THE INFLUENCE OF TOTAL DOSES, TIME AND SPLITING OF NITROGEN ON THE GRAIN PROTEIN CONTENT OF TWO ROW SPRING BARLEY (*Hordeum vulgare* L., conv. Distichum Alef.)

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Abstract: *Two-year field experiments (2008-2010) were conducted at the Didactic and Experimental Station in Timişoara. Research objects were the influence of total doses, time and spliting of nitrogen on the protein content of Annabell two row spring barley variety. The various treatments were laid down in a randomized complete block design with three replications in the climatic conditions of Timişoara. We monitored four nitrogen doses, three splits applications (% from total nitrogen doses) and two times of nitrogen application in growth stage. The experimental crop was top dressed with the following amounts of nitrogen: 40, 60, 80 and 100 kg ha-1, then at growth stage these dosages were divided (30%, 50%, 70%), and the application have been made in one-stage: two leafs and three-stages: two leafs; boot stage; soothing stage. The control variant was not top dressed. Two row spring barley has to fulfill several quality demands to be fully accepted and paid by the malting industry. The variety has to be selected by the industry, the vitality of the lot has to be high, the husk has to be uninjured and the grain protein content has to be at the proper level and as even as possible. The average data obtained after two years of study indicate an increasing in protein content with increase in split doses of nitrogen and time of (N) applications. In 2008/2009 year crop, the lowest protein content was obtained in the variants fertilized with low doses of (N) in one-stage application while in three-stages application, the increase in protein content was on average by 11,3% (N0) to 13.2% (N100). In the 2009/2010 year crop we can see that, depending on the split nitrogen doses and timing of (N) applications, we could record an amplitude of the variation between 11.1% (N0) to 13.2 % (N100).*

Key words: *spring barley, nitrogen timming, grain protein content, split applications*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth cereal crop in the world, and the most adaptable: there is barley varieties suited to temperate, to subarctic as well as to subtropical climatic conditions. To achieve good yields from spring-sown barley, the best environment is a temperate moist climate with a growing period of at least 90 days.

A good quality barley for the brewing industry is obtaining more in humid areas where the accumulation of protein in barley grain is lower. (PÎRSAN et al.,2006) Compared to six barley, two row spring barley has a lower protein content and high starch (BÎLTEANU et al., 1991).

The malting quality of barley is very complex and is controlled by many genes and is strongly influenced by the environment (FOX et al., 2003). Malting barley, used in the brewing industry must have a protein content of 10-12%, which can be obtained in a temperate moist climate, which promote protein accumulation at the expense of starch accumulation. (PÎRSAN et al.,2003).

High grain protein is correlated with low carbohydrate content and low malt extract, thus prolonging the malting process and affects the final beer quality (ZHANG et al., 2001). Low grain protein results in limited amino acids available for yeasts during brewing (FOX et al., 2003).

Grain protein content is affected by the rate and time of N fertilizer application, available N in the soil (CHEN Et al., 2006), water availability and temperature (RILEY et al., 1998).

The application of nitrogen (N) fertilizer to malting barley is essential to obtain high yields but in amounts that do not affect malting quality (THOMPSON Et al., 2004). Malt quality is influenced by kernel protein content (ŠIMIC et al., 2007) and kernel protein content is affected by the rate and timing of N fertilizer application (RILEY et al., 1998). High protein content reduces water uptake during germination and lowers malt extract levels. In the brewery, high protein content in kernels have a longer filtration time, beer develops cloudiness and has a shorter shelf life. Insufficient levels of protein limit yeast growth during fermentation and causes beer foam to cling to the side of the glass (EMEBIRI et al., 2005).

MATERIAL AND METHODS

Field experiments were conducted to investigate the influence of nitrogen (N) rate and split application for N in different proportions (30% , 50%, 70%) at growth stage on the quantity and quality of spring barley as well as the effects of timing aplication with low rates of nitrogen (stage 1, stage 3) at the growth stage.

The influence of nitrogen (N) rate and time of application on the content of protein in spring barley (*Hordeum vulgare* L.) grain were studied at the experimental station of Banat's University of Agricultural Siences and Veterinary Medicine from Timişoara (Lat: 45,74; Lon: 21,22; altitude 91 m). The soil of the experimental fild is cambic chernozem slightly gleyied, slightly hyposalic and moderately hyposalty under 100 cm, slightly decarbonized on loess moderate fine deposits medium clay loam / medium clay loam. According to its composition, the soil falls within the class texture "fine textured", subclass medium clay loam, undifferentiated in profile. In the first part of the profile the soil reaction is neutral (pH 7.03-7.18) and in the second half the reaction is slightly alkaline (pH 8.25-8.49). The reserve of humus is low (62.98-75.65 t / ha) in the processed horizon and very low under 60 t / ha for theunderlying horizons.

