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Influence of genotype, nitrogen fertilisation and weather conditions on yield variability and grain quality in spring malting barley

Uticaj genotipa, azotne ishrane i vremenskih uslova na varijabilnost prinosa i kvalitet zrna kod jarog pivskog ječma

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ABSTRACT

Production traits and grain quality of spring malting barley as the major raw material in malt and beer production were evaluated over a period of three years (2012–2014) in the region of Požarevac, Serbia. Four cultivars of two-rowed spring barley released in the Republic of Serbia ('Novosadski 448', 'Novosadski 456', 'Dunavac' and 'Jadran') were used. The objective of this research was to assess variability in grain yield and yield components in genetically divergent cultivars of two-rowed spring barley grown at different nitrogen application rates, as dependent on precipitation and air temperature during the growing season. The results showed significant variations in grain yield, yield components and grain protein content across genotypes (G), N application rates (N) and experimental years (E). Significant interdependence was found between grain yield and yield components. Increasing the N application rate up to 135 kg/ha had a positive effect on grain yield, plant height, number of spikes per unit area, spike length and grain protein content. Number of grains per spike and 1,000-kernel weight increased significantly at N application rates up to 105 kg/ha and 75 kg/ha, respectively.

Keywords: barley genotype, nitrogen fertilisation, protein content, variability, yield components

IZVOD

Tokom trogodišnjeg perioda (2012–2014) na području Požarevca (Srbija) proučavane su produktivne osobine i kvalitet zrna četiri sorte jarog dvoredog ječma, selekcionisane u Republici Srbiji („Novosadski 448", „Novosadski 456", „Dunavac" i „Jadran"), kao glavne sirovine u proizvodnji slada i piva. Cilj ovog rada bio je da se proceni varijabilnost prinosa zrna i komponenti prinosa genetički različitih sorti jarog dvoredog ječma pri različitim nivoima ishrane mineralnim azotom u zavisnosti od količina padavina i temperature vazduha tokom godine. Dobijeni rezultati su ukazali na značajno variranje prinosa zrna, komponenti prinosa i sadržaja proteina u zrnu između genotipova ječma (G), primenjenih doza azota (N) i eksperimentalnih godina (E). Utvrđene su značajne međuzavisnosti između prinosa zrna i komponenti prinosa. Prinos zrna bio je u pozitivnoj korelaciji sa brojem klasova po m^2, visinom biljke, brojem zrna po klasu, dužinom klasa i masom 1000 zrna. Povećanje primene azota do135 kg/ha je imalo pozitivan uticaj na prinos zrna, visinu biljke, broj klasova po jedinici površine, dužinu klasa i sadržaj proteina u zrnu. Broj zrna po klasu značajno se povećavao do doze od 105 kg/ha, a masa 1000 zrna do doze od 75 kg/ha.

Ključne reči: genotip ječma, azotna ishrana, sadržaj proteina, varijabilnost, komponente prinosa

INTRODUCTION

Choosing appropriate cultivars is the first step in the successful production of malting barley (Leistrumaitë and Paplauskienë, 20005). Modern barley breeding is largely directed towards the development of genotypes characterised by increased yield potential, wide adaptation and high responses to agronomic inputs (Pržulj et al. 2014). However, some studies have shown that grain yield is more dependent on environmental conditions during the growing season than on genotypic effect (Ayranci et al., 2014; Ahmadi et al., 2016).

Grain yield in barley is a complex trait with an intricate genetic background. It is the resultant interaction between cultivar, cultural practices and environmental conditions throughout the life cycle of a barley plant (Biberdžić et al., 2012). Grain yield is determined by three major components: number of spikes per m^2, number of grains per spike and 1,000-kernel weight. These components undergo complex and, often, antagonistic interactions, with maximum yield occurring as the result of their most favourable balance.

