THE INFLUENCE OF CHEMICAL FERTILIZERS WITH NITROGEN AND PHOSPHORUS ON GLUTEN STRUCTURE AT THE WHEAT

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Abstract: *This paper presents the results of the research on nitrogen and phosphorus fertilization over the gluten quality to the winter wheat. The research was carried out between 2015-2016 at the Agricultural Research Station Lovrin, in a long-term experience with chemical fertilizers. Nitrogen and phosphorus fertilizers, both alone and in interaction, directly influence the quality of wheat (Triticum aestivum L.). From the total protein, gluten proteins have the most important role in ensuring the rheological properties of the dough. In this work, we highlight the influence of chemical fertilizers on the accumulation of gliadins and glutenins, HMW and LMW-glutenin subunits and on the gliadin-glutenin ratio. The nitrogen doses used are 30.60.90,120 kg active substance N / ha, applied to a phosphorus support 80 kg P_2O_5/ ha.*

Keywords: *winter wheat, fertilizers, glutenin, gliadin, content of gluten.*

INTRODUCTION

The long-term fertilization system, utilized in stationary experiments, is the most complex method of highlighting the quantitative and qualitative degree of supply and soil nutrient balance. Also, its effect on productivity, production quality and plant health is well known (HERA, 1980, POPESCU, 1981).

Wheat flour is used to make a wide variety of products including cakes, pasta, bread, noodles, and biscuits, which is possibly because of the gluten proteins in wheat, which consist of gliadins and glutenins (SHEWRY ET AL., 2009,DOLORES, 2017).

The gluten proteins are the most important storage proteins of a wheat grain, to be found exclusively in the starchy endosperm, the part of the grain that is ground into flour (GOESAERT ET AL.,2005).. During the ripening process the proteins combine with each other forming large polymers and when the flour is mixed with water while being worked into dough they build a continuous network of proteins. This network of protein molecules gives flexibility and viscositu to the dough, making it possible for instance to produce raised bakery products (TOSI AT AL.,2011, HORVATH, 2014).

Wheat proteins are conventionally assigned to two different groups: the density and extensibility of the dough is determined by the monomeric gliadins, while its flexibility is determined by polymeric gluteins (HORVATH, 2014).

Although some authors have associated specific gliadin alleles with the quality of the final product (bread), it has been agreed that these proteins do not have a direct impact on the wheat quality in terms of dough strength. It is assumed that this is a result of close genetic linkage between
low molecular weight glutenin subunits (LMW−GS) and gliadins (GIANIBELLI ET AL., 2001; TOMIC,2011).

Glutenins are polymeric proteins. They consist of two types of molecules: high molecular weight glutenin subunits (HMW−GS) and low molecular weight glutenin subunits (LMW−GS) (Song et al., 2007) which are interconnected with disulphide bonds and possess elastic properties (TOMIS,2011).

Glutenin subunits of large molecular weight are quantitatively minor components, but are crucial in the process of breadmaking since they determine the elasticity of gluten. LMW−GS compose about one−third of the total protein content of grain, and approximately 60% of the total glutenin. Despite their large quantity, they have been given less attention in research than the HMW−GS. This is mainly a consequence of the difficulties that arise in their identification of the one−dimensional SDS−PAGE gels (GIANIBELLI ET AL., 2001) and newer techniques, such as automated capillary chip electrophoresis allow improved characterization of all the glutenin complex subunits (TOMIS,2011).

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

Each year, from the installation of the experimental device, the following doses of fertilizers were applied: nitrogen (N_0, N_{30}, N_{60}, N_{90}, N_{120})(and phosphorus (P_0, P_{40}, P_{80}, P_{120}, P_{160}).

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

In this experience samples from the following experimental variants were tested: N_0P_0, N_0P_{80}, $N_{30}P_{80}$, $N_{60}P_{80}$, $N_{90}P_{80}$, and $N_{120}P_{80}$.

The phosphorus was applied autumn, under basic plowing and the nitrogen was applied fractionally: ½ to the resumption of vegetation and ½ at the elongation of the stem. The precursor culture was soybean and this is why the nitrogen was not applied to sowing.

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 30mg 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 (ŽIVANČEV, 2015).

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

RESULTS AND DISCUSSIONS

RAGASITS ET AL., 2000, CITID BY HORVATH in 2014, show that increasing the amount of the P and the N fertiliser has a remarkable impact on the quantity, composition and quality of the yield. These impacts depend on the agro-ecological features of the area concerned. Even the application of a smaller dose of fertiliser triggers an increase in yield, while a higher dose generates a quality improvement as well.

Table 1 shows the variation of the two grain quality indexes (protein and wet gluten) on the six fertilization levels analyzed. The percentage of protein varies between 10.9% and 15.8%. The variation from the non-fertilized

control is distinctly significant in the fertilized variant with 120 kg N / ha and 80 kg P / ha and significant, starting at a nitrogen dose of 60 kg N / ha.

It is well known that with the increase in protein percentage increases and the percentage of wet gluten. The variation in wet gluten from 21.4% to 36.2% demonstrates the decisive role of fertilization on wheat quality.

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).

