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## RESEARCH ARTICLE

**EFFECTS OF ENVIRONMENT ON OPTIMIZING NITROGEN NUTRITION ON  
CONTENT OF FIRST-CLASS SEEDS IN WINTER BARLEY CULTIVARS  
(*Hordeum vulgare* L.)**

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**Abstract**

In this paper was investigated variability of percentage content of seeds first-class (thickness of seeds >2.5mm) in four divergent barley cultivars (Jagodinac, Premium, NS 489 and NS 495) originated from different breeding centres in Serbia. These cultivars investigated during two years on experimental field on plots 5m<sup>2</sup> and four replications under four different rate of nitrogen treatments (control N<sub>0</sub>=0, N<sub>1</sub>=20, N<sub>2</sub>=40 and N<sub>3</sub>=60 kg ha<sup>-1</sup>). The variability of content of seeds first-class was established in both years of experiment and under all nitrogen rate of supplied N fertilizer. According to percentage of content first-class (thickness of seeds >2.5mm) were found differences among the cultivars in each years of experiment and all variant of nitrogen nutrition. In average, content of seeds first-class for all cultivars and all treatments was higher in second year 82.34% than in first experimental year 80.03%. The average value of percentage content of seeds first-class in both growing seasons and under N treatment was the highest in barley cultivar NS 595 (82.70%) and the lowest in barley cultivar Premium (78.79%). For all investigated cultivars, content of seeds first-class increased with increasing nitrogen rate. This indicate that content of seeds first-class mostly affected by mineral nitrogen nutrition and to a much lesser degree by cultivar.

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**INTRODUCTION**

Generally the main objectives of barley breeding programs are to create new cultivars with high seed yield, malting quality and resistant to environmental stress factors (Paunović et al., 2008; Knezevic et al., 2011). The genetic factors are determine seed yield components value, for example for seed mass spike<sup>-1</sup> are located at the 3H, 7H chromosome (Ren et al., 2013). Investigation of Fox et al., (2006) showed that genetic effect on seed size was greater than environmental as well high value of heritability (89-98%) of seed size on different site of investigation. Numerous genes controlling seed and plant traits which are associated to seed size. Genetic association between seed size and number of plant genes including earliness (*esp*), photoperiod response (*ppd*) inflorescence (*vrs*), dwarf

(*denso*) and semi dwarf (*sdw*) Coventry et al. (2003), as well association between waxy starch (*wx*), naked (*n*) and short awned (*lk2*) genes. The barley yield formation based grain yield into spikes per unit area, content of seeds first-class, number of seeds per unit, weight of seeds spike<sup>-1</sup>, weight of thousand seeds (Madic et al., 2009). Also, very important whether is correlation among these components of yield, positive or negative. Study of Paunovic et al. (2006) showed positive correlation between spike length and content of first-class seeds, weight of seeds spike<sup>-1</sup> and seeds number spike<sup>-1</sup>, spike number and seed protein content, while negative correlation between spike number m<sup>-2</sup> and length of spike as well first-class of seeds. Also, the content of first-class seeds was negatively correlated protein content, as well grain yield.

The genotype and environment (climatic and soil conditions) and genotype/environment interaction have influence to variation of yield and quality (Atlin et al., 2000). Generally, these two complex traits yield and quality are in negative correlation. Considering that correlation of yield and quality is negative the very important is developing optimal growing technology. Application of optimal scientific farming measures have great significance for achieving high and profitable grain yield and good technological traits (Koutna et al., 2003). Also, the size of seeds in barley is different and depends of genetic and environmental factors. In breeding process of cultivar creation on the base of seed thickness determined three classes of seeds (I class: >2.5 mm, II class: from 2.2 to 2.5 mm and III class <2.2 mm (EBC rules). For malting barley 90% and higher percent of first-class seeds is criteria to be classified as a good quality cultivar. Except of noted components, the seed yield depends from sowing density, length of vegetative period, resistance to lodging, resistance to low temperatures, pest, diseases. Increasing of sowing density implicate to increasing of number of spike m<sup>-2</sup> as a results of induced tillering. The increasing number of spike contributed to smaller seeds size which have mostly high protein content and low starch accumulation (Madic et al., 2006). To attain higher effectiveness of crop management practices, conducted numerous studies of irrigation (Jolankai et al., 2008) time and density of sowing (Paunovic et al., 2008; Křen et al., 2014), application of nitrogen nutrients (Kovacevic et al., 2006). The efficiency of absorption and utilization of nitrogen associated to genotype roots capacity as well amount of available nutrient and soil moisture (Veigh and Rajkai, 2006). Barley plants by root system are use nitrogen and utilize for biomass production and protein synthesis and deposition in seed (Knezevic et al., 2008). According to nitrogen absorption, were found differences among barley cultivars (Gorny, 2001; Rashid and Khan, 2008; Knezevic et al., 2014) as well among wheat (Knezevic et al., 2007). The environmental condition and applied N nutrition has an significant influence to increasing yield components as well seed yield of barley (Knezevic et al., 2015).

