits (HMW) (MaRichie, 1980).

Houbner and Wall (1976) and Bottomleyet et al. (1982) separated glutenin into two fractions: high molecular weight subunits (HMW) and low molecular weight subunits (LMW), and found a direct relationship between the proportion of HMW subunits and bakery attributes for some wheat varieties. Similar results have also been obtained by Singh et al. (1990), a poor quality of wheat correlates with the low amount of HMW glutenin.

LMW subunits, although having genes similar to those of gliadin, positively influence the quality of the meal, to a greater extent than gliadin (Singh et al., 190).

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 with a mobile P content of 75.7 ppm, mobile K of 205 ppm and a humus content of 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.

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The chemical nitrogen fertilizers applied had the following graduations: N0, N30, N60, N90 and N120. These were applied in a fractional manner: ½ on early spring and ½ at the straw elongation. The precursor culture was soybean and this is why the nitrogen was not applied at sowing.

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

Pentru extractia gliadinelor si a gluteninelor s-a folosit Lab-on-a-Chip (LoaC) technique. 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.

Interperetarea statistica a rezultatelor s-a facut dupa metoda analizei variantei (ANOVA).

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

RESULTS AND DISCUSSIONS

Nitrogen fertilization decisively influences the quality of wheat. It is known that the type and amount of proteins accumulated during grain filling have a genetic component, but, also, the environmental growing conditions, particularly N fertilization, may cause changes in the quantity and distribution of these proteins (Garcia-Molina, 2017). Wieser and Seilmeier (1998), Garcia-Molina (2017) reported that the composition of the protein fractions was more influenced by the N nutrition than by temperature.

Between 2015 and 2016 the percentage of protein varies between 11.5% and 15.1%, and wet gluten registers values from 24.8% on the unfertilized variant to 35.1% on the variant fertilized with 120 kg of active substance/ha of nitrogen.The influence of nitrogen on the two qualitative indices is presented in Table 1.

Table 1

Influence of nitrogen on the percentage of protein and wet gluten

Nitrogen Kg/ha	Protein					Wet gluten				
	2015	2016	2015-2016	Diff.	Semniff.	2015	2016	2015-2016	Diff.	Semniff.
N_0	10.9	12.1	11.5	mt		21.4	28.1	24.8	mt	
N_{30}	11.2	12.7	11.9	0.4		24.1	30.5	27.3	2.5	
N_{60}	11.7	13.4	12.6	1.1	*	25.9	32.3	29.1	4.3	*
N_{90}	15.1	14.4	14.8	3.3	***	34.5	32.3	33.4	8.6	***
N_{120}	15.5	15.1	15.3	3.8	***	35.8	34.4	35.1	11.3	***

Protein: DL 5%=1.66; DL 1%=2.76; DL 0.1 %=5.16. Gluten:DL 5%=0.65; DL 1%=0.89; DL 0.1 %=1.21.

Analyzing Table 2, we can assert that the regression for protein and gluten (regression equation) is statistically assured, according to test t, at $\alpha = 0.1\%$.

According to the regression coefficient we can state that nitrogen influences positively the accumulation of protein and gluten; by increasing with 1 kg of N protein increases by 0.03% (regression coefficient = 0.0309) and gluten by 0.09% (regression coefficient = 0.09017).

When talking about the quality of wheat, it is inappropriate to refer only to the percentage of protein or wet gluten.

The quality of gluten is due to the presence of the two gluten proteins.

Table 2

Examining the multiple equation of regression - protein and gluten – 2015-2016

	Correlation Coefficient	Standard Correlation Coefficient Error	Regression Coefficient (b)	Standard Regression Coefficient Error	Test t	
					Value	Semniffication
Control			24.60200	0.849315	28.96687	***
N - gluten	0.792965	0.078733	0.09017	0.008953	10.07160	***
Control			11.32400	0.237084	47.76361	***
N - protein	0.837459	0.067731	0.03090	0.002499	12.36451	***

The gliadins, which provide extensibility and viscosity to the dough, are monomeric and they are classified into three structural fractions: a/b-, &-,and g-gliadins. The glutenins, responsible for dough elasticity, are present as polymeric complexes linked by disulphide bonds and comprise two types of subunits; the HMW and the LMW subunits of glutenin.

Under the action of nitrogen, the accumulation of gliadins and glutenins changes, also the accumulation of glutenin subunits HMW. Table 3 lists these results.

Gliadines correlate positively with nitrogen, in the unfertilized control, their value being 26.9 g/100 g flour and progressively increases up to 34.5 g/100 g flour, in the variant fertilized with 120 kg/ha of nitrogen.