Table 1

The influence of chemical fertilizers with nitrogen and phosphorus on grain quality indices

Variant	Content of gluten %	%	Diff.	Signif.	Content of protein %	%	Diff.	Signif.
V1 – control (unfertilized)	21.4	100	-	-	10.9	100	-	-
V2 – fertilized with N_0P_{80}	21.6	101	0.2	n.s.	11.0	101	0.1	n.s.
V3 – fertilized with $N_{30}P_{80}$	27.6	129	6.2	n.s.	12.6	116	1.7	n.s.
V4 – fertilized with $N_{60}P_{80}$	29.4	137	8.0	*	13.2	121	2.3	*
V5 – fertilized with $N_{90}P_{80}$	30.1	141	8.7	***	13.6	125	2.7	*
V6 – fertilized with $N_{120}P_{80}$	36.2	169	14.8	***	15.8	145	4.9	***

Protein: **DL 5% = 2.12 : DL 1% = 2.86 ; DL 0.1% = 3.83;** Gluten: **DL 5% = 6.34 : DL 1% = 8.58 ; DL 0.1% = 11.46**

Table 2 shows the influence of fertilizers with nitrogen, on a general phosphorus agrofond of 80 kg P_2O_5 / ha, on the accumulation of the two gluten proteins: gliadin and glutenin. Glutenin variation takes place between 6.62 and 22.3 g / 100 g flour, variance statistically ensured as significant in variants 3 and 4 and distinctly significant in variants 5 and 6. In variant 2, by applying only 80 kg P_2O_5 / ha and in the absence of fertilizers with nitrogen, the value of glutenin increases to 14.9 g / 100 g flour, compared to 6.62 g / 100 g flour in the control variant. With the increase in the amount of glutenin, there is a decrease in the amount of gliadin, decrease assured statistically as significant. In control, the gliadin records a value of 38.5 g / 100 g flour, reaching in fertilized variant with 120 kg N / ha at 28.6 g / 100 g flour, with 26% less than control.

Table 2

The influence of chemical fertilizers on the accumulation of gluten proteins

Variant	Glutenins g/100 g flour	Significance	Gliadins g/100 g flour	Significance
V1 – control (unfertilized)	6.62		38.5	
V2 – fertilized with N_0P_{80}	14.9	*	63	***
V3 – fertilized with $N_{30}P_{80}$	17.3	**	22.3	00
V4 – fertilized with $N_{60}P_{80}$	20.7	**	27.1	0
V5 – fertilized with $N_{90}P_{80}$	23.2	***	25.2	0
V6 – fertilized with $N_{120}P_{80}$	22.3	***	28.6	0

Glutenins DL 5% =5.78 **; DL 1%** =9.06 **; DL 0,1%** = 15.41
Gliadins DL 5% =9.05 **; DL 1%** 14.20**; DL 0,1%** =24.15

For high quality of bakery products,a high wheat content in the protein is not enough. Dough rheology is largely influenced by the presence of HMW and LMW glutenin subunits and also by their proportion in the glutenin structure.

With the increase in nitrogen doses and in the presence of phosphorus, the proportion of HMW subunits increases from 0.48 to 6.39 g / 100 g of flour (Table 3), increased statistically assured only in fertilized variants with 90 kg N / ha and 120 kg N / ha and, of course, in the presence of phosphorus.

The accumulation of LMW glutenin subunits is statistically ensured in all experimental variants and shows an increase from 1.94 g / 100 g flour to 17.46 g / 100 g flour.

Between the chemical fertilizers applied and the accumulation of glutenin, is established a significant positive correlation (r = 0.84 *), statistically assured at α = 5%, and with the proportion of the glutenin HMW subunits a very significant positive correlation (r = 0.898 ***) statistically assured at α = 0.1 % (Table 4).

Table 3

The influence of fertilization on glutenin structure

Variant	Glutenins ng/µl		Glutenins g/100 g flour							
	HMW	LMW	HMW	%	Diff.	Signif.	LMW	%	Diff.	Signif.
V1 – control (unfertilized)	64.2	259.5	**0.48**	**100**	**mt**		**1.94**	**100**	**mt**	
V2 – fertilized with N_0P_{80}	220.1	1774.6	**1.65**	343.8	1.17		**13.30**	685.6	11.36	**
V3 – fertilized with $N_{30}P_{80}$	346.1	1973.6	**2.59**	539.6	2.11		**14.80**	762.9	12.86	**
V4 – fertilized with $N_{60}P_{80}$	499.2	2263.2	**3.74**	779.2	3.26		**16.97**	874.7	15.03	***
V5 – fertilized with $N_{90}P_{80}$	760.1	2328.4	**5.70**	1187.5	5.22	*	**17.46**	900.0	15.52	***
V6 – fertilized with $N_{120}P_{80}$	852.3	2114.6	**6.39**	1331.3	5.91	**	**15.85**	817.0	13.91	**

HMW - DL 5% =3.53**; DL 1%** =5.53**; DL 0,1%** = 9.41
LMW - DL 5% =5.60**; DL 1%** =8.78**; DL 0,1%** =14.93

Table 4

The matrix of correlation coefficients

	NP	Glutenin	Gliadin	HMW	LMW	Gliadin/glutenin
NP	1.00	0.84*	-0.60	0.98***	0.65	-0.77
Glutenin		1.00	-0.47	0.91**	0.95**	-0.93**
Gliadin			1.00	-0.53	-0.33	0.70
HMW				1.00	0.75	-0.82*
LMW					1.00	-0.90*
Gliadin/glutenin						1.00

Between glutenin and the accumulation of HMW and LMW subunits there is a significant positive correlation ($r=0.91^{**}$ for HMW and $r=0.95^{**}$ for LMW). Between the glutenin/gliadin ratio and glutenin is established a significant negativ corelation ($r=-0.93^{**}$), statistically assured at the level $\alpha=0.1$ %.

Acknowledgements

The research was performed in ``Horia Cernescu`` Research Laboratories (established by infrastructure project POSCCE SMIS 2669) with financial support of research project 10/19.06.2016.

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