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Full Length Article

Genotype × Environment × Management (G×E×M) Impacts on Grain Yield and Quality of Spring Malting Barley (*Hordeum vulgare*)

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Abstract

Agronomic management and environment affect malting barley yield and quality. The objective of this study was to determine optimum agronomic practices (cultivar, fertilization, and seeding rate) for yield and quality of malting barley. A study was conducted during 2012–2014 in the region of Požarevac, southeastern Serbia, to evaluate the weather-dependent effect of seeding rate (S_1=350, S_2=450 and S_3=550 seeds m^{-2}) and nitrogen fertilization rate (N_1=45, N_2=75, N_3=95 and N_4=135 kg N ha^{-1}) on the yield and quality of spring malting barley cultivars ('Novosadski 448', 'Novosadski 456', 'Dunavac' and 'Jadran'). Increasing seeding rate had a significantly negative effect on the quality, whereas the effect on yield was dependent upon weather during the growing season. Grain yield and grain protein content significantly increased with an increase in nitrogen rate up to 135 kg N ha^{-1}. The optimum nitrogen rate for the average thousand-kernel weight and percentage of kernels ≥ 2.5 mm in all years was 75 kg N ha^{-1}, and for test weight 105 kg N ha^{-1}. Germinative energy depended on genotype and weather conditions, whereas seeding and nitrogen rates had a significant effect only during the first year. Results indicated that seeding rates above 350 seeds m^{-2} and nitrogen rates above 75 kg N ha^{-1} led to substantial grain quality deterioration in barley cultivars. © 2021 Friends Science Publishers

Keywords: Seeding rate; Nitrogen; Protein contents; Temeprature; Cultivars

Introduction

Barley (*Hordeum vulgare* L.) is the major cereal in many dry areas of the world and is vital for the livelihoods of many farmers. In Serbia, in 2018, barley was grown on 105740 ha of land, with a total annual production of 410138 t and an average yield of 3.9 t ha^{-1} (FAOSTAT 2020). Over the last five years, about 50% of all barley produced has been used as livestock feed, and the rest for the malting and brewing industry (Kandić 2015). However, the malting and brewing industry sets very strict grain quality requirements for malting barley, primarily including plump kernels of uniform size, with a high germination rate and a protein content range of 9.5–11.5% (Pettersson 2006), maximum 12.5% (Paunović and Madić 2011). Environment of Serbia is significantly different from the barley belt of Western and Central Europe. High amounts of precipitation and temperatures on fertile soils facilitate the uptake of nitrogen at levels higher than required by spring barley, thus leading to an increase in grain protein concentration, either directly or, even more so, indirectly through plant lodging. Moreover, spring barley mostly experiences, during grain filling period in particular, the moisture deficit and elevated temperature (Pržulj and Momčilović 2002; Zhang *et al.* 2020). In cereals, this causes accelerated and forced maturation, directly resulting in a shortened grain filling duration, decreased grain weight and size resulting in less yield of poor quality (Pržulj *et al.* 2000; Wajid *et al.* 2004). The first step in successful malting barley production is the proper choice of cultivars (Leistrumaitë and Paplauskienë 2005). Nitrogen is one of essential nutrients required to harvest potential grain yields (Shafi *et al.* 2011). Given that barley plants uptake nitrogen almost until the very end of the growing season, its excessive concentration in the soil can lead to a high rate of uptake by plants and, hence, to an increase in grain protein concentration (Paunović and Madić

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2011). Seeding rate is another important factor in malting barley production. Low-density and excessively dense stands have an adverse effect on grain yield and quality. Reduced seeding rate prolongs tillering, thus increasing the number of spikes per plant and percentage of small grain fractions, causing non-uniform maturation (Paunović *et al.* 2009). Overly dense stands are particularly risky since they significantly decrease grain yield, first-class grain yield and thousand-kernel weight, and increase the presence of prevalent diseases (Malešević and Starčević 1992). Proper seeding rate and mineral nutrition enable the formation of an optimum number of spikes to obtain high grain yields (Paunović *et al.* 2007). This study therefore evaluated the influence of different seeding and nitrogen fertilization rates on the grain yield and quality of spring barley in southeastern Serbia.

