Szent István University

Impact of nitrogen supply on the performance of quality parameters and protein compounds of five winter wheat (*Triticum aestivum* L.) varieties

Doctoral (PhD) Thesis

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1. BACKGROUND AND AIMS

Wheat is one of the most important cereals in the world. Its role is crucial in the alimentation of the human race. Wheat production of the Earth has exceeded 700 million tons in 2013. Because of its favourable properties bread made from wheat is a basic food all over the world. It is essential to achieve as higher yield as possible and producing an outstanding quality flour in order to meet the growing consumers' needs. Yield potential and quality are genetically determined characteristics of all crops. Quality can be spoiled or manifested by agronomic traits, but never improved. The basis of good quality depends on the production of a proper variety. The expression of the genetically determined good properties are influenced by environmental factors. The goal of the application of the agronomical methods is the enforcement of expression of the preferential genetic aptitude.

Among the environmental factors plant nutrition has an outstanding role; the fertilisation is an effective tool to promote the expression of the properties of quality. In case of winter wheat the harmonic nutrition – even beside good water and nutrient management – is a cardinal yield increasing element. The effectiveness of fertilisation is influenced by agro-ecological, biological and agro-technical factors. According to the literature among the plant nutrient products the N fertilisers play a prominent role.

The nitrogen fertilisation increases the crop yield, farinograph value, grain protein and wet gluten content, the vitreousness, the thousand-grain weight and even the starch content. N fertilisation has an impact on the growth of albumin and globulin content and on the amount of gliadins and glutenins as well. N treatments are suitable to influence the gluten content, the stability and the shelf life of the flour. During the formation of the dough more disulphide bonds are being formed, resulting in a higher degree of polymerisation. The increasing and – according to the literature – the multiple doses of nitrogen top dressing results in quality improvement even under adverse environmental conditions.

Gluten, the material known for almost 300 years, consist of proteins, the most important storage substances of the ripe wheat grain. The baking quality is determined by the quantity and quality of these proteins. The wheat proteins can be separated by their size. The wheat flour contains 45% of glutenins, 45% of gliadins and 10% of soluble proteins commonly. The density and extensibility of the dough is determined by the monomeric gliadins, while its flexibility and strength is determined by polymeric glutenins. Proteins are concentrated predominantly in the subaleuron layer of the ripe grain. Accordingly, the endosperm is not a homogeneous tissue and its key components (proteins, starch and cell-wall polysaccharides) show a qualitative and a quantitative gradient as well. The application of nitrogen fertilisers however, affects the expression patterns within the grain. Similarly to other wheat storage proteins its prolamins are also polymorphic, encoded by multigene families that are present in the form of homeologous alleles on three genomes.

Considering the important role of the top dressing in determination of wheat quality, and it's relatively high cost, we should find the most effective solution for supplying nutrients to serve useful information for practical agriculture. Although there were many studies done on this topic in Hungary, it still seemed to be expedient to update our knowledge through testing less studied varieties of winter wheat. In addition to the traditional quality indicators, the quantitative development of the components of gluten proteins, gliadins and glutenins should be observed. The role of the latter are crucial in the determination of the quality of winter wheat varieties, however the scientists spent less energy recently on studying of the changing ratio of these proteins as a result of fertilisation.

We set the objective to investigate the change in qualitative parameters within the proportion and composition of storage proteins of different wheat varieties due to different doses of nitrogen fertiliser – in undivided and split treatment ¬ of several crop years. Since the practical use of near infrared (NIR) devices has an increasing importance, we planned to compare the results derived from those

NIR tools with the data come from measurement of quality parameters of winter wheat with Kjeldahl analytical method.

2. MATERIALS AND METHODS

2.1 The circumstances of the field trials

In a long term field trial a wide range of high milling and baking quality winter wheat (*Triticum aestivum* L.) varieties were examined under identical agronomic conditions and different levels of N fertiliser. The small plot trials were run at the Nagygombos 5 hectares of experimental field of the Szent István University, Crop Production Institute, Hungary. Soil type of the experimental field is chernozem (calciustoll).

The size of each plot was 10 m^2 (1x10 m), the experiments were conducted in split-plot design with three replications. Various identical agronomic treatments were applied to plots.