The experiment was organized after the method of subdivided parcels using threefactorial fild trials, in three replication with the following experimental factors:

Factor A – total nitrogen (N) doses (kg/ha), with the graduations : a_1- N_{40}; a_2- N_{60}; a_3- N_{80}; a_4- N_{100}.

Factor B – split applications of nitrogen dose (percent from total nitrogen dose at sowing time and growth stage): b_1- 30%; b_2- 50%; b_3- 70%.

Factor C – times of topdressing of split (N) doses at different growth stage: c_1 - stage1 application: - two leaf stage; c_2 – stage 3 application: -two leaf stage;boot stage; soothing stage

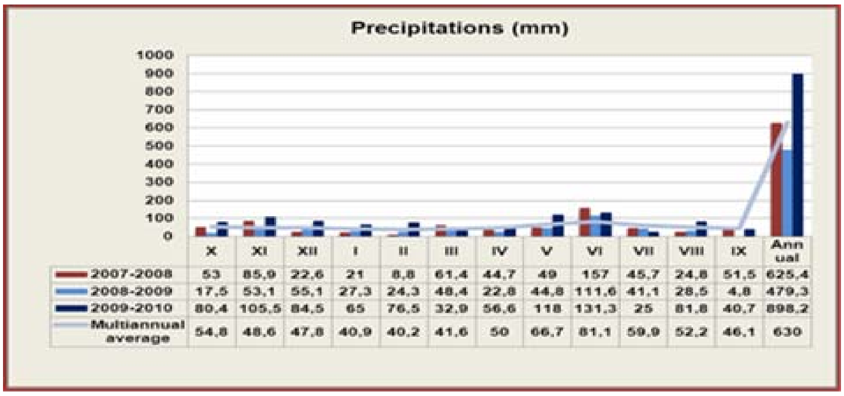
Treatment without nitrogen (control N_0) was included for comparison.

Experimental plot size was of 28.8 m^2 and the experimental field size was of 2419.2 m^2. Protein content was measured by the Kjeldahl method.

The variety used in this research was Annabell (German origin). This variety is one of the most popular malting barley varieties in Europe. Its moderate proteolysis and good cytolysis meet malsters and brewers demands. Annabell has a very high tillering and fits in all regions because of a very high compensation competence .

Malting quality: fullsize kernel yields (> 2,5 mm) -medium-high 94,0% ; hectoliter weight- medium-high 69,2 kg; malt extract- high 83,0 % d.m; protein content-low-very low 9,9 % ; viscosity- low-very low 1,45 mPa x s; friability- high-very high 93,8 %; kolbach index-medium-high 45,2 % ; apparent attenuation limit- high-very high 82,5 % .

The top dressing was performed in March, using ammonium nitrate, applying the following quantities of nitrogen: 40, 60, 80 and 100 kg ha^{-1} then, in the growth stage, nitrogen rates were splited and the application was made in I stage respectively III stages. Because of its short growing season, the requirements of two row spring barley regarding the humidity in our country in most years are covered by precipitation. In the two years, the annual amounts of rainfall were 479,3 mm and 898,2 mm. These levels were lower by 24% in the first crop year and higher by 42,5 % in the second crop year, than the long-term average (630 mm) (figure1).

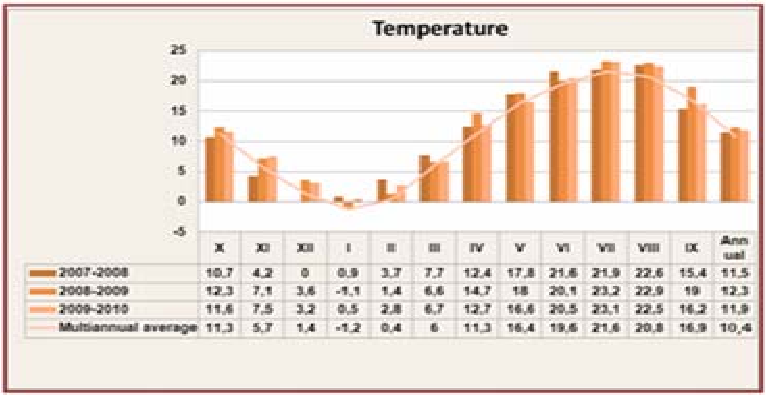


	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	Annual
2007-2008	53	85,9	22,6	21	8,8	61,4	44,7	49	157	45,7	24,8	51,5	625,4
2008-2009	17,5	53,1	55,1	27,3	24,3	48,4	22,8	44,8	111,6	41,1	28,5	4,8	479,3
2009-2010	80,4	105,5	84,5	65	76,5	32,9	56,6	118	131,3	25	81,8	40,7	898,2
Multiannual average	54,8	48,6	47,8	40,9	40,2	41,6	50	66,7	81,1	59,9	52,2	46,1	630

