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Original scientific paper

INFLUENCE OF GENOTYPE AND NITROGEN NUTRITION ON GRAIN SIZE VARIABILITY IN SPRING MALTING BARLEY

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Grain size is an important quality parameter of malting barley, which depends on genotypes, environmental factors and their interactions. Also, grain size is governed by the efficiency of assimilation and translocation of mineral nutrients (mainly nitrogen) during grain endosperm development, which affects grain yield. The aim of this study was to evaluate variability in the percentage of three different grain size classes: class I (thickness ≥2.5 mm), class II (2.2-2.5 mm) and class III (<2.2 mm) in spring malting barley genotypes ('Novosadski 448', 'Novosadski 456', 'Dunavac' and 'Jadran'). The experiment was conducted during three years (2012-2014) in a randomized complete block design with three replications at different rates of nitrogen fertilization (N_1=45, N_2=75, N_3=105 and N_4=135 kg ha^{-1}). The presence of different grain sizes in barley cultivars in all N fertilization treatments after harvest was

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investigated. The proportion of the three grain classes was dependent upon year, cultivar and nitrogen fertilization rate. The highest percentage of class I grains was recorded in 'Novosadski 456', and that of class II and class III grains in 'Dunavac'. The percentage of class I grains increased significantly with increasing nitrogen rates up to 75 kg ha^{-1}, stagnated at 105 kg ha^{-1}, and decreased significantly as the nitrogen level was further increased to 135 kg ha^{-1}. Class II and class III grain contents decreased at nitrogen rates up to 105 kg ha^{-1}, but increased significantly at 135 kg ha^{-1}. The best response to favorable environmental conditions and the highest percentage of class I grain in all years were recorded in 'Novosadski 456'. The most favorable effect on grain size in the studied spring malting barley genotypes was exhibited by the nitrogen rate of 75 kg ha^{-1}.

Keywords: barley, genotype, grain size, nitrogen, variability

INTRODUCTION

In malting barley production, grain size is the primary grain quality trait, as small grains generally have low starch and high protein levels, thus reducing the extract value (KAYA and AYRANC, 2016), which is not suitable for the beer industry. Breeding to create new cultivars involves grouping grains into three classes according to their thickness (class I: >2.5 mm, class II: 2.2-2.5 mm, and class III: <2.2 mm (EBC) (KNEZEVIC *et al.*, 2015). Grain size fractions of >2.5 mm thickness are used in malt production, whereas fractions <2.5 mm in thickness are used for feeding (PRŽULJ *et al*., 2014). In most countries, the brewing industry requires more than 85% of grains >2.5 mm in thickness for two-rowed barley varieties (FOX *et al*., 2008). Barley is considered to be of uniform quality if it contains more than 85% of grains larger than 2.5 mm. Such barley has a higher malting performance, and ensures a more homogenous malt quality (PAUNOVIĆ and MADIĆ, 2011).

Grain yield is more dependent on environmental conditions during the growing season than on genotypic effect (AYRANCI *et al*., 2014; AHMADI *et al.,* 2016). BRAZIENE (2007) observed that grain yield of spring barley positively correlated with the amount of rainfall in May, and negatively with total rainfall in June and July. In regions with less favorable environments such as southern and eastern Europe, the Pannonian zone and other regions with similar environments, it is more difficult to produce high quality malting barley (OLESEN *et al.*, 2011). In these regions, barley yield and quality are limited by water availability, heat stress and the duration of the grain filling period (PRŽULJ and MOMČILOVIĆ, 2012). Drought and high temperatures during grain filling cause an increase in the percentage of low-sized grains (small grains) and a simultaneous increase in grain protein concentration in barley (SYLVESTER-BRADLEY and KINDRED, 2009; MAGLIANO *et al.,* 2014). High temperatures induce heat stress, thus affecting starch synthesis during grain filling and inhibiting the translocation of dry matter after flowering (GARCÍA DEL MORAL *et al.,* 2003). Grain filling rate is dependent on both grain filling duration and grain position in the spike. In drought-tolerant bread wheat cultivars, the distribution of photosynthetic products is less oriented towards the ends of the spikelet, with acceptable grain size maintained in the middle of the spike (YILDIRIM, 2013).