2.2 The studied winter wheat varieties

Five high baking quality winter wheat varieties have been investigated. Beside of intensive cultivars from Maronvásár (Mv Magdaléna, Mv Suba, Mv Toborzó, Mv Toldi) a semi intensive winter wheat variety, Alföld-90 was involved in the study.

2.3 Meteorological features of the investigated crop years

1. Table Meteorological features of the first crop year

Year	2012				2013							
Season	Autumn			Winter			Spring			Summer		
Month	9.	10.	11.	12.	1.	2.	3.	4.	5.	6.	7.	8.
Mean tempera-ture	***18-19***	***11-12***	7-8	-1,5 - (-2)	-0,5 - (-1)	***+2 - (+3)***	3-4	***12-13***	16-17	20-21	***22-23***	***22-23***
Precipi-tation	50-55	60-70	***15-20***	50-55	55-70	70-80	90-100	***25-30***	90-100	100-120	***5-10***	80-90

LEGEND
The value corresponds to the average of many years
At least 20% more rainfall, or 1°C lower temperature
At least 20% less rainfall, or 1 ° C higher temperature

At the experimental field in the first investigated year (1. Table) at sowing and germinating time adequate amounts of rain fell, and this – together with relatively high temperature – was favourable at the early stages of development. Apart from the arid period in April and July there was sufficient rain at the site, and the temperature was average or a bit higher. There was rather hot summer, and no precipitation was experienced during the period of harvest.

2. Table Meteorological features of the second crop year

Year	2013				2014							
Season	Autumn			Winter			Spring			Summer		
Month	9.	10.	11.	12.	1.	2.	3.	4.	5.	6.	7.	8.
Mean temperature	***14-15***	***12-13***	7-8	+1-(+2)	+2-(+3)	***+4-(+5)***	9-10	***12-13***	15-16	19-20	***22-23***	***19-20***
Precipitation	25-30	35-40	***60-70***	5-10	40-45	60-65	5-10	***30-35***	90-95	30-35	***85-90***	80-90

The second crop year was much drier (**Hiba! A hivatkozási forrás nem található.**) compared to the first one. There was extremely little rain in September and in December, and in the spring in March and April. In addition to all this the temperature was well above the long-term average at the experimental field.

2.4 Treatments: top dressing with nitrogen fertiliser

The aim of the top dressing treatment was to evince the impact of nitrogen fertiliser on quality and quantity of crop. N topdressing variants were applied by single and repeated topdressings in the years of 2013 and 2014. In case of undivided N supply, fertilisation was done during tillering stage. The divided treatment was performed during tillering and in the stage of heading. The experiments were conducted in three replications, but in 2013 – because of loss treatment of other but otherwise identical experiments – there was an opportunity to evaluate nine replications. The applied fertiliser was granular ammonium nitrate with 34% content of active ingredient.

N topdressing variants were applied by single and repeated topdressings representing 5 levels: 0, 80 120, kg/ha N in single applications, whereas 80+40 and 120+40 kg/ha N in two applications. We did all the treatments on all varieties to study the performance of these cultivars under different agronomical impacts.

2.5 Investigations

Among the typical quality parameters the grain protein, including proportion of gliadins and glutenins and the wet gluten content were determined. Parameters of the storage proteins were compared to the applied treatments.

2.5.1 The crop yield, the hectolitre- and the thousand-grain weight

The crop yield, the hectolitre- and the thousand-grain weight was defined and evaluated according to the Hungarian standards.

2.5.2 The grain protein

The protein content was determined by three different methods. Two of them were near infra-red (NIR) quick test and the third was the Kjeldahl analytical test. In this way, there was an opportunity to compare the reliability of those different methods.

2.5.2.1 The protein yield

The protein yield was computed from the crop yield on the basis of the proportion of grain protein determined by the Kjeldahl analytical method and the NIR methods.

2.5.2.2 PAGE – definition of gliadin and glutenin proteins

The examination of gliadin and glutenin proteins of the grain samples took place in the laboratory of the National Food Chain Safety Office with polyacrylamide gel electrophoresis (PAGE).