The aim of this paper is study of variability of percentage content of seeds first-class, in four genetically divergent winter barley cultivars grown under three different dose nitrogen nutrition (20kg N ha<sup>-1</sup>, 40kg N ha<sup>-1</sup>, 60kg N ha<sup>-1</sup>) and different environmental conditions during two experimental years.

## Materials and methods

Field experiment was conducted in the 2010/11 and 2011/12 growing seasons. The variability of grain mass spike<sup>-1</sup> in two-row winter barley (Jagodina, Premium, NS 489 and NS 495) were investigated. The cultivars grown under nitrogen treatments which included various rates of applied nitrogen: control N<sub>0</sub>=0, N<sub>1</sub>=20, N<sub>2</sub>=40 and N<sub>3</sub>=60kg ha<sup>-1</sup>. The experiment was set up as a randomized block design in four replications on 5m<sup>2</sup> size of unit plot. Nitrogen in the form of the mineral fertilizer KAN (27% N) was applied at the stage of stem elongation. The experiment carried out on the soil type pseudogley having poor physical properties, an acid pH (pH<sub>H2O</sub> = 4.2) and the following content: humus 2.31%, readily available phosphorus 7.6 mg 100g<sup>-1</sup> soil and potassium 14.6 mg 100<sup>-1</sup> g soil. In full stage of maturity of 80 plants (20 plants per replication) were used for analysis of content of seeds first-class. Experimental data analysis was made using the EBC rules. The analysis of variance was calculated according to randomized complete block design with three factors: A (cultivars), B (year) and factor C (N-dose), using ANOVA (MSTAT-C program, 1989). The significant differences among the means were estimated by least significant difference (LSD) test.

## Climatic conditions during growing seasons

The values of temperature and precipitation were different between first and second years of investigation. These values of temperature and precipitation of each year, were different from average values of previous ten years (table 1). During period October/June the average values of temperature was 8.7<sup>0</sup>C in first and 8.1<sup>0</sup>C in second year of experiment. In both years of experiment the average temperature was lower than in long term period (9.08<sup>0</sup>C). The precipitation were favorable in 2010/11 with sums of 520.5mm, than in 2011/12 (474.7mm) year. In second year (April-May months) during seed filling stage was higher amount of precipitation (174.6mm) than in first (120mm).

**Table 1 (here)!****Results and Discussion**

The content of seeds first-class is an important component of total seed yield of barley, which is in dependence to number of florets, number of seeds spike<sup>-1</sup>, efficiency of seed filling (Barczak and Majcherczak (2008). Similar to other traits, the content of seeds first-class is under genetic control and its value, also affected by environment and scientific farming factors (Madic et al., 2012). In this investigation, nitrogen application had significant effects on percentage content of seeds first-class.

The percentage values of content of seeds first-class in investigated barley cultivars were significantly different in two growing seasons. In general, the higher average of content of seeds first-class were established in second growing season of barley genotypes (Table 1). In the second year of investigation, during seed filling stage (April-May) the amount (174mm) and distribution of precipitation (April-69.1mm and May-105.5mm) was more favorable which is an important factor of expressing higher content of seeds first-class in all barley cultivars. Investigation of Poulsen et al., (2000) showed that environmental conditions as well sowing data and nitrogen application have impact on seed size. Another studies showed that high temperature causing heat stress that have effect on starch synthesis in period of seed filling (Savin and Nichols, 1999).

In average for all variant of supplied N and both growing seasons, the highest content of seeds first-class had cultivar NS 595 (82.70%) as well as mainly on each variant of N nutrition. Percentage content of seeds first-class increased with N increasing in all investigated cultivars, and was the highest in N<sub>3</sub> variant (Table 1). The maximal average value of content of seeds first-class was in N<sub>3</sub> variant at cultivar NS 595 (83.90%) in 2011/12 year, and minimal was in N<sub>0</sub> (control) in barley cultivar Premium (71.15%) in 2010/11 year. Two years average value of content of seeds first-class was highest in NS 595 (82.70%) the least in barley cultivar Premium (78.79%).