Mineral nutrition has a large impact on grain yield and quality of malting barley. Nitrogen is the key element in achieving consistently high yields in cereals (SHAFI *et al.*, 2011; KNEŽEVIĆ *et al.,* 2016). Also, grain yield in spring barley is affected by sowing density (PAUNOVIĆ *et al.,* 2006a; POPOVIĆ *et al.,* 2011). However, grain yield and quality are generally negatively correlated. Nitrogen fertilization can increase grain yield and grain protein concentration, and reduce the proportion of large grains (MCKENZIE *et al.*, 2005; O'DONOVAN *et al*., 2012; MAGLIANO *et al.*, 2014).

The objective of this study was to assess variability in the contents of class I, class II and class III grains in spring barley cultivars ('Novosadski 448', 'Novosadski 456', 'Dunavac' and 'Jadran') grown at different nitrogen fertilization rates.

MATERIAL AND METHODS

Experimental material

The research was conducted over a period of three years in 2012, 2013 and 2014 in the region of Požarevac, Serbia (44° 36' 55" N and 21° 10' 57" E), at an altitude of 94 m. The experiment was laid out as a randomized complete block design with three replications and a plot size of 5 m^2 (1 x 5 m) on a noncalcareous vertisol. The agrochemical analysis of the soil sampled at 0-0.3 m depth showed that the soil was slightly acidic reaction (pH KCl - 6.13; pH H_2O - 6.9), slightly calcareous (1.72% $CaCO_3$), with a low humus content (2.9%), moderately supplied with nitrogen (0.15%), poor in phosphorus (8.0 mg 100^{-1} g) and poor supply in potassium (13.0 mg 100^{-1} g). Agrochemical soil testing was performed as follows: soil pH - potentiometrically, $CaCO_3$ content – volumetrically after Scheibler, humus content – by Tjurin's method, total nitrogen – by Kjeldahl's method, and the content of available K_2O and available P_2O_5 – by the Egner-Riehm AL method. Four cultivars of two-rowed spring malting barley were used i.e. 'Novosadski 448' (G_1), 'Novosadski 456' (G_2), 'Dunavac' (G_3) and 'Jadran' (G_4). Basic fertilization involved 300 kg ha^{-1} NPK 15:15:15, i.e. 45 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5 and 45 kg ha^{-1} K_2O. Experimental plots were sown by hand at a spacing of 10 cm between the rows on 12 March 2012, 24 March 2013 and 7 March 2014. Nitrogen was applied during tillering at 0, 30, 60 and 90 kg ha^{-1} (N_1=45, N_2=75, N_3=105 and N_4=135 kg ha^{-1}) as calcium ammonium nitrate CAN (27% N). The crop was harvested at full harvest maturity stage on 9 July 2012 in the first experimental year, on 25 July 2013 in the second and on 18 July 2014 in the third year. Samples of 1.0 m^2 per plot were collected for analysis of the content of different grain size fractions. The contents of class I, class II and class III grains were measured by a Tehnica ET-1111 scale (120/0.01 g), after manual screening over sieves 2.2 and 2.5 mm in diameter.

Weather conditions

Graph 1 and Table 1 show weather data for the Požarevac site obtained from the Veliko Gradište weather station (44° 45′ 14.4″ N, 21° 30′ 29.4″ E, altitude 68 m). The growing season in 2012 was characterized by a high amount of rainfall during April and May, and drought and high air temperatures during grain filling and maturation until harvest. The high amounts of rainfall in April and May led to early lodging of the barley crop. March 2013 was humid and cold, with a thick blanket of snow, due to which sowing date was postponed until the end of March. Lack of rainfall and high temperature extremes occurred from mid April to the third ten days in May. Drought and high temperatures continued from the beginning of June until harvest. Weather conditions during the 2014 growing season were characterized by high amounts of uniformly distributed rainfall and almost daily rains during grain filling and maturation.