2.5.3 Determination of farinographic value

A Valorigraph instrument was used. The equipment is useful for determination simultaneously of kneaded dough's formation time, the gluten's quality and the gluten's over mixing time.

2.6 Statistical evaluation

The data were evaluated with MS Excel software package. There was calculated the dimensionless Pearson's correlation coefficient (r) (its value can be between -1, 0 and 1, 0 included the borders as well). We determined if there is a significant difference with one-way analysis of variance. With other words this proved if there is any significant correlation between the observed parameter and the treatment.

3. RESULTS

3.1 Correlation between the N treatment and the quantitative and qualitative properties

3. Table Correlation between the N top dressing treatment and the quantitative and qualitative properties of the investigated five cultivars in 2013

Correlation of N top dressing treatment and the qualitative properties of the investigated five cultivars in 2013																	
Cultivars	[t/ha]	Hecto-litre weight [kg/hl]	thousa nd-grain weight [g]	Determined with Mininfra method				Determined with Instalab method			Farino-graph value	Determined with Kjeldahl method					
				Grain protein [%]	Grain protein yield [kg/ha]	Wet gluten [%]	Zeleny number [ml]	Grain protein [%]	Grain protein yield [kg/ha]	Wet gluten [%]		Grain protein [%]	Grain protein yield [kg/ha]	Gliadin	Gluteni n	Other proteins	Ratio of Gli/Glu
r (Alföld-90)	**0,9837**	*0,9979*	**0,5640**	**0,9962**	**0,9909**	**0,9931**	**0,9806**	**0,9924**	**0,9900**	**0,9793**	**0,8287**						
r (Mv Magdaléna)	**0,9883**	*0,9750*	*0,9563*	*0,4003*	**0,9901**	*0,2015*	*0,8071*	*0,3648*	**0,9887**	*0,5542*	**0,4105**						
r (Mv Suba)	**0,9936**	*0,9144*	*0,9432*	**0,1305**	**0,9990**	**0,1414**	**0,8023**	*0,3852*	**0,9973**	*0,3913*	**0,7215**						
r (Mv Toborzó)	**0,9452**	*0,7715*	*0,4595*	**0,9509**	**0,9661**	**0,9220**	**0,9124**	**0,9542**	**0,9625**	**0,9530**	**0,5729**	**0,9056**	**0,9911**	**0,9164**	*0,6811*	*0,9740*	**0,8324**
r (Mv Toldi)	**0,9802**	*0,9556*	*0,7422*	**0,6782**	**0,9863**	**0,6306**	**0,7531**	**0,7922**	**0,9869**	**0,7486**	**0,8892**						

Legend:

0,7506	significant correlation
0,7506	reversed significant correlation
0,1078	insignificant correlation

4. Table Correlation between the N top dressing treatment and the quantitative and qualitative properties of the investigated five cultivars in 2014

Correlation of N top dressing treatment and the qualitative properties of the investigated five cultivars in 2014