Precipitation limitation limited nitrogen used by crops and as a consequence significantly decrease yield (FAO, 2013).

In this study the amount of precipitation and temperature values were more favorable in year (2011/12) of experiment, what is effected to increase value of first-class of seeds in each barley cultivars and all variant of nitrogen dose of applied fertilizer (tab. 2)

Seed size is a morphological character of barley seeds, that are genetically determined within environmental condition (Fox et al., 2006). Large seeds usually contain a higher level of starch with a decreased level of protein, what is suitable for malting industry. Environmental factors, especially high temperature which can cause heat stress, have influence on key mechanisms of biosynthesis (starch, protein, etc.) particularly during phase of seed filling. The expected results of growing plants under stress condition is decreasing of weight of seeds as well as reducing size of seeds.

By analysis all variants of N nutrition and years the average values of content of seeds first-class in average for all investigated barley cultivars was the highest in both year on N<sub>3</sub> variant (82.34%). The increase of nitrogen rate on average is best responded to the cultivar NS 595, which had the largest increase in content of seeds first-class in N<sub>3</sub> compared with N<sub>0</sub> variant.

Table 1. Monthly and mean temperatures and monthly and cumulative precipitation

Tem&Precpt	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
<sup>0</sup> C 2010/11	9.2	11.1	2.7	0.3	0.6	6.6	12.2	15.6	20.4	8.74	78.7
<sup>0</sup> C 2011/12	10.4	3.2	3.3	-0.1	-4.2	8.8	12.7	16.0	23.1	8.12	73.3
2000-2010	12.2	7.0	2.0	0.9	2.4	7.6	12.0	17.2	20.4	9.08	81.7
(mm)2010/11	93.6	34.1	64.9	28.1	59.2	48.9	37.1	82.9	71.7	57.8	520.5
(mm)2011/12	30.4	1.7	63.7	107.1	54.9	24.5	69.1	105.5	17.8	52.7	474.7
2000-2010	64.3	57.4	48.5	42.8	44.7	52.5	66.6	74.9	92.2	60.3	543.8

Table 2. Average values of content of seeds first-class in barley cultivars

Cultivar	Year	Content of seeds first-class
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		N <sub>0</sub>	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	Mean
Jagodinac	2010/11	77.35 <sup>l</sup>	81.85 <sup>defg</sup>	82.45 <sup>bcdef</sup>	82.78 <sup>abcde</sup>	81.11 <sup>d</sup>
	2011/12	80.0 <sup>ijk</sup>	80.75 <sup>ghi</sup>	82.45 <sup>bcdef</sup>	83.,2 <sup>abc</sup>	81.60 <sup>cd</sup>
	Average	78.68	81.3	82.45	82.99	81.36
Premium	2010/11	71.15 <sup>n</sup>	75.25 <sup>m</sup>	77.70 <sup>l</sup>	79.35 <sup>jk</sup>	75.86 <sup>e</sup>
	2011/12	80.85 <sup>ghi</sup>	81.25 <sup>fghi</sup>	82.03 <sup>cdefg</sup>	82.75 <sup>abcde</sup>	81.72 <sup>cd</sup>
	Average	76.00	78.25	79.86	81.05	78.79
NS 589	2010/11	79.1 <sup>k</sup>	81.25 <sup>fghi</sup>	81.5 <sup>efgh</sup>	82.45 <sup>bcdef</sup>	81.07 <sup>d</sup>
	2011/12	82.0 <sup>cdefg</sup>	82.4 <sup>bcdef</sup>	83.15 <sup>abc</sup>	83.35 <sup>ab</sup>	82.72 <sup>ab</sup>
	Average	80.55	81.82	82.32	82.90	81.90
NS-595	2010/11	80,5 <sup>hij</sup>	81,75 <sup>efgh</sup>	82,7 <sup>abcde</sup>	83,4 <sup>ab</sup>	82,09 <sup>bc</sup>
	2011/12	82,7 <sup>abcde</sup>	83,1 <sup>abcd</sup>	83,55 <sup>ab</sup>	83,9 <sup>a</sup>	83,31 <sup>a</sup>
	Average	81.60	82.42	83.12	83.65	82.70
Average for all cultivars and treatments	2010/11	77.02	80.02	81.09	81.99	80.03
	2011/12	81.39	81.88	82.79	83.30	82.34
	Average	79.20	80.95	81.94	82.64	81.18