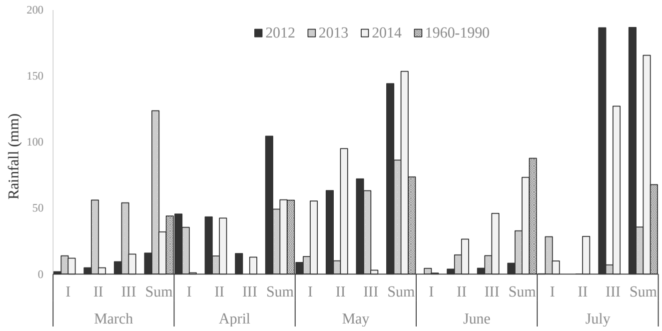

Figure 1. Rainfall totals per 10-day period and month (mm) for 2012-2014 and long-term average (1960-1990)

Table 1. Mean monthly temperatures (°C) for the 2012-2014 period and long-term average (1960-1990)

Year	Month		March	April	May	June	July
2012	Average absolute monthly temperature	Max	15.4	20	23.2	29.9	33.3
		Min	-0.4	6.8	10.4	14.3	17.1
	Average monthly temperature		7.4	13.2	16.5	22.7	25.3
2013	Average absolute monthly temperature	Max	10.4	20.4	24.5	26.4	30.9
		Min	1.1	5.8	12.2	13.9	13.6
	Average monthly temperature		5.4	13.2	18.5	20.3	22.8
2014	Average absolute monthly temperature	Max	15.4	18.2	21.7	26.3	28.6
		Min	3.6	8.2	10.6	13.6	16.6
	Average monthly temperature		9.5	13.6	16.3	20.1	22.2
1960 - 1990	Average absolute monthly temperature	Max	11.2	17.4	22.4	25.4	27.5
		Min	1.7	6.2	10.9	13.6	14.5
	Average monthly temperature		6.0	11.6	16.4	19.3	20.8

Statistical analysis

Results were subjected to both a three-way ANOVA (year, cultivar, fertilization) for the entire experimental period and a two-way ANOVA (cultivar, fertilization) across experimental years using the statistics package Statistica/w 10.0. (StatSoft, Inc., Tulsa, USA 2010). The significance of difference between the means was assessed by Duncan's multiple range test at the 95% level. Prior to calculating the analysis of variance, the proportion of each fraction was transformed using the formula:

$$\text{arc sin}\sqrt{\%fraction} * \frac{180}{\pi}$$

RESULTS AND DISCUSSION

Class I grain content is an important yield component in barley dependent upon number of flowers, number of kernels per spike and grain filling efficiency (BARCZAK and MAJCHERCZAK, 2008). In addition to being genetically controlled, grain size is affected by environmental conditions and production method (sowing date, seeding rate and mineral fertilization). In this study, the percentage of class I grains was significantly dependent on cultivar, year and mineral nitrogen fertilization (Table 2). The content of class I grains in the studied genotypes was highest (85.6%) in the third growing season (Table 3), which was characterized by moderate temperatures, high rainfall totals and uniform distribution of rainfall during the grain filling period (May-June - 226.8 mm). The rainfall amount of 248.6 mm during April and May in the first growing season caused early lodging in the barley crop, resulting in a significant decrease in class I grain content compared to the third growing season without lodging. The lowest content of class I grains in the studied genotypes was recorded in the second growing season (76.13%), due to late sowing and adverse climatic conditions (drought and high temperatures) at the grain filling stage. Rainfall totals during May and June in the second experimental year were 119.1 mm, and were unfavorably distributed (May-86.3, June-32.8 mm). The important effect of rainfall amount during grain filling on the proportion of class I grains was also reported by MAGLIANO *et al.* (2014) and KNEZEVIC *et al.* (2015). The cultivars exhibited significant differences in the average proportion of class I grains, and showed different responses under diverse environmental conditions ($p<0.01$). 'Novosadski 456' had a significantly higher content of class I grains in all three growing seasons compared to the other cultivars. In the first two experimental years, 'Dunavac' had a significantly lower content of class I grains compared to the other cultivars, but in the third growing season the content was significantly lower than in 'Novosadski 456'. 'Novosadski 456' gave a greater average spike length (7.03 cm) and a lower average number of kernels per spike (15.63) compared to 'Dunavac' (6.97 cm, 17.60). The fact that class I grain content is a genotypic trait largely dependent on spike length and kernel number per spike was confirmed by PAUNOVIĆ *et al.* (2006b), who observed that class I grain content positively correlated with spike length and negatively with kernel number per spike. The important differences in class I grain content across cultivars were also reported elsewhere (PAUNOVIĆ *et al.*, 2009; PRŽULJ *et al.*, 2014 and KNEZEVIC *et al.*, 2015).