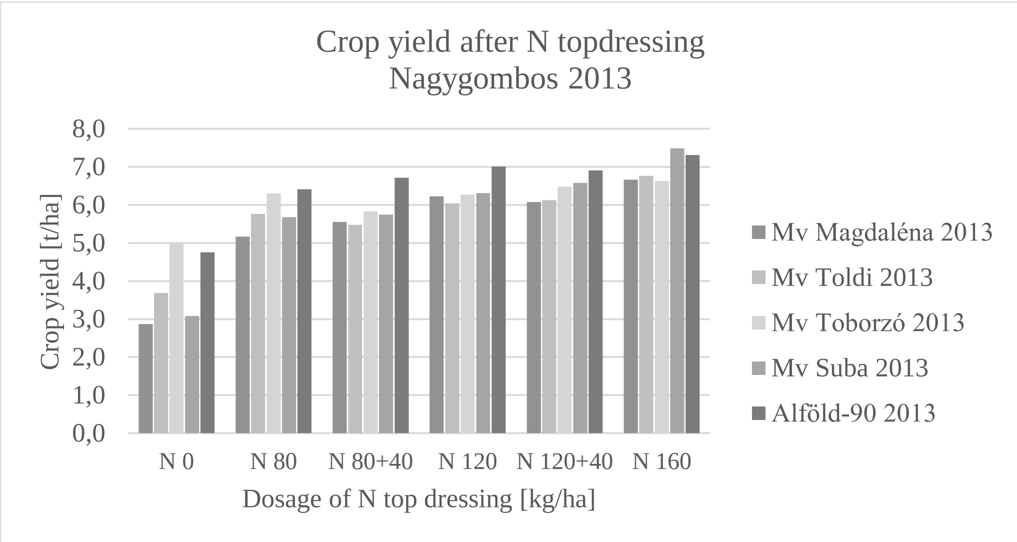
Cultivars	[t/ha]	Hecto-litre weight [kg/hl]	thousand-grain weight [g]	Determined with Mininfra method				Determined with Instalab method			Farino-graph value	Determined with Kjeldahl method					
				Grain protein [%]	Grain protein yield [kg/ha]	Wet gluten [%]	Zeleny number [ml]	Grain protein [%]	Grain protein yield [kg/ha]	Wet gluten [%]		Grain protein [%]	Grain protein yield [kg/ha]	Gliadin	Gluteni n	Other proteins	Ratio of Gli/Glu
r (Alfõld-90)	**0,4578**	*0,7055*	*0,3952*	**0,9877**	**0,8826**	**0,9924**	**0,9974**	**0,8621**	**0,7928**	**0,7858**	**0,1078**	**0,9017**	**0,9419**	**0,9661**	*0,9066*	*0,8300*	**0,9652**
r (Mv Magdaléna)	**0,9463**	**0,9174**	*0,0151*	**0,9954**	**0,9828**	**0,9917**	**0,9753**	**0,9911**	**0,9814**	**0,9894**	**0,6823**	**0,9353**	**0,9701**	**0,9483**	*0,8386*	*0,9779*	**0,9049**
r (Mv Suba)	**0,5977**	*0,8952*	**0,7827**	**0,9463**	**0,8592**	**0,9405**	**0,9513**	**0,9941**	**0,8377**	**0,9914**	**0,7415**	**0,9957**	**0,8428**	**0,9267**	*0,9642*	**0,2698**	**0,9417**
r (Mv Toborzó)	**0,5716**	*0,6887*	*0,8961*	**0,9536**	**0,8645**	**0,9453**	**0,9662**	**0,9456**	**0,8972**	**0,9314**	*0,6863*	**0,9575**	**0,9088**	**0,8994**	*0,4932*	*0,9585*	**0,8161**
r (Mv Toldi)	**0,7506**	*0,7831*	*0,8343*	**0,9729**	**0,9308**	**0,9757**	**0,9838**	**0,9832**	**0,9540**	**0,9836**	**0,9927**	**0,9850**	**0,9581**	**0,9441**	*0,8791*	*0,4583*	**0,9424**

Legend:

0,7506	significant correlation
0,7506	reversed significant correlation
0,1078	insignificant correlation

3.2 The impact of the N top dressing on crop yield of winter wheat

In accordance with expectations the increased nitrogen doses resulted in rising of crop yield by all the used varieties in both crop years (1. Figure). Along with that, the impact of varieties and that of the crop year was observed. We found that the more extensive wheat varieties resulted in poorer harvest than those of the intensive ones, however in case of poorer nutrient supply – as proven by the control treatments – extensive varieties had better crop yields.



1. Figure The average crop yields of the five studied wheat cultivars according to the N top dressing in 2013

3.3 A hectolitre- and thousand grain weight

In case of crop year 2013 the hectolitre weight showed a negative correlation with the N supply, as in 2014 the interrelation was much weaker. The correlation between the thousand-grain weight and N supply was unclear between crop years and varieties as well. While for instance Mv Magdaléna from that point of view showed a significant positive correlation in 2013, in 2014 there was barely interrelation between those two parameters. On the other hand while thousand-grain weight of Mv Suba and the N supply showed a significant negative correlation in 2013, in 2014 we get an opposite result.