Materials and Methods

Study site, treatments, experimental design and crop management

Field trials on four cultivars of two-rowed spring barley, viz. 'Novosadski 448' (G1), 'Novosadski 456' (G2), 'Dunavac' (G3) and 'Jadran' (G4), were set up during 2012–2014 in Požarevac, southeastern Serbia (44° 36' 55" N and 21° 10' 57" E, 94 m a.s.l.). The experiment was conducted in in a randomized block design in nest plot size measuring 1 m × 5 m with three replications. Each experimental plot had 10 cm spaced 10 rows. The soil used in the trial was classified as Vertisol developed on loamy sands overlying loamy material. Primary tillage was carried out each year in autumn and involved plowing to a depth of 25 cm. Secondary tillage was performed in spring before seeding using a cultivator. Maize was the preceding crop in all years. Before seeding, the soil was treated with 300 kg ha^{-1} $N_{15}P_{15}K_{15}$, i.e. 45 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5 and 45 kg ha^{-1} K_2O. Three seeding rates were used: 350 (S_1), 450 (S_2) and 550 (S_3) seeds m^{-2}. Seeding was performed on 12 March, 24 March and 7 March in 2012, 2013 and 2014, respectively. At tillering, calcium ammonium nitrate CAN fertilizer (27% N) was applied at 0, 30, 60 and 90 kg/ha (N_1=45+0, N_2=45+30, N_3=45+60, and N_4=45+90 kg N ha^{-1}, respectively). Fertilization was performed on 27 April, 5 May and 10 May in 2012, 2013 and 2014, respectively.

Sampling and measurements

At the harvest maturity, the crop was harvested on 9 July, 25 July and 18 July during the first, second and third experimental years,respectively. Plant samples were taken from the middle 1 m^{-2} (0.4 m × 2.5 m, *i.e.*, 4 rows × 2.5 m) of each plot for analysis of yield and quality traits. Grain yield and 1000-grain weight were corrected to a 14% moisture concentration basis. Thousand grains were counted by grain counter machine and the thousand counted grain was weighed and taken as thousand grain weight. The following parameters were determined: grain moisture by ISO 712:2009, thousand-kernel weight by ISO 520:2010, test weight (using a *Dickey* analyzer) by ISO 7971-1:2009, and the percentage of kernels ≥ 2.5 mm after manual screening over 2.5 mm diameter sieves in three replications. Germinative energy was assessed by germination in petri dishes on filter paper (5 replicates of 100 seeds each) at a temperature of 30/20°C in a closed germination chamber (ECD01E Snijders Scientific) as stipulated by the Rulebook on Seed Quality of Agricultural Crops (Official Gazette of the RS 2013). Crude protein content was determined by the Kjeldahl method.

Soil sampling and weather conditions

A composite soil sample was taken before sowing to determine the threshold level of plant nutrients in the soil. Soil samples were randomly collected in a diagonal pattern before sowing from a depth of 0–20 cm. The soil samples were air dried and passed through a 2 mm sieve for physico-chemical analysis. The soil was analyzed soil total nitrogen, available phosphorous and potassium, pH, $CaCO_3$, and humus content, before sowing (on plot bases) (Table 1). Total soil N was analyzed by Kjeldhal digestion method with sulphuric acid (Jackson 1962). Soil pH was determined from the filtered suspension of 1:2.5 soils to water ratio using a glass electrode attached to a digital pH meter, potentiometer (FAOSTAT 2020). The experimental soil was slightly acidic with a low humus content having moderate nitrogen and potassium contents and poor in phosphorus. Rainfall data were obtained from the nearest recording station, which was generally within 1 km of the respective sites. The average weather conditions during the experimental period relative to the long-term average are presented in Table 2. Spring 2012 had significantly above-average rainfall and moderate average temperatures. The highest amount of precipitation (186.8 mm) during the 2012 growing season was recorded in the third ten-day period of July (after the harvest). The prolonged snow cover and heavy precipitation in spring 2013 were the reasons for the postponement of spring barley seeding until the third ten-day period of March. Rainfall totals during June and July were, respectively, three times and twice lower than the long-term average, whereas average temperatures were well above the long-term average. In terms of agro-meteorological conditions, 2013 was the least favorable year for the production of spring malting barley. Weather conditions in 2014 were characterized by large amounts of rainfall and their even distribution in the later part of the growing season, with average temperatures above the 30-year average.