Table 2. Analysis of variance of the proportion of grains of different size classes in spring barley

Source of variation	d.f.	Class I (≥2.5 mm)			Class II (2.2-2.5 mm)			Class III (<2.2 mm)		
		SS	MS	Expl. SS(%)	SS	MS	Expl. SS(%)	SS	MS	Expl. SS(%)
Treatments (T)	47	12675.16	81665.00		6473.26	2461.27		2104.41	752.55	
Genotypes (G)	3	4040.64	1346.88**	31.88	1675.82	558.61**	25.89	711.22	34.605**	33.80
Fertilizer (N)	3	515.43	171.81**	4.07	116.50	38.83**	1.80	265.07	12.897**	12.60
Environm. (E)	2	6809.80	3404.90**	53.73	3302.65	1651.33**	51.02	751.49	54.847**	35.71
G x E	6	926.08	154.35**	7.31	1189.62	198.27**	18.38	216.28	5.262**	10.28
N x E	6	76.04	12.67ns	0.60	19.42	3.24ns	0.30	40.02	0.974ns	1.90
G x N	9	116.59	12.95ns	0.92	28.50	3.17ns	0.44	35.49	0.576ns	1.69
G x N x E	18	190.58	10.59ns	1.50	140.75	7.82ns	2.17	84.84	0.688ns	4.03
Error	384	5012.30	13.05		2871.57	7.48		216.28	5.262	
Total	431	17687.46	5128.00		9344.83	2468.75		4735.13	759.40	

SS – sum of squares; MS – mean squares; Expl. SS (%) - explained sum of squares (%)

** F - test significant at the 99% level; * F - test significant at the 95% level; ns - non-significant

Over the experimental period, the highest average percentage of class I grains was obtained under treatment with N_3=105 kg ha^{-1} nitrogen (82.78%), and the lowest under N_1=45 kg ha^{-1} (80.31%). The application rate of 75 kg ha^{-1} in all three years led to a significant increase in class I grain content compared to the control. Increasing the nitrogen rate from 75 to 105 kg ha^{-1} resulted in a slight increase in class I grain content in the first two years, and a slight decrease in the third year. The highest nitrogen rate (135 kg ha^{-1}) applied brought about a significant decrease in the average proportion of class I grains in all three experimental years. In the first and third years, there was no significant difference in the average percentage of class I grains between the lowest nitrogen fertilization rate (N_1) and the highest nitrogen level (N_4). In the second year, the average content of class I grains at the highest nitrogen rate N_4 (75.9%) was significantly lower than under treatment with N_3 (77.8%), but significantly higher compared to N_1 treatment (74.2%). The present results are in agreement with the findings of STANKOVIĆ *et al.* (2000) and KNEZEVIC *et al.* (2015).

The average content of class II grains over the experimental period varied significantly with year, cultivar and nitrogen application rate ($p<0.01$). Across growing seasons, the percentage of class II grains was highest in 2013 (17.3%), which had the lowest amount of rainfall and high temperatures during grain filling, and lowest in the favorable 2014 growing season (10.6%). The studied cultivars showed significant differences in the average percentage of class II grains, and responded differently under diverse environmental conditions ($p<0.01$). 'Novosadski 456' had the lowest content of class II grains in all three experimental years. 'Dunavac' exhibited a significantly higher content of class II grains than the other cultivars, except in the second year, when no significant differences were observed in comparison to 'Jadran'. The highest nitrogen application rate in 2012 and 2014 caused a significant increase in the proportion of class II grains compared to treatments with 75 and 105 kg ha^{-1} nitrogen. No important effect of nitrogen fertilization was observed in the second year. STANKOVIĆ *et al.* (2000) also found an increase in class II grain content under treatment with nitrogen at rates above 100 kg ha^{-1}.