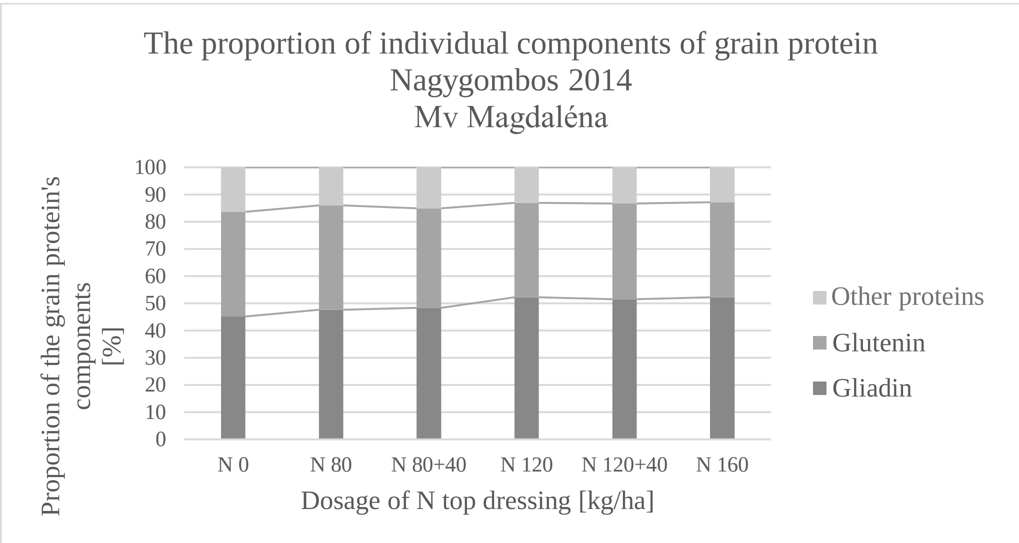
3.4 Grain protein content

The grain protein content was determined by three different methods. Two of them were NIR quick tests and the third was the Kjeldahl analytical test. We could use the last one to study all involved varieties solely in 2014, and just one variety of five was investigated with this method in 2013. During our test three varieties' responses were significantly positive in 2013 by the two others this could not be observed. The rise of nutrient supply showed clear correlation with the increase of nutrient supply in 2014.

3.5 The grain protein yield

The yield of grain protein showed a definite positive correlation with the nitrogen supply. The one-way analysis of variance calculations proved that regarding the protein yields, the impact of the rise of the N fertiliser's doses– at all investigated levels – was significant.

3.6 The proportion of the individual components of grain protein

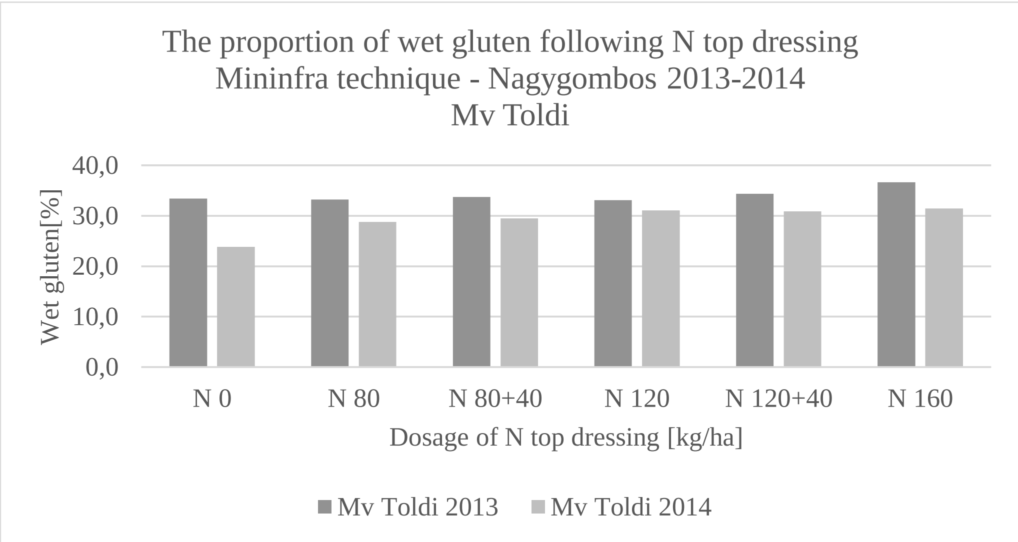


2. Figure The proportion of different type of grain proteins depending of N doasage – Mv Magdaléna, 2014

We studied the individual components of grain protein, and the results analysed by statistical probes demonstrated that proportion of gliadin proteins clearly rise, the proportion of glutenin protein conversely changes and the proportion of the rest group of the storage proteins correlate mainly negatively with the increment of N supply (4. Table). This can be observed as well by the Mv Magdalena cultivar's protein composition change (2. Figure).