Environmental conditions and N fertilisation are significantly effective in increasing yield components and seed yield in barley (Knezevic et al., 2015). Nitrogen is the key element in achieving consistently high yields in cereals, with the rate of uptake and partitioning of N being largely determined by supply and demand during various stages of plant growth (Shafi et al., 2011). The efficiency of nitrogen absorption and utilisation is associated with genotype's root capacity as well as with the amount of available nutrients and soil moisture (Veigh and Rajkai, 2006). El Metwally et al. (2010) observed that increases in barley grain yield with increasing nitrogen application rate can be attributed to the stimulating effect of nitrogen on vegetative growth, photosynthetic activity, number of spikes per plant, number of spikelets per spike, spike length and number of grains per spike. Although increased nitrogen application rates can promote grain yield, they can indirectly lead to undesirable grain quality traits in malting barley (smaller grains, increased grain protein content). In some situations, at the highest N application rates, yield may decline while grain protein concentration continues to increase (Benzian and Lane, 1979). The complexity of nitrogen fertilisation is largely the result of climatic conditions during the growing season (total precipitation, distribution of precipitation and air temperature). Nitrogen and water availability interact strongly in crop development.

To provide good quality raw material for the beer industry, barley grains should be uniform in size, with the absolute weight of over 40 g and total protein below 12% (Malešević et al., 2010). One of the main tasks of a barley breeding programme is to increase barley productivity while preserving and improving grain quality (Valcheva et al., 2013).

The objective of this study was to examine variability in grain yield and yield components in genetically divergent cultivars of two-rowed spring barley grown at different nitrogen application rates, as dependent on precipitation and air temperature during the growing season.

MATERIALS AND METHODS

The research was conducted during 2012–2014 in the region of Požarevac, Serbia (44° 36' 55" N and 21° 10' 57" E, 94 m a.s.l) on the same main plot divided in 2 subplots for maize–spring barley rotation. Maize was used as the preceding crop in each experimental year. The experiment was conducted on a Vertisol having a relatively high clay content and unfavourable physical properties. Major physicochemical properties of the soil: pH_{KCl} – 6.13, humus – 2.9%, $CaCO_3$ – 1.72%, N – 0.1%, P_2O_5 – 0.08 g/kg and K_2O – 0.13 g/kg. The experiment was laid out in a randomised complete block design with three replications. Each treatment was planted in plots of 5.0 m^2 area, consisting of ten 5.0 m long rows and spaced 0.1 m between rows. Seeds were sown by hand into the experimental plots on 12 March 2012, 24 March 2013 and 7 March 2014, at a seeding rate of 450 germinating seeds per m^2. Four spring barley cultivars, 'Novosadski 448' (G_1), 'Novosadski 456' (G_2), 'Dunavac' (G_3) and 'Jadran' (G_4), were the first factor. Nitrogen application rates were the second factor (N_1=45+0, N_2=45+30, N_3=45+60 and

N_4=45+90 kg N/ha). Before seeding, the soil was treated with 45 kg N/ha, 45 kg P_2O_5/ha and 45 kg K_2O/ha (300 kg/ha $N_{15}P_{15}K_{15}$). The remaining N (30, 60 and 90 kg N/ha) was applied in the form of calcium ammonium nitrate (KAN – 27% N) at the stage of tillering of barley plants (27 April 2012, 5 May 2013 and 10 May 2014). Barley was harvested at full maturity on 9 July 2012, 25 July 2013 and 18 July 2014. A sample consisting of 30 randomly selected plants was taken from middle rows of each plot (2 rows x 2.5 m). Average values for plant height, spike length and grain number per spike are presented as averages for 90 plants (three replications). Plant height was measured, starting from the tillering node to the last grain of the spike in the longest stem, and expressed in cm. Spike length was determined by measuring from the base to the last grain of the spike without awn, and expressed in cm. Grain number per spike was determined by counting grains in each spike of the plant in the same sample. At harvest, grain yield in each plot was measured from an area of 1 m^2 (4 middle rows x 2.5 m length), and calculated as yield in kg/ha at 14% moisture. Grain moisture was determined by drying to a constant weight at 105°C in a drying oven in three replications. Thousand-kernel weight was determined from bulk harvested grains for each treatment and replication (SRPS EN ISO 520:2012) using a Tehnica technical balance (120/0.01 g). Crude protein content was assessed by the Kjeldahl method and in accordance with SRPS EN ISO 20483:2009 using a Vapodest 30 (Gerhardt) unit at the Public Health Institute, Požarevac.