The average content of class III grains was significantly affected by year, cultivar and nitrogen application rate, with cultivars responding differently across years ($p<0.01$). The unfavorable conditions during grain filling in 2013 promoted the content of class III grains (6.6%) compared to 2012 (3.7%) and 2014 (3.8%). 'Novosadski 456' had the lowest content of class III grains in all three experimental years. In the first and second years, the highest content of class III grains was found in 'Novosadski 448' (4.2 and 9.3%, respectively), whereas in the third year 'Dunavac' (5.4%) outperformed the other cultivars. Nitrogen rates of 75 and 105 kg ha^{-1} in 2012 and 2014 resulted in a significant decrease in the average content of class III grains compared to treatments with 45 and 135 kg ha^{-1}. In 2013, under treatment with 75 kg ha^{-1}, a significantly lower percentage of class III grains was obtained compared to the other nitrogen fertilization treatments. The results of the present experiment comply with those of STANKOVIĆ *et al.* (2000).

Grain size is primarily a cultivar-specific trait formed due to genotype x environment interactions. The expression of this trait is affected by the stability and variability of climatic factors. For the three grain size fractions analyzed, the values of the average content differed significantly across cultivars, fertilization treatments and experimental years (seasons). The observed differences indicate the genetic specificity of the studied barley cultivars in terms of morphophysiological properties and the capacity to uptake, assimilate and translocate nitrogen into reproductive organs, the grain. The highest content of class I grain was determined in 'Novosadski

456' and the lowest in 'Dunavac'. 'Dunavac' exhibited the highest percentage of class II and class III grains. The percentage of class I grains increased significantly with increasing nitrogen rates up to 75 kg ha^{-1}, stagnated at 105 kg ha^{-1}, and decreased significantly as the nitrogen level was further increased to 135 kg ha^{-1}. Class II and class III grain contents decreased at nitrogen rates up to 105 kg ha^{-1}, but increased at 135 kg ha^{-1}. The most favorable effect on grain size in the studied spring malting barley genotypes was exhibited by the nitrogen rate of 75 kg ha^{-1}.

Table 3. Percentage of class I, class II and class III grains (%) as dependent upon genotype and nitrogen application rate

Treatment		Cultivar				Nitrogen fertilization (kg N ha^{-1})				Average
Parameter	Year	G_1	G_2	G_3	G_4	N_1	N_2	N_3	N_4	
Class I grain ≥ 2.5 mm (%)	2012	81.7 c	86.3 a	78.8 d	84.7 b	82.0 b	84.0 a	84.1 a	81.5 b	82.9 B
	2013	76.1 c	81.5 a	68.8 d	78.1 b	74.2 c	76.6ab	77.8 a	75.9 b	76.1 C
	2014	85.0 b	89.3 a	83.8 b	84.2 b	84.7 b	86.6 a	86.5 a	84.4 b	85.6 A
Average		80.9 c	85.7 a	77.2 c	82.3 b	80.3 b	82.4 a	82.8 a	80.6 b	81.5
Class II grain 2.2–2.5 mm (%)	2012	14.1 b	11.4 c	16.2 a	11.9 c	13.5ab	13.0 b	12.9 b	14.3 a	13.4 B
	2013	14.2 c	14.7 c	23.9 a	16.1 b	17.9ns	16.8ns	16.8ns	17.9ns	17.3 A
	2014	11.2ab	8.4 c	10.7 b	11.9 a	10.3 b	10.0 b	10.5 b	11.6 a	10.6 C
Average		13.3 b	11.5 c	16.9 a	13.3 b	13.8 b	13.3 b	13.4 b	14.6 a	13.8
Class III grain < 2.2 mm (%)	2012	4.2 a	2.3 b	5.0 a	3.4 ab	4.5 ab	3.0 c	3.0c	4.2 a	3.7 B
	2013	9.7 a	3.8 d	7.3 b	5.8 c	7.9 a	6.6 ab	5.4 b	6.2 ab	6,6 A
	2014	3.8 b	2.3 c	5.5 a	3.9 b	5.0 a	3.4 bc	3.0 c	4.0 ab	3.8 B
Average		5.9a	2.8 c	5.9 a	4.4 b	5.8 a	4.3 b	3.8 b	4.8 a	4.7