Data analysis

Results were statistically analyzed by the analysis of variance (ANOVA) over the experimental years and for the total period using the statistics package Statistica/w 10.0. Differences were tested at a significance level of $\alpha = 0.05$ by the Duncan test (Statistica 2010).

Results

Agronomic management and environment affect malting barley yield and quality. Weather conditions are often un-favorable for malting barley quality in Central Serbia, but agronomic practice may improve the probability of attaining acceptable quality. Dry and warm weather in March 2012 allowed timely sowing and favored the germination and emergence of spring barley. In April, total rainfall was 104.4 mm, which along with moderate temperatures ensured good tillering and abundant spike formation. In May, the additional 144.2 mm of rainfall intensified shoot elongation and induced early lodging (Table 2). The large number of spikes per m^{-2} and plant lodging caused a non-significant reduction in thousand-kernel weight and percentage of kernels ≥ 2.5 mm, whereas the grain protein concentration was significantly higher than in 2014. However, dry and warm weather during grain filling did not affect test weight and germinative energy which were significantly higher than in the other years. This may be attributed to sufficient soil water reserves. Most of April and May 2013 had a drying effect on young barley plants, resulting in few plants per m^{-2}. The low-density stand was partially compensated for by a somewhat higher percentage of total and productive tillers. Delayed seeding, drought and high temperatures caused a faster rate of progress through all developmental stages, with plants consequently producing significantly shorter spikes and fewer kernels per spike compared to the other experimental years. Cultivar, seeding rate and nitrogen fertilization rate differed in their effect on grain yield and quality traits in spring malting barley as dependent on weather conditions (Table 3 and 4). In 2012, which was marked by pronounced plant lodging, the highest grain yield was exhibited by the shortest cultivar (‘Novosadski 448'), but there were no significant differences relative to the grain yield of 'Novosadski 456' and 'Dunavac'. The lowest grain yield was obtained by 'Jadran', which had the fewest plants and spikes per unit area and the lowest 1000-kernel weight. In 2013, which had the most unfavorable conditions, grain yield was highest in 'Novosadski 456', due to the highest values for general tillering, productive tillering, number of spikes per unit area and 1000-kernel weight. The highest variation in grain yield induced by weather conditions was exhibited by 'Jadran'. The cultivar had significantly loess grain yields than the other cultivars during the first two years, and in the third year it gave the highest yield (differences in grain yield among the cultivars in the third year were not significant) (Table 4). Dry weather and high temperatures in 2013 had a far greater effect on grain protein concentration in cultivars with lighter and smaller kernels ('Novosadski 448' and 'Dunavac'). Depending on production conditions, seeding rates had different effects on grain yield and quality. As the seeding rate increased, the average grain yield in almost all cultivars significantly increased up to a seeding rate of 450 seeds m^{-2}. However, grain yield increased significantly with increasing seeding rate up to 450 seeds m^{-2} in 2012, and up to 550 seeds m^{-2} in 2013. Seeding rate had no significant effect on grain yield in 2014, with cultivars showing different responses (cultivar × seeding rate interaction). Increasing seeding rate significantly reduced the average thousand-kernel weight and test weight. Also, the increase in seeding rate significantly decreased the percentage of kernels ≥ 2.5 mm, except in 2013, when no significant difference was observed between the seeding rates of 350 and 450 seeds m^{-2}. As seeding rate increased, the average grain protein concentration increased in all three years. The increase in nitrogen fertilization rate resulted in increased grain yield up to the highest rate applied. However, in the first growing season, grain yield significantly increased with each increasing nitrogen rate, whereas in the second and third years the increase in grain yield was significant up to the nitrogen rate of 105 kg ha^{-1}. In all three years, thousand-kernel weight and the percentage of kernels ≥ 2.5 mm increased significantly at nitrogen rates up to 75 kg ha^{-1}. Grain test weight increased significantly at nitrogen rates up to 75 kg ha^{-1} in 2012, and up to 105 kg ha^{-1} in 2013 and 2014. Germinative energy depended on genotype and weather conditions, whereas seeding and nitrogen rates had a significant effect only in the first year. The highest variation in grain protein concentration was induced by nitrogen application. The lowest values in the total experimental period were in the control, and the highest under treatment with the highest nitrogen rate. Cultivar, 'Novosadski 456' had the highest values for thousand-kernel weight, test weight, percentage of grains ≥ 2.5 mm and grain protein concentration. In contrast, 'Dunavac' had significantly lower values for thousand-kernel weight, test weight, percentage of kernels ≥ 2.5 mm, and grain protein concentration.