Figure. 1. Monthly rainfall, annual and multiannual average (mm) Meteorological station Timişoara

The rainfall over the multi annual average in March (2009) and April (2010), have created favorable growing conditions for spring barley. In the first year, the dry period that coincided with the highest barley requirement, lead to higher protein content, the year being considered less favorable. In the second year of study, the total rainfall and distribution per barley phenophases were more favorable than in the first year.

In the 2009/2010 crop year the monthly temperature distribution during barley growing season was within the limits of the long-term average for the region of Timişoara. In the 2008/2009 crop year, in spring the temperature were higher, and the precipitations under the multiannual average the year being considered less favorable (Figure 2).



	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	Annual
2007-2008	10,7	4,2	0	0,9	3,7	7,7	12,4	17,8	21,6	21,9	22,6	15,4	11,5
2008-2009	12,3	7,1	3,6	-1,1	1,4	6,6	14,7	18	20,1	23,2	22,9	19	12,3
2009-2010	11,6	7,5	3,2	0,5	2,8	6,7	12,7	16,6	20,5	23,1	22,5	16,2	11,9
Multiannual average	11,3	5,7	1,4	-1,2	0,4	6	11,3	16,4	19,6	21,6	20,8	16,9	10,4

Figure 2. Monthly temperature ^{o}C, annual and multiannual averageMeteorological station Timişoara

High temperatures tended to reduce grading percentages and to increase grain protein content. Therefore the weather conditions during the growing period are among the factors of primary importance which impact malting barley yield and especially grain and malt quality. Minimum temperature required to browse barley vegetation cycle is about 4 0C in May, 10 -11 0C in June, July 14 0C, 12 0C to 7 0C in August and September. During baking, barley requires minimum daily average temperature of 10 0C, the minimum temperature for germination of barley is 1-2 0C, barley emergence in optimal condition occurs at a temperature of 15- 20 0C. Under our country conditions tow row spring matures in 90 -150 days.

RESULTS AND DISSCUTIONS

The average data obtained after two years of study indicate an increasing in protein content with increase in split doses of nitrogen and time of (N) applicatios in three-stages (stage 3) .The results showed that splitting of recommended dose of nitrogen, recorded higher total protein content as compared to control . Thus, for the Annabell variety, the average of the years studied (2008-2010), in terms of protein content had values by 11.1% (N0) to 12.7% (N100) for split doses of nitrogen and time of (N) applications in one-stage (stage I), compared with three-stages application (stage 3) which recorded a higher protein content betwen 11% (N0) and 13.4% (N100).

Results concerning the average of timming (N) applications showed that the lowest protein content was recorded in (stage 1) application in the year 2009/2010 with a value of 11.8%, compared with the year 2008/2009, when the protein content registered a value of 12.4%. In (stage 3) application, the lowest average in protein content was recorded in the year 2009/2010 with a value of 12.4% compared with the year 2008/2009 when the value registered was 12.6%. (Table 1.)

Table 1

Effect of level and time of nitrogen application on grain protein content of two row spring barley 2008/2010

Year		2008/2009	2009/2010	$\overline{X}$	2008/2009	2009/2010	$\overline{X}$
Nitrogen proportions		30%, 50%, 70%	30%, 50%, 70%		30%, 50%, 70%	30%, 50%, 70%	
Timming of (N) application		Stage 1	Stage 1		Stage 3	Stage 3	
Nitrogen doses (N)	N0	11,8	11,1	11,4	12,0	11,3	11,6
	N40	12,2	11,4	11,8	12,6	12	12,5
	N60	12,4	11,7	12,0	12,8	12,6	12,7
	N80	12,7	12,2	12,4	13,1	12,9	13,0
	N100	12,9	12,6	12,7	13,6	13,2	13,4
	$\overline{X}$	12,4	11,8	12,1	12,6	12,4	12,5

Figure 3 show the evolution of protein content in the 2008/2009 year crop. Analyzing the influence of the interaction between fertilization x time of (N) application, we can see that in 2008/2009 year crop, the lowest protein content was obtained in variants fertilized with low doses of (N) in (stage 1) application. Thus, the increase in protein content, in (stage 1) application was by 11.8% (N0) to 12.9% (N100) with a difference of 0.4% (N40) to ,1% (N100), the lowest protein content compared with the control variant occurred in variant of fertilization (N40) with a value of 12.2%. In (stage 3) application, the increase in protein content was on average, by 12% (N0) to 13.6% (N100), with a difference of 0.6% (N40) to 1.6% (N100), while the low protein content, compared with the control was recorded in variant fertilized with (N40), with a value of 12.6%.