3.7 Changes in wet gluten content

The wet gluten content was defined by two types of NIR instruments. Investigating the 2013 crop yields we can see that the increased level of nitrogen fertiliser caused a significant rise of wet gluten proportion at three varieties of winter wheat. The results of the next year was even more clear because the wet gluten content of the crop harvested in 2014 correlated strongly with the rise of the dose of nitrogen fertiliser at the five studied winter wheat varieties, considering the results of both instruments (3. Figure, 3. Table, 4. Table). At the same time the increase of wet gluten content was influenced by the different genotypes.



3. Figure The proportion of wet gluten following N top dressing
Mininfra technique, Mv Toldi, 2013. and 2014.

3.8 Farinograph value

Regarding the farinographic value the direction of change was not clear. In 2013 there was a positive correlation between the rises of N supply to plants and this parameter, but clear improvements were just in case of two of the five varieties examined. According to the observations of 2014 the results were less clear, since negative, significant and slightly positive as well as not significant results were found in this crop year.

3.9 Comparison of NIR instruments with the analytical method

The NIR instruments proved to be reliable compared to the analytical method, however we have to pronounce that for their safe use regular periodical calibration is necessery. The following table (5. Table) shows the accuracy of the used two NIR quick tests and the Kjeldahl analytical test to each other

5. Table Comparison of the methods of the determination of proteins during 80 kg/ha applied N fertiliser treatment

N treatment 80 [kg/ha] 2013	NIR quick test				Analytical method
	Mininfra		Instalab		Kjeldahl
	Grain protein [%]	Wet gluten [%]	Grain protein [%]	Wet gluten [%]	Grain protein [%]
Alföld-90	100,0%	100,0%	101,4%	93,6%	
Mv Magdaléna	100,0%	100,0%	99,2%	89,3%	
Mv Suba	100,0%	100,0%	103,8%	95,1%	
Mv Toborzó	100,0%	100,0%	101,7%	95,5%	86,6%
Mv Toldi	100,0%	100,0%	101,4%	93,5%	

N treatment 80 [kg/ha] 2014	NIR quick test				Analytical method
	Mininfra		Instalab		Kjeldahl
	Grain protein [%]	Wet gluten [%]	Grain protein [%]	Wet gluten [%]	Grain protein [%]
Alföld-90	112,6%	100,0%	134,1%	101,6%	100,0%
Mv Magdaléna	98,6%	100,0%	112,9%	90,6%	100,0%
Mv Suba	100,0%	100,0%	106,2%	84,3%	100,0%
Mv Toborzó	96,2%	100,0%	103,4%	89,5%	100,0%
Mv Toldi	92,3%	100,0%	103,6%	95,7%	100,0%

3.10 The impact of single and split doses of fertilisation

At two levels of fertilisers we investigated whether there is any impact on quantity and quality of crop yields of split doses of N fertilisers compared to single plant nutrient applications. According to our data none of the studied parameters depended on that if we spread the plot with the same amount of fertiliser in one or in two parts.

3.11 New scientific results

We have determined the quantitative and qualitative reaction of five winter wheat cultivars (Alföld 90, Mv Magdaléna, Mv Suba, Mv Toborzó és Mv Toldi) on applied variants of N topdressing doses in single and split form.

We have determined that the yield of semi-extensive variety Alföld-90 was usually the smallest in 2013, however in 2014 in control and most of other treatments with a few exceptions it's harvest was greater than the intensive winter wheat cultivars'.