G – cultivars: G_1=Novosadski 448; G_2=Novosadski 456; G_3=Dunavac; G_4=Jadran
N– nitrogen application rates: N_1=45 kg ha^{-1} + 0 kg ha^{-1}/control/; N_2=45 kg ha^{-1} + 30 kg ha^{-1} = 75 kg ha^{-1}; N_3=45 kg ha^{-1} + 60 kg ha^{-1} = 105 kg ha^{-1}; N_4=45 kg ha^{-1} + 90 kg ha^{-1} = 135 kg ha^{-1}

The means for cultivars and nitrogen application rates designated by different lowercase letters across years (rows) are significantly different at 95% according to Duncan's test. The average values for years designated by different capital letters across classes (the last column) are significantly different at 95% according to Duncan's test.

CONCLUSION

Based on the study of the percentage of class I, II and III grain contents, differences were observed among the barley genotypes. Significant differences were found in the percentage of - class I, II and III grains as affected by years, which were characterized by different weather factors and different nitrogen application rates. The highest percentage of class I grain was recorded in 'Novosadski 456', and the highest content of class II and III grain in 'Dunavac' . The content of class I grain increased significantly as the N rate increased up to 75 kg ha^{-1}. Nitrogen rates above 75 kg ha^{-1} led to a decrease in the percentage of class I grain. Class II and III grain contents increased at rates up to 135 kg N ha^{-1}. The development of new genotypes with stable grain size and optimization of nitrogen fertilizer rates are important tasks in breeding malting barley for the purpose of satisfying the interests of producers, the brewing industry, and end users. The rate of 75 kg N ha^{-1} in three different climatic years had a favorable effect on grain size in the studied spring malting barley genotypes.

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UTICAJ GENOTIPA I ISHRANE AZOTOM NA VARIJABILNOST VELIČINE ZRNA KOD JAROG PIVSKOG JEČMA

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Izvod

Veličina zrna je važan pokazatelj kvaliteta pivskog ječma, koja zavisi od genotipa, uslova sredine i njihove interakcije. Takođe, veličina zrna zavisi od efikasnosti asimilacije i translokacije mineralnih hranljivih elemenata (uglavnom azota) tokom perioda razvoja endosperma, što ima uticaj i na prinos zrna. Cilj ovog rada je proučavanje varijabilnosti u procentnom sadržaju tri različite klase veličine zrna: I klasa (debljina ≥2.5 mm), II klasa (2,2-2,5 mm) i III klasa (<2,2 mm) kod genotipova jarog pivskog ječma (Novosadski 448, Novosadski 456, Dunavac i Jadran). Eksperiment je izveden tokom trogodišnjeg perioda (2012-2014) po potpuno slučajnom blok sistemu u tri ponavljanja, primenom različitih doza mineralne ishrane azotom (N_1=45, N_2=75, N_3=105 i N_4=135 kg ha^{-1}). Nakon žetve analiziran je sadržaj zrna različite veličine sorti ječma na svim varijantama đubrenja azotom (N). Sadržaj sve tri klase zrna je zavisio od godine, sorte i doze azota. Najveći sadržaj zrna I klase je zabeležen kod sorte Novosadski 456, a II i III klase kod sorte Dunavac. Procenat zrna I klase je značajno porastao sa povećanjem doze azota do 75 kg ha^{-1}, stagnirao na 105 kg ha^{-1}, zatim se značajno smanjio, s obzirom da je nivo azota dodatno povećan na 135 kg ha^{-1}. Sadržaj II i III klase zrna smanjivao se do doze azota od 105 kg ha^{-1}, ali je značajno povećan na 135 kg ha^{-1}. Najbolji odgovor na povoljne uslove spoljašnje sredine i najveći procenat zrna I klase tokom svih godina ispitivanja je imala sorta Novosadski 456. Najpovoljniji uticaj na krupnoću zrna ispitivanih genotipova jarog pivskog ječma je ispoljila doza azota od 75 kg ha^{-1}.

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