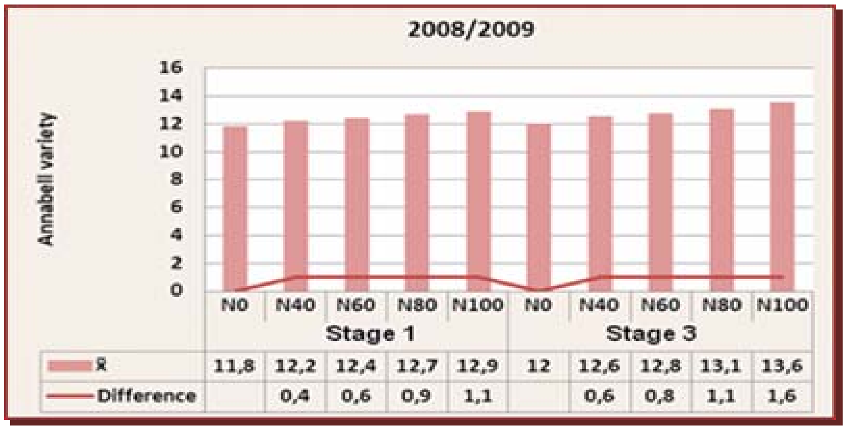


Figure 3. Variation of spring barley protein content (%) depending on doses time and splitting of nitrogen from Timisoara in 2008/2009

Figure 4 show the evolution of protein content in the 2009/2010 year crop. We can see that, depending on the nitrogen doses and timing of (N) applications, we could record an amplitude of the variation between 11.1% (N0) to 13.2 (N100) %. Thus, the increase in protein content was on average by 11,1% (N0) to 12,6% (N100) in (stage 1) application with a difference from 0,4 % (N40) to 1,5% (N100), the lowest protein content compared with the control was recorded in the variant fertilized with (N40) with a value of 11,4%. In (stage 3) application, the increase in protein content was on average by 11,3% (N0) to 13.2% (N100), with a difference of 0.7% (N40) to 1.9% (N100), while the low protein content, compared with the control was recorded in variant fertilized with (N40), with a value of 12.%.

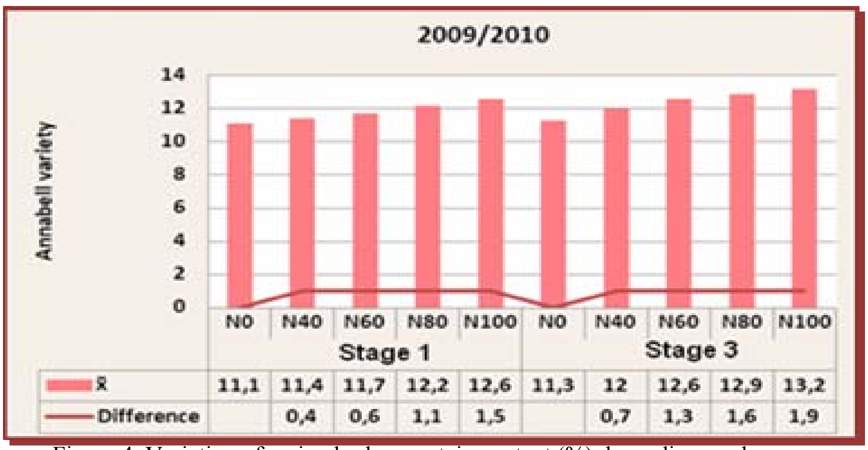


Figure 4. Variation of spring barley protein content (%) depending on doses time and splitting of nitrogen from Timisoara in 2009/2010

CONCLUSIONS

1. At the interactions between nitrogen (N40, N60) fertilization and split application in one stage, the protein content is lower compared to the same dose applying in three stages and the grains can be used to obtain malt and beer in the end.

2. By getting on average, for the two years of study, a low protein content in spring barley grains that does not exceed 12.5% according to European Malting barley requirements, in terms of climatic conditions from Banat -Timisoara, we can conclude that malting barley for beer production is suitable for cultivation in this climate area.

3. The conclusion that emerges from the analysis of economic efficiency is that nitrogen fertilization in low doses and split application in one stage is efficient in all the years, the grain protein content is consistent with European Malting barley requirements

4. Fertilization with higher doses of nitrogen (N80, N100) and their divided into three stages are implemented protein content increase above the recommended limits of European Malting barley being inefficient in economic terms.

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