Table 1: Physico-chemical properties of the soil at the experimental site (0-30 cm)

pH_{KCl}	pH_{H2O}	Humus (%)	$CaCO_3$ (%)	N (%)	P_2O_5 (g kg^{-1})	K_2O (g kg^{-1})
6.13	6.9	2.9	1.72	0.15	0.08	0.13

Table 2: Weather conditions during the growing periods

Month	March	April	May	June	July	Average
Year	Mean monthly air temperature (°C)					
2012	7.4	13.2	16.5	22.7	25.3	17.0
2013	5.4	13.2	18.5	20.3	22.8	16.0
2014	7.4	13.3	17.1	21	23.5	16.5
1981-2010	6.2	11.8	17.0	19.9	21.9	15.4
Year	Monthly rainfall (mm)					Sum
2012	16	104.4	144.2	8.3	186.8	459.7
2013	123.7	49.1	86.3	32.8	35.6	327.5
2014	32	56.3	153.5	73.3	165.7	480.8
1981-2010	41.5	57.2	59.8	81.6	61.4	301.5

Table 3: Analysis of variance for the tested parameters over a three-year period

Factors	df	Mean squares - MS					
		Grain yield (t ha^{-1})	Thousand-kernel weight (g)	Test weight (kg hL^{-1})	Kernel ≥ 2.5 mm (%)	Germinative energy (%)	Total protein (%)
A	3	8.96×10^{7}**	25.71**	1940.75**	340490**	10715,49**	2,61**
B	2	1.81×10^{6}**	565.83**	169.14**	1346,88**	6,969**	7,02**
C	3	1.01×10^{7}**	177,55**	220.14**	508,23**	16,219**	10,43**
D	3	5.34×10^{7}**	152.825**	110.78**	171,81**	5,509**	73,22**
A × B	6	2.62×10^{6}**	53.70**	74.37**	154,35**	50,078**	2,96**
A × C	9	5.15×10^{6}**	1.09^{ns}	11.84**	6,60^{ns}	4,041**	3,26**
A × D	6	5.41×10^{5ns}	1.369^{ns}	10.05**	11,80^{ns}	3,14*	2,206**
B × C	6	6.07×10^{5ns}	7.629*	8.385**	12,67^{ns}	3,43**	0,206ns
B × D	9	7.04×10^{5ns}	2.129^{ns}	3.65^{ns}	12,95^{ns}	0,82^{ns}	0,376*
C × D	6	8.68×10^{5}*	14.88**	9.62**	32,42*	2,28^{ns}	0,19ns
A × B × C	12	7.06×10^{5}*	3.17^{ns}	8.73**	9,18^{ns}	2,44*	1,99**
A × B × D	18	2.61×10^{5ns}	4.71*	5.62**	10,59^{ns}	2,19*	0,70**
A × C × D	12	1.84×10^{5ns}	1.62^{ns}	1.92^{ns}	7,14^{ns}	3,08**	0,29ns
B × C × D	18	1.99×10^{5ns}	2.28^{ns}	1.88^{ns}	6,44^{ns}	1,77^{ns}	0,07ns
A × B × C × D	36	3.98×10^{5ns}	2.45^{ns}	1.81^{ns}	9,274^{ns}	1,12^{ns}	0,33**
Error	288	3.80×10^{5}	2.69	2.62	10,61	1,12	0,19