Table 3

Influence of nitrogen on the gluten structure

Doza azot	Gliadin		Glutenins		HMW		LMW	
	g/100 g flour	Semnif	g/100 g flour	Semnif	g/100 g flour	Semniff.	g/100 g flour	Semniff.
N_0	**26.9**		**7.6**		**0.4**		**5.1**	
N_{30}	**32.7**		**8.6**		**1.0**		**7.6**	
N_{60}	**24.8**		**11.8**		**2.1**		**9.8**	*
N_{90}	**29.8**		**13.9**	*	**3.4**		**10.3**	*
N_{120}	**34.5**	*	**17.1**	**	**4.8**	*	**12.0**	*

Table 4

The matrix of correlation coefficients

	N	Glutenin	Gliadin	HMW	LMW
N	1.00	0.95*	0.89*	0.90*	1.00
Glutenin		1.00	0.95*	0.99***	0.97**
Gliadin			1.00	0.95*	0.90*
HMW				1.00	0.92*
LMW					1.00

With increasing the fertilizer dose, the proportion of glutenin, the protein responsible for the dough elasticity, also increases. In the control variant, the gluten value is 7.6 g/100 g flour and increases to 17.1 g/100 g flour in the N120 variant.

This increase is statistically assured as very significant at $\alpha = 0.1\%$.The accumulation of glutenin HMW subunits increases with increasing the nitrogen dose. In the N120 version, their value is 4.8 g/100 g flour, 4.4 g more than in the unfertilized version where their value is only 0.4 g/100 g flour.The LMW subunits range from 5.1 to 12 g/100 g flour, the increase being statistically significant starting with the N60 nitrogen dose.

Analyzing the two electroforeforms (Fig.1, Fig.2) corresponding to glutenin for 2015, one can observe its distribution at the molecular level. HMW subunits are considered to be between 100 and 240 Kda. In the control variant, glutenin is only available in the range of 4.5 - 6.3 Kda, while in the variant fertilized with 120 kg nitrogen/ha, the total HMW subunits is 674.20 ng/µl, ie 28.7% of the total 2348.9 ng/µl glutenin.

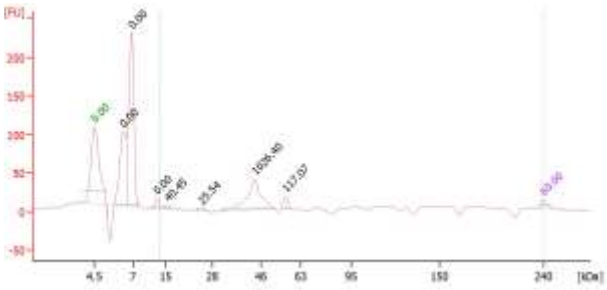

Figure 1. **The glutenin electrophoregram for the variant fertilized with N0 (2015)**

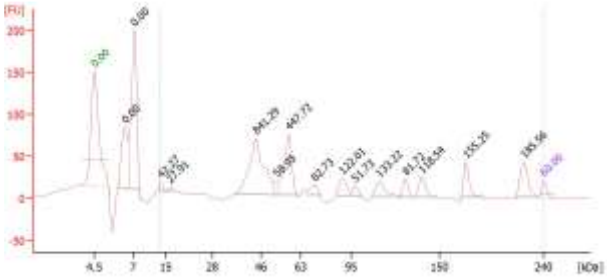

Figure 2. **The glutenin electrophoregram for the variant fertilized with N120 (2015)**

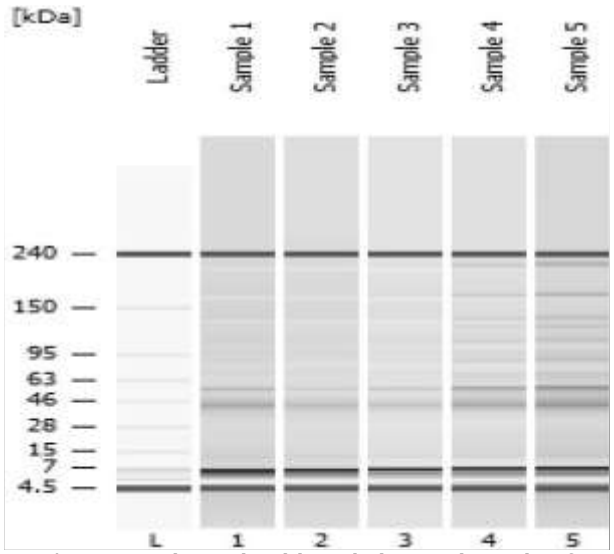

Figure 3. **Polyacrylamide gel electrophoresis of glutenin on the five agrofonds**

CONCLUSIONS

Nitrogen chemical fertilizers decisively influence the quality of wheat, correlating positively with the percentage of protein and gluten, which are statistically assured as distinctly significant.

The structure of gluten is also influenced by nitrogen fertilizers. Accumulation of the two storage proteins and the distribution of glutenin subunits at different levels of molecular weight is also correlated positively with the applied nitrogen dose.

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