Table 3. Analysis of variance for content of seeds first-class in barley cultivars

Source	DF	MS	F	LSD <sub>0.05</sub>	LSD <sub>0.01</sub>
Repetitions	3	0.156	0.238	-	-
Genotype (A)	3	91.389	139.475 <sup>**</sup>	0.6439	1.182
Year (B)	1	170.201	259.755 <sup>**</sup>	-	-
AB	3	46.633	71.170 <sup>**</sup>	0.9106	1.671
N-dose (C)	3	71.240	108.723 <sup>**</sup>	0.6439	1.182
AC	9	3.596	5.488 <sup>**</sup>	0.9154	1.315
BC	3	15.457	23.590 <sup>**</sup>	0.9106	1.671
ABC	9	2.541	3.878 <sup>**</sup>	1.295	1.860
Error	93	0.655	-	-	-
Total	127	-	-	-	-

Increased nitrogen rates induced highly significant differences in general content of seeds first-class between the nitrogen rates of 60 kg ha<sup>-1</sup> and the control variant, as noted in investigation (Paunovic et al., 2007). Large size of seeds providing reduced content of protein and increased content of starch what is important especially for malting spring barley and brewing industry. Also, for various purposes of barley seed handling, the market required large, plump and uniform seed size.

In this investigations, content of seeds first-class increased significantly with increasing nitrogen fertilization dose (tab. 2 and 3).

However, investigation of four spring barley cultivars showed that decreasing sowing densities in all three research years resulted in increasing first-class grain percentage (Paunovic et al., 2010). In their investigation the highest percentage of large grains was determined at the lowest density of 300 seeds m<sup>-2</sup>. Also, the increasing N rates nutrition (50, 80 and 110 kg N ha<sup>-1</sup>) did not follow increasing content of first-class seed of barley, while they found that the highest first-class seed percentage was produced at the lowest N rate (50 kg N ha<sup>-1</sup>) in the tested barley cultivars. Similar results obtained in previous investigations for these traits and seed yield of barley (Paunovic et al., 2007). By increasing level of fertilization the number of tillers linearly increased, which is consistent with previous research (Rashid and Khan, 2008). This trait directly influenced spike density and seed yield of barley genotypes.

Also, nitrogen status is in relation to leaf senescence, chlorophyll content (Schildhauer et al., 2008) as well with seed filling in dependence of temperature and precipitation regime.

Analysis of variance showed highly significant differences among cultivars (A) for content of seeds first-class. Differences between investigated years (B), N levels (C) and all analyzed interactions (A x B, A x C, B x C, A x B x C) were also high significant for this trait. The strongest influence to this trait had year, than cultivar and N-doses, but less interaction between factors (Table 3).

Seed size has breeding target, which required by the market and industry. Indeed, seed size, density, and uniformity are important attributes for determining the market value of barley seeds since they influence the quality and yield malting and milling performance. Investigation of other cereal species showed that seed size in wheat was associated with various characteristics of flour, such as protein content and hydrolytic enzymes activity, which in turn determine quality and end-use suitability (Evers, 2000; Gegas et al., 2010). For predicting genetic effect for this as well other traits is very important to understand interaction of genotype/environment and on the base of that develop improved breeding program which accelerate genetic progress. On the base of knowledge, the breeding of barley cultivars is necessary directed to create genotypes which maintain large, stable seed size across a range of environments (Fox et al., 2006).

## Conclusions

Nitrogen application had significant effect on content of seeds first-class. The content of seeds first-class was in dependence of cultivars and years. Interactions between cultivars, applied nitrogen doses and years were also highly significant, which means that studied cultivars are positive reacted to applied N rate. The best response to N increasing rate expressed NS 595 cultivar. For this reason, it is necessary to find a compromise between different production technology factors which would by their interaction contribute to achieving high and profitable yield and good quality of barley cultivars in certain soil and climatic conditions. Seed size is important trait which indicate level of seed yield and quality. Breeders selecting cultivars of barley with large seed size in the aim to satisfy requirement of producers, industry and end users. Genetic impact of variation in seed size and shape in barley is base direct investigation to improve yield potential and processing performance, especially in the current climate changes in the aim to satisfy requirements of worldwide market.

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