A – Year; B – Cultivar; C – Seeding rate; D – Nitrogen fertilization rate

Table 4: Average values of grain yield and quality of malting barley

Factors		Cultivars				Seeding rate (seeds m^{-2})			Nitrogen fertilization rate (kg N ha^{-1})				Average
Parameters	Year	G_1	G_2	G_3	G_4	S_1	S_2	S_3	N_1	N_2	N_3	N_4	
Grain yield	2012	5.70 ^{a}	5.49 ^{a}	5.47 ^{a}	5.02 ^{b}	5.06 ^{b}	5.68 ^{a}	5.52 ^{a}	4.51 ^{d}	5.28^{c}	5.80 ^{b}	6.11 ^{a}	5.42 ^{B}
(t ha^{-1})	2013	4.42 ^{a}	4.52 ^{a}	4.37 ^{a}	3.91 ^{b}	3.69 ^{c}	4.40 ^{b}	4.81 ^{a}	3.48 ^{c}	4.21 ^{b}	4.61 ^{a}	4.91 ^{a}	4.30 ^{C}
	2014	5.84 ^{ns}	5.67 ^{ns}	5.69 ^{ns}	6.10 ^{ns}	5.88 ^{ns}	5.82 ^{ns}	5.77 ^{ns}	4.77 ^{c}	5.66 ^{b}	6.44 ^{a}	6.44 ^{a}	5.82 ^{A}
Average		5.32 ^{a}	5.30 ^{ab}	5.25 ^{ab}	5.24 ^{b}	4.88 ^{b}	5.30 ^{a}	5.37 ^{a}	4.25 ^{d}	5.05 ^{c}	5.61 ^{b}	5.82 ^{a}	5.18
Thousand-	2012	37.62 ^{b}	42.08 ^{a}	37.84^{b}	37.49 ^{b}	39.78 ^{a}	38.66 ^{b}	37.83 ^{c}	38.04 ^{b}	39.50 ^{a}	39.69 ^{a}	37.80 ^{b}	38.76 ^{B}
kernel weight	2013	35.66 ^{d}	43.26^{a}	37.21 ^{c}	39.31 ^{b}	40.10 ^{a}	38.86 ^{b}	37.63 ^{c}	37.79 ^{b}	39.54 ^{a}	39.79 ^{a}	38.33 ^{b}	38.86 ^{B}
(g)	2014	37.39 ^{c}	41.29 ^{a}	39.00 ^{b}	40.47 ^{a}	40.74 ^{a}	39.37 ^{b}	38.51 ^{c}	38.26 ^{b}	40.90 ^{a}	41.06 ^{a}	37.92 ^{b}	39.54 ^{A}
Average		36.89 ^{d}	42.21 ^{a}	38.02 ^{c}	39.09 ^{b}	40.21 ^{a}	38.96 ^{b}	37.99 ^{c}	38.03 ^{b}	39.98 ^{a}	40.18 ^{a}	38.02 ^{b}	39.05
Test weight	2012	64.14 ^{c}	68.15 ^{a}	64.15 ^{c}	67.25 ^{b}	66.84 ^{a}	66.07 ^{b}	64.86 ^{c}	65.15 ^{b}	66.24 ^{a}	66.55 ^{a}	65.75 ^{ab}	65.92 ^{A}
(kg hL^{-1})	2013	60.06 ^{b}	61.01 ^{a}	58.11 ^{c}	56.78 ^{d}	60.50 ^{a}	58.94 ^{b}	57.52 ^{c}	57.94 ^{c}	59.03 ^{b}	60.81 ^{a}	58.16 ^{c}	60.05 ^{C}
	2014	61.13 ^{c}	61.94 ^{b}	60.86^{c}	63.27 ^{a}	63.03 ^{a}	61.66 ^{b}	60.71 ^{c}	60.73 ^{c}	62.35 ^{b}	63.14 ^{a}	60.97 ^{c}	61.80 ^{B}