Meteorological conditions

Weather conditions during the growing seasons in 2012, 2013 and 2014 are presented in Figure 1. The average mean monthly air temperature for the three growing seasons (March–July) was 16.5°C i.e. it was higher by 1.1°C than the long-term average (1981–2010) (15.4°C). The total precipitation level during the spring barley growing season (March–July) was higher than the 30-year average (301.5 mm), as follows: in 2012 by 158.2 mm, in 2013 by 26.0 mm, and in 2014 by 179.3 mm. The distribution of precipitation during the first research year was non-uniform: the highest amount of precipitation was between March and end of May, and a rainfall deficit occurred at grain filling and maturation, when air temperatures were highest on average, which resulted in accelerated grain maturation in the tested cultivars. The highest air temperature during the spring barley growing season was recorded in 2012 (17.0°C), with mean air temperatures in June (22.7°C) and July (25.3°C) significantly higher than the 30-year average (June 21.9°C and July 21.5°C). In the second experimental year, seeding was delayed by 20–30 days relative to the optimal date due to prolonged winter and high snow depth. The long cold winter period was abruptly followed by high air temperatures during April and May (13.2°C and 18.5°C, respectively), which were above the long-term average (11.8°C and 17.0°C, respectively). Towards the end of the growing season of spring malting barley in 2013, precipitation levels during June were only 40.2% of the long-term average. The third experimental year had low amounts of precipitation at the beginning of the growing season (March, April) and extremely high levels in May (153.5 mm), June (73.3 mm) and July (165.7 mm).

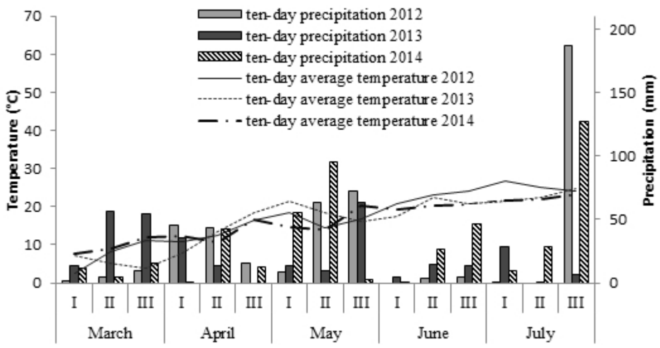

Figure 1. The average air temperatures and precipitation totals of ten-day periods during the growing seasons in 2012, 2013 and 2014

Statistical analysis

Results were subjected to a three way ANOVA (year, cultivar, fertilisation) for the experimental period using the statistical package Statistica 10 (StatSoft Inc., 2011). Means were compared using Duncan's multiple range test at the 95% level. The correlation between grain yield and its components was determined by correlation analysis (Pearson's correlation coefficient, R).

RESULTS AND DISCUSION

Results (ANOVA) showed that all tested factors (G–genotypes, N–fertiliser, E–environments) had a significant effect on grain yield and its biological components, as well as on grain quality traits i.e. grain protein content (Table 1). Weather conditions during the experimental period were the dominant factor of variability in plant height (72.07%), spike length (62.07%), grain number per spike (59.48%) and grain yield (47.83%), whereas genotype traits were the dominant factor of variability in 1,000-kernel weight (63.36%). Nitrogen fertiliser rate had the strongest effect on grain protein content (78.22%) and number of spikes per m^2 (48.71%). Interaction effects of factors had a significantly lower impact on the traits analysed, with their partial proportion ranging from 0.16% to 12.03%.

Grain yield is a complex trait governed by the optimal relationship among its biological components: plant height, number of spikes per m^2, number of grains per spike and 1,000-kernel weight. The contribution of these components to grain yield formation is dependent on weather conditions during the critical stages of growth and development (particularly water stress), but also on cultural practices (Blue et al., 1990), especially seeding date, seeding rate, fertiliser timing and rates of nutrients, particularly nitrogen.