The reliability of NIR quick tests was evaluated during our studies. We have concluded that the protein levels measured with NIR instruments in most cases were within the borders of confidence compared to the parameters derived from the measurements fulfilled with the traditional analytical method, however occasionally there can be significant differences between the data measured with different methods. Thus in any case is justifiable the calibration of NIR instruments before every harvest time. Our studies have shown that the increasing level of N nutrient had strong positive correlations with the wet gluten content of winter wheat cultivars regardless of the crop years. The different reaction of the involved wheat sorts was identified and we have define the degree of that.

Regarding the farinographic value the direction of change was not clear and showed differences between crop years as well. The farinographic value under given conditions between the single varieties was mostly evincible.

The grain protein content of the wheat varieties was consequently increased in varying degrees by the increased level of nitrogen fertiliser. Within the increased amount of storage proteins at all of the investigated varieties changes have been observed in the proportion and the amount of gliadin and glutenin components. It was ascertained that the change of gliadin proteins was significantly positive and the glutenin proteins rate change was counter with it.

The impact of nitrogen fertiliser applied in a single and in split dose was analysed. It was revealed that this difference in the spreading method was without any impact on grain yield, the N content and the wet gluten content of the crop neither in 2013 nor in 2014 crop year. Under given conditions and wheat varieties it could not be confirmed the widely accepted quality improving impact of the split method of top dressing, known from the literature.

4. CONCLUSIONS AND RECOMMENDATIONS

We have determined the quantitative and qualitative reaction of five winter wheat cultivars on applied variants of N topdressing doses in single and split form. We found that the more extensive wheat varieties resulted in poorer harvest than those of the intensive ones, however in case of poorer nutrient supply – as proven by the control treatments – extensive varieties had better crop yields. We suggest that farmers should take into consideration the applied level of nutrients in accordance with the wheat varieties applied.

From methodological point of view the reliability of the applied methods and laboratorial instruments were assessed. It was revealed that the protein parameters measured with NIR equipment can be considered the same value as the data determined with analytical method. However on the basis of comparison with the Kjeldahl method we should draw attention the calibration of this NIR instruments if possible before every harvest time, to the interest of as precise as possible determination of the different parameters real values. Like this it can be minimised the debates with serious financial implications around the wheat quality.

Regardless on crop year the rising doses of N fertiliser correlated positively with the wet gluten content of wheat cultivars. The degree of the gluten volume growth was different among the studied varieties. It can be suggested that farmers take into consideration the nitrogen demand of different wheat varieties, and use appropriate varieties for different levels of fertiliser use.

According to our investigations regarding the farinographic value of the studied varieties the direction of change was not clear. It can be concluded as well that the farinographic value can be considered variety-specific.

Our results confirmed that the increasing level of N nutrient consequently rose the protein content of the studied wheat varieties. Within the increased amount of storage protein at all the investigated sorts has changed the proportion and the amount of gliadin and glutenin components. It was ascertained that with rise of nitrogen fertiliser the amount of gliadin proteins has risen significantly within the group of storage proteins. In order to achieve better baking quality, we suggest farmers to apply suitable, in other words greater dosage levels of N fertilisers.

The split top dressing dosage did not cause change of the quality parameters compared to the identical but undivided dosage treatment. According to our data none of the studied parameters depended on that if we spread the plot with the same amount of fertiliser in one or in two parts. Since the greater part of the specific literature provide results opposite to this data we recommend further studies of this aspect of the fertilisation.

The appropriate nitrogen supply is particularly important to ensure a good quality of winter wheat. The genetically determined quality parameters of different wheat sorts are going not to be expressed beside insufficient N supply. Through our tests we defined great difference in quality parameters of varieties. We clarified too, that within gluten proteins the proportion of gliadins and glutenins could be determinative to baking quality, and this parameter was easy to influenced by applied nitrogen treatment in case of all varieties.

5. RELATED PUBLICATIONS OF THE AUTHOR

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5. Horváth Cs. (2014): A búza (Triticum aestivum L.) tartalékfehérjéi, az ezek minőségét és mennyiségét befolyásoló ökológiai hatások, különös tekintettel a nitrogén tápanyag-ellátásra. Növénytermelés. 63. 3. 95-125 pp

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