Average		61.77 ^{d}	63.70 ^{a}	61.04 ^{b}	62.43 ^{c}	63.46 ^{a}	62.22 ^{b}	61.03 ^{b}	61.28 ^{c}	62.54 ^{b}	63.50 ^{a}	61.63 ^{c}	62.59
Kernel	2012	81.69 ^{c}	86.33 ^{a}	78.84 ^{d}	84.70 ^{b}	84.90 ^{a}	82.70 ^{b}	81.08 ^{c}	82.02 ^{b}	84.02 ^{a}	84.06 ^{a}	81.46 ^{b}	82.89 ^{B}
≥ 2.5 mm	2013	76.13 ^{c}	81.46 ^{a}	68.81 ^{d}	78.11 ^{b}	77.52 ^{a}	76,62 ^{a}	74.24 ^{b}	74.17 ^{c}	76.64^{ab}	84.06 ^{a}	75.91 ^{b}	76.13 ^{C}
(%)	2014	84.95 ^{b}	89.28 ^{a}	83.83 ^{b}	84.19 ^{b}	87.50 ^{a}	85,82 ^{b}	83.36 ^{c}	84.74 ^{b}	86.60 ^{a}	84.06 ^{a}	84,38 ^{b}	85.56 ^{A}
Average		80.92 ^{c}	85.69 ^{a}	77.16 ^{d}	82.33 ^{b}	83,30 ^{a}	81,71 ^{b}	79,56 ^{c}	80.30 ^{b}	82.42 ^{a}	82.79 ^{a}	80.58 ^{b}	81.53
Germinative	2012	97.24 ^{c}	98.07 ^{b}	97.94^{b}	98.56 ^{a}	98.60 ^{a}	97.98 ^{b}	97.27 **c**	98.18 ^{b}	98.55 ^{a}	97.79 ^{c}	97.30 ^{d}	97.95 ^{A}
energy	2013	84.20 ^{a}	83.09 ^{b}	78.51 ^{d}	82.23 ^{c}	82.80 ^{ns}	82.58 ^{ns}	82.43 ^{ns}	82.85 ^{ns}	82.64 ^{ns}	81.87 ^{ns}	81.80 ^{ns}	82.61 ^{C}
(%)	2014	97.35 ^{b}	96.43 ^{c}	98.27 ^{a}	96.44 ^{c}	97.29 ^{ns}	97.07 ^{ns}	97.00 ^{ns}	97.27^{ns}	96.94^{ns}	97.25^{ns}	97.03^{ns}	97.12 ^{B}
Average		92.93 ^{a}	92.52 ^{b}	91.57 ^{b}	92.41 ^{b}	92.91 ^{a}	92.54 ^{b}	92.23 ^{c}	92.76^{a}	92.71^{a}	92.3 ^{b}	92.04 ^{c}	92.56
Total	2012	12.04 ^{c}	12.98 ^{a}	11.97 ^{c}	12.66 ^{b}	11.85 ^{c}	12,49 ^{b}	12.90 ^{a}	11.33^{d}	12.16 ^{c}	12.80 ^{b}	13.37 **a**	12.41 ^{B}
protein	2013	12.65 ^{ns}	12.50 ^{ns}	12.56 ^{ns}	12.49 ^{ns}	12.42 ^{b}	12.39 ^{b}	12.83 ^{a}	11.64 ^{d}	12.32 ^{c}	12.87 ^{b}	13.38 ^{a}	12.55 ^{A}
(%)	2014	11.99 ^{c}	12.73 ^{a}	12.04 ^{c}	12.35 ^{b}	12.15 ^{ns}	13.38 ^{ns}	13.31 ^{ns}	11.27 ^{d}	11.95 ^{c}	12.68 ^{b}	12.90^{a}	12.28 ^{C}
Average		12.23 ^{c}	12.73 ^{a}	12.19 ^{c}	12.50 ^{b}	12.14 ^{c}	12.42 ^{b}	12.68 ^{a}	11.41 ^{d}	12.14 ^{c}	12.78^{b}	13.32^{a}	12.41

The means followed by different lowercase letters across years (rows) for cultivars, seeding rates and nitrogen fertilization rates are significantly different at 95% according to Duncan't test. The average values for years (the last column) followed by different capital letters are significantly different at 95% according to Duncan't test

G – Cultivars: G_1 ='Novosadski 448'; G_2='Novosadski 456'; G_3='Dunavac'; G_4='Jadran'. S – Seeding rate: S_1=350 seeds m^{-2}; S_2=450 seeds m^{-2}; S_3=550 seeds m^{-2}

N – Nitrogen rate**:** N_1 = 45 kg N ha^{-1} N_2 = 75 kg N ha^{-1}; N_3 = 105 kg N ha^{-1}; N_4 = 135 kg N ha^{-1}

Discussion

Agronomic management and environment affect malting barley yield and quality. There is little published information from Serbia on the effects of agronomic practices such as seeding and N rates on yield and quality of malting barley and the relative response of different cultivars to these factors especially over the range of variable edaphic and climatic conditions that prevail across the region. Yield is reduced mostly when drought stress occurs during heading or flowering and soft dough stages (Taheri *et al.* 2011). Drought stress during maturity results in about 10% decrease in yield, while moderate stress during the early vegetative period has essentially no effect on yield (Bauder 2001; Rajala *et al.* 2011). Heat stress has the strongest negative effect on barley yield if it occurs at the beginning of the grain filling period *i.e.*, 10–14 days after flowering (Savin and Nicolas 1999). The significant effect of climatic conditions on the yield and quality of barley was also observed by Křen *et al.* (2014) and Meng *et al.* 2016). Seeding rate influences grain quality, but has little effect on yield (McKenzie *et al.* 2005). The increase in seeding rate had a positive effect on grain yield in 'Novosadski 448', 'Novosadski 456' and 'Dunavac', and a strongly negative effect on 'Jadran'. The results of the present study show that the highest seeding rate gave the highest number of spikes per unit area, but the lowest number of kernels per spike and the lowest kernel weight per spike. This led to grain yield stagnation at seeding rates between 450 and 550 seeds m^{-2}. High seeding rates (> 300 seeds m^{-2} or > 3.0 million seeds ha^{-1}) resulted in reduced grain weight, plumpness, and protein concentration of two-row barley (McKenzie *et al.* 2005; O'Donovan *et al.* 2011). However, high seeding rates were associated with increased grain uniformity (O'Donovan *et al.* 2012). The present results are partially consistent with the findings of O`Donovan *et al.* (2012), who reported stagnating yields at seeding rates between 300 and 400 seeds m^{-2}, and decreasing yields at higher seeding rates. Yield stagnation at seeding rates between 450 and 550 seeds per m^{-2} in the present experiment may also result from a somewhat smaller number of emerged plants. As seeding rate increased, the average grain protein concentration increased in all three years. Increasing the seeding rate is implicated in increasing the number of spikes m^{-2} as a result of induced tillering (Knezevic *et al.* 2015). The increase in the number of spikes leads to a reduction in seed size, with seeds mostly having a high protein content and low starch accumulation (Madic *et al.