Variations in the average grain yield were primarily induced by the effect of E (47.83%), followed by the N application rate (42.71%) and G (1.22%). Significant differences ($P<0.05$) were observed in grain yield among years.The highest average grain yield was obtained in

Table 1. Results of the analysis of variance and the proportion of the explained sum of squares

Source of variation	d.f.	Plant height (cm)	Number of spikes per m^2	Spike length (cm)	Number of grains per spike	1,000 kernel weight (g)	Grain yield (kg/ha)	Grain protein content (%)
		Sum of squares						
G	3	8525**	412606**	18,20**	743,3**	1697,5**	5.43 x 106 *	21.07**
N	3	4515**	1454525**	85,01**	223,3**	458,5**	1.60 x 108 **	219.66**
E	2	44364**	879581**	225,37**	1569,3**	51,4**	1.76 x 108 **	5.21**
G x N	9	261**	20300ns	46,8ns	5.20**	19,1ns	3.33 x 106 ns	3.37 ns
G x E	6	3228**	74954ns	15,04**	13,3ns	322,2**	1.57 x 107 **	17.76**
N x E	6	96ns	43073ns	11,02**	8,8ns	45,7ns	3.24 x 106 ns	1.23 ns
G x N x E	18	571**	101286ns	4,40ns	33,6ns	84,9ns	1.88 x 106 ns	12.57 *
		Explained sum of squares (%)						
G		13.85	13.82	5.01	28.17	63.36	1.45	7.5
N		7.33	48.71	23.41	8.46	17.11	42.71	78.22
E		72.07	29.45	62.07	59.48	1.92	47.83	1.86
Gx N		0.42	0.68	1.11	1.76	0.71	1.69	1.2
G x E		5.24	2.51	4.14	0.5	12.03	4.2	6.32
N x E		0.16	1.44	3.04	0.33	1.71	0.87	0.44
G x N x E		0.93	3.39	1.21	1.27	3.17	1.25	4.46

G – Genotypes; N – Fertiliser; E – Experimental years

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

2014 (5,824.2 kg/ha), which was characterised by the highest average level of precipitation and its favourable distribution (Table 2).

Delayed seeding, lack of precipitationand high temperatures in 2013 decreased grain yield by 26.1% and 20.7% compared with 2014 and 2012, respectively. High temperatures and lack of soil moisture and air humidity during heading lead to a decrease in grain yield by 18 to 35%, depending on cultivar tolerance to water and temperature stress conditions (Paunović et al., 2008).

The tested cultivars showed significant differences in grain yield and different responses to environmental conditions (G x E interaction) (Table 3). Regardless of year, the highest grain yield was achieved by 'Novosadski 448' and the lowest by 'Jadran', whose yield exhibited the highest variations across years. Averaged over the years and nitrogen application rates, 'Novosadski 456' gave the highest average number of spikes, the lowest number of grains per spike and the highest thousand-kernel weight. The highest average values for spike length and number of grains per spike were recorded in 'Novosadski 448'. However, the difference in grain yield among 'Novosadski 448', 'Novosadski 456' and 'Dunavac' was not significant.

On average for all cultivars, plant height, number of spikes per m^2 and spike length increased significantly until the N application rate reached maximum. Considering the important role of plant height in cereal production and its strong association with lodging susceptibility, tall plants often have low yields and poor grain quality (Berry et al., 2007). In our study, plant height varied across cultivars, ranging from 74.18 cm ('Novosadski 448') to 86.54 cm ('Jadran'). The effect of nitrogen application rate on plant height is conditional upon cultivar specificity and soil moisture. Plant height in 'Novosadski 448' and 'Jadran' significantly increased at N rates up to 105 kg/ha, and in 'Novosadski 456' and 'Dunavac' at rates up to the maximum of 135 kg/ha (G x N interaction). Increasing the N rate from 75 to 105 kg/ha had no significant effect

Table 2. Averages for grain yield, yield components and grain protein content in spring barley genotypes at different nitrogen application rates over three years