* 2006). Paunović *et al*. (2009) and Noworolnika (2010), also reported that grain protein concentration in 'Nadek', 'Sebastian' and 'Mauritia' was not significantly affected by increasing seeding rates, as opposed to the significant increase in grain protein concentration in 'Widawa', 'Kirsty', 'Toucan' and 'Nagradowicki'. Contrary to the present results, Koutná *et al*. (2003), Mckenzia *et al*. (2005) and O'Donovana *et al*. (2011, 2012), reported a reduction in grain protein concentration with increasing seeding rates. O'Donovan *et al*. (2012) observed the highest decrease in grain protein concentration at seeding rates between 100 and 300 seeds m^{-2}, whereas further increase in seeding rate had little or no effect on grain protein concentration. The different effects of seeding rate on grain protein content in barley may be attributed to different cultivar characteristics and different environmental conditions under which barley is produced. Grain protein concentration is a key quality criterion in malting barley production. Nitrogen fertilizer application rate is the most influential agronomic factor controlling both grain yield and quality of malting barley (McKenzie *et al.* 2005; Sainju *et al.* 2013). Nitrogen fertilizer application increases grain protein concentration and decreases kernel plumpness (Petrie *et al.* 2002; Sainju *et al.* 2013). A challenge facing malting barley growers is how to use nitrogen (N) fertiliser to increase crop yields without compromising the quality of grain for malting by increasing the grain protein content. Recommendations for N fertiliser are dependent on the yield potential, cultivar sown, the nitrogen status of the soil and the end use of the crop (Lieffering *et al.* 1993). Grain of cultivars with higher stems ('Novosadski 456' and 'Jadran') had a significantly higher protein concentration in the years with higher amounts of precipitation. Comparison between the cultivar having the highest grain protein concentration ('Novosadski 456') and the cultivar with the lowest ('Dunavac') suggests that grain protein concentration is primarily a cultivar-specific trait. The highest variation in grain protein concentration was induced by nitrogen application. The difference between the control and the treatment with the highest nitrogen rate was 2.04, 1.74 and 1.63% in 2012, 2013 and 2014, respectively. The highest difference in the first year may be due to plant lodging at increased nitrogen rates (the correlation coefficient r = 0.721, P < 0.01), whereas the considerably smaller differences in the second year were attributed to the increased protein concentration under all nitrogen treatments due to drought and high temperatures during ripening. Numerous studies have reported the effect of applied N fertiliser at differing rates and timing on malting barley grain yields (Ramos *et al.* 1995; Ruiter and Brooking 1996; Małecka and Blecharczyk 2008; Malešević *et al.* 2010; Janković *et al.* 2011; Shafi *et al.* 2011; Křen *et al.* 2014; Tahir *et al.* 2019).

Conclusion

Agronomic practices had significant effects on malting quality and may be useful to increase the probability of achieving acceptable malting quality under more typical climatic conditions. The analysis of the present results shows that optimum seeding and nitrogen rates that give high yields, good grain quality and optimum grain protein concentration are largely dependent on production conditions. Also, results indicate that seeding rates above 350 seeds m^{-2} and nitrogen rates above 75 kg ha^{-1} lead to substantial grain quality deterioration in the studied spring barley cultivars.

Author Contributions

Vladanka Stupar: Conceptualization, Investigation, Writing–Original Draft; Ivica Đalović: Conceptualization, Writing–Original Draft, Writing–Review & Editing; Desimir Knežević: Conceptualization; Methodology, Writing–Review & Editing, Project; Milomirka Madić: Conceptualization; Methodology, Writing–Original Draft; Aleksandar Paunović: Conceptualization; Methodology, Writing–Review & Editing; Supervision, Project.

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