	Source of variation	Plant height (cm)	Number of spikes per m^2	Spike length (cm)	Number of grains per spike	1,000 kernel weight (g)	Grain yield (kg/ha)	Grain protein content (%)
G	G1	74.18 ^{d}	756.48 ^{b}	7.46 ^{a}	19.34 ^{a}	36.89 ^{d}	5319.6 ^{a}	12.23 ^{c}
	G2	79.44 ^{c}	794.58 ^{a}	7.02 ^{b}	15.63 ^{c}	42.21 ^{a}	5226.9 ^{a}	12.73 ^{a}
	G3	78.44 ^{b}	763.25 ^{ab}	6.97 ^{b}	17.60 ^{b}	38.02 ^{c}	5174.1 ^{ab}	12.19 ^{c}
	G4	86.54 ^{a}	708.26 ^{c}	6.98 ^{b}	17.56 ^{b}	39.09 ^{b}	5010.8 ^{bc}	12.50 ^{b}
N	N1	74.83 ^{d}	676.28 ^{d}	6.47 ^{d}	16.48 ^{c}	38.03 ^{b}	4249.4 ^{d}	11.41 ^{d}
	N2	78.91 ^{c}	734.59 ^{c}	6.95 ^{c}	17.26 ^{b}	39.98 ^{a}	5047.9 ^{c}	12.14 ^{c}
	N3	81.30 ^{b}	776.83 ^{b}	7.34 ^{b}	18.15 ^{a}	40.18 ^{a}	5612.6 ^{b}	12.78 ^{b}
	N4	83.56 ^{a}	834.87 ^{a}	7.66 ^{a}	18.24 ^{a}	38.02 ^{b}	5821.5 ^{a}	13.32 ^{a}
F	E1	85.59 ^{b}	811.0 ^{a}	6.84 ^{b}	17.21 ^{b}	38.76 ^{b}	5422.4 ^{b}	12.41 ^{ab}
	E2	65.38 ^{c}	700.5 ^{c}	6.39 ^{c}	15.38 ^{c}	38.86 ^{b}	4301.8 ^{c}	12.55 ^{a}
	E3	87.97 a	755.5 ^{b}	8.10 ^{a}	20.01 ^{a}	39.54 ^{a}	5824.2 ^{a}	12.28 ^{b}

G – Genotypes: G_1 = 'Novosadski 448'; G_2 = 'Novosadski 456'; G_3 = 'Dunavac'; G_4 = 'Jadran'
N –Nitrogen application rates: N_1=45 kg/ha/control /; N_2=75 kg/ha; N_3=105 kg/ha; N_4=135 kg/ha
E – Experimental years: E1 = 2012; E_2 = 2013; E_3 = 2014
* Mean values in columns followed by different lowercase letters indicate significant differences according to Duncan's test (P≤0.05)

on plant height in 'Novosadski 456'. When compared with the control, the highest N rate led to an increase in plant height by 7.73 cm in the first growing season, when lodging occurred, by 8.88 cm in the second, dry season, and by 9.59 cm in the third, wettest year.

As a result of the positive association with grain number per spike, spike length is one of the most important yield components (Madic et al., 2009). The average spike length in this study was 7.13 cm. On average, 'Novosadski 448' had the highest spike length, whereas the lowest was recorded in 'Dunavac'. Spike length increased significantly at N rates up to 105 kg/ha in the first growing season, and at rates up to the maximum 135 kg/ha (N x E interaction) in the second and third years.

Increased N rates adversely affected grain number per spike and thousand-kernel weight. At 75 kg N/ha, grains per spike and thousand-kernel weight increased significantly compared with the control. At a higher nitrogen rate (105 kg N/ha), grain number per spike increased significantly, but thousand-kernel weight stagnated, whereas the rate of 135 kg N/ha led to a slight decrease in the average number of grains per spike and a significant reduction in the average thousand-kernel weight.

The significance of the G x N interaction indicates different responses of genotypes (cultivars) to increased rates of nitrogen regarding grain number per spike. The highest number of grains per spike in cvs. 'Dunavac' and 'Jadran' was obtained at 135 kg N/ha, whereas 'Novosadski 456' and 'Novosadski 448' gave the highest values at 105 kg N/ha. Moreno et al. (2003) and Malesevic et al. (2010) found that the number of grains per spike increased at nitrogen application rates up to 100 kg/ha. In contrast, Gozdowski et al. (2012) observed no significant effect of nitrogen fertilisation on grain number per spike in spring barley cvs. 'Rastik' and 'Rasbet', which was most likely due to different climatic conditions.

Grain protein content is a major indicator of malting barley quality. This cultivar trait is largely dependent on climatic conditions (rainfall and temperature during ripening) (Ottman, 2011; Sedlář et al., 2011 and Pržulj et al., 2014). The average grain protein content across cultivars ranged from 12.19% ('Dunavac') to 12.73% ('Novosadski 456'). As the result of their higher stems and higher tendency to lodging, 'Novosadski 456' and 'Jadran' had significantly higher grain protein contents in high-rainfall years compared with the shorter-stemmed cultivars 'Novosadski 448' and 'Dunavac' (G x E interaction).

A very weak positive correlation (r=0.19, P<0.01) was found between plant height and grain protein content, and a weak positive correlation between lodging and grain protein content (r=0.35, P<0.01, results not shown).

Differences in grain protein content in the unfavourable year 2013 among cultivars were not significant. However, 'Novosadski 448' and 'Dunavac', which gave smaller grains and lower thousand-kernel weights, had significantly higher grain protein contents in 2013 than in 2012 and 2014. This may indicate that stress due to heat and water deficit during grain filling has a considerably higher impact on cultivars with lighter and smaller grains. Small grains have a low degree of starch and extract accumulation and a high protein content, which is not suitable for the beer industry (Madić et al., 2006). Stress during the grain filling periodhas a stronger effect on starch synthesis than on protein accumulation, which is the main reason for the low starch concentration and elevated protein content (Passarella et al., 2002).

The amount of nitrogen fertiliser has a direct effect on grain protein content. Apart from directly inducing an increase in grain protein content, nitrogen has a much greater indirect effect, leading to an increase in protein content through lodging (Paunović et al., 2009). Also, high N rates can cause moisture stress due to intensive vegetative growth, which involves depletion of moisture reserves for subsequent grain filling (Madić et al., 2011). As the rate of nitrogen fertiliser increased, the average grain protein content significantly (P<0.05) increased at N rates up to 135 kg/ha (Table 2). When compared with the control, the highest amount of N in the first growing season, when crop lodging occurred, gave an increase in grain protein content by 2.03%.

Table 3. Means for grain yield, yield components and grain protein content in spring barley genotypes at different nitrogen application rates over three years (genotype x nitrogen, year x genotype and year x nitrogen interactions)

	Source of variation	Plant height (cm)	Number of spikes per m^2	Spike length (cm)	Number of grains per spike	1,000 kernel weight (g)	Grain yield (kg/ha)	Grain protein content (%)
G_1	N_1	77.69^{j}	688.9	6.85	18.44^{cd}	36.08	4587.6	11.32
	N_2	73.25^{i}	753.0	7.24	19.00^{bc}	37.69	5081.8	12.22
	N_3	75.57^{g}	773.9	8.87	20.47^{a}	38.21	5888.0	12.54
	N_4	77.25^{g}	825.1	7.89	19.45^{b}	35.58	5720.8	13.10
G_2	N_1	75.30^{h}	709.6	6.56	15.05^{h}	41.11	4306.5	11.60
	N_2	79.09^{ef}	767.9	6.87	15.39^{h}	43.34	5117.3	12.47
	N_3	80.04^{de}	825.1	7.18	16.07^{g}	42.95	5684.0	13.14
	N_4	83.32^{c}	875.7	7.49	16.02^{g}	41.42	5799.8	13.74
G_3	N_1	72.05^{ij}	687.9	6.27	16.36^{g}	36.88	4181.8	11.24
	N_2	77.91^{fg}	749.9	6.81	17.23^{f}	38.94	5089.9	11.99
	N_3	80.30^{de}	772.8	7.17	18.04^{d}	39.49	5453.0	12.64
	N_4	83.50^{c}	842.4	7.63	18.76^{c}	36.76	5961.6	12.90
G_4	N_1	81.31^{d}	618.7	6.22	16.07^{g}	38.05	3911.6	11.51
	N_2	85.39^{b}	686.6	6.89	17.41^{ef}	39.94	4902.6	12.16
	N_3	89.30^{a}	735.5	7.15	18.00^{e}	40.07	5425.2	12.88
	N_4	90.16^{a}	793.3	7.65	18.74^{c}	38.31	5803.8	13.55
E_1	G_1	76.28^{d}	823.3	7.30^{c}	18.86	37.62^{e}	5703.2^{bc}	12.04^{d}
	G_2	84.42^{c}	838.3	6.43^{e}	15.38	42.08^{b}	5494.4^{bc}	12.98^{a}
	G_3	84.46^{c}	827.6	6.70^{d}	17.22	37.84^{e}	5468.3^{c}	11.97^{d}
	G_4	97.20^{a}	754.7	6.92^{d}	17.38	37.49^{e}	5023.7^{d}	12.66^{bc}
E_2	G_1	62.71^{f}	699.3	6.92^{d}	17.09	35.66^{f}	4415.0^{e}	12.65^{bc}
	G_2	65.23^{e}	746.2	6.33 e	13.69	43.26^{a}	4516.9^{e}	12.49^{bc}
	G_3	66.68^{e}	719.8	6.27^{ef}	15.59	37.21^{e}	4370.1^{e}	12.56^{bc}
	G_4	66.92^{e}	636.5	6.05^{f}	15.13	39.31^{d}	3905.4^{f}	12.49^{bc}
E_3	G_1	83.54^{c}	746.8	8.17^{ab}	22.07	37.39^{e}	5840.4^{ab}	11.99^{d}
	G_2	88.66^{b}	799.2	8.32^{a}	17.83	41.29^{bc}	5669.4^{bc}	12.73^{ab}
	G_3	84.19^{c}	742.4	7.93^{b}	19.98	39.00^{d}	5683.8^{bc}	12.04^{d}
	G_4	95.50^{a}	733.6	7.97^{b}	20.16	40.47^{c}	6103.3^{a}	12.35^{bc}
E_1	N_1	81.69	720.4	6.46^{h}	13.39	38.03	4511.1	11.33
	N_2	84.75	796.6	6.63^{fg}	16.76	39.50	5278.0	12.14
	N_3	86.49	832.6	7.01^{e}	17.74	39.69	5729.1	12.80
	N_4	89.42	894.4	7.24^{e}	18.05	37.80	6108.4	13.36
E_2	N_1	60.06	638.2	5.39^{i}	14.18	37.79	3480.3	11.60
	N_2	64.92	684.3	6.29^{h}	15.28	39.54	4207.7	12.32
	N_3	67.63	717.2	7.77^{d}	15.98	39.79	4607.3.	12.86
	N_4	68.94	762.1	7.12^{e}	16.07	38.33	4912.1	13.38
E_3	N_1	82.73	670.3	7.57^{d}	18.87	38.26	4756.8	11.27
	N_2	87.07	722.9	7.94^{c}	19.74	40.90	5658.0	11.95
	N_3	89.78	780.7	8.25^{b}	20.82	41.06	6438.2	12.68
	N_4	92.32	848.09	8.63^{a}	20.61	37.92	6443.9	12.90

G – Genotypes: G_1 = 'Novosadski 448'; G_2 = 'Novosadski 456'; G_3 = 'Dunavac'; G_4 = 'Jadran'.
N–Nitrogen application rates: N_1=45 kg/ha/control/; N_2=75 kg/ha; N_3=105 kg/ha; N_4=135 kg/ha.
E – Experimental years: E_1 = 2012; E_2 = 2013; E_3 = 2014.
* Mean values in columns followed by different lowercase letters indicate significant differences according to Duncan's test (*P*≤0.05).

In the second, dry growing season, grain protein content at the highest N rate was higher by 1.78% than in the treatment without N. The lowest increase in grain protein content (1.63%) was recorded in the third year, which had the highest rainfall amount and a favourable distribution of precipitation, with no crop lodging, which is consistent with the findings of Magliano et al. (2014). The lower grain protein content in 2014 was due to the highest grain yield and larger grains with higher starch contents, as attributed to the protein dilution effect. In regions with less favourable environments, such as southern and eastern Europe, barley yield and quality is limited by water availability, heat stress and the duration of the grain filling period (Pržulj and Momčilović, 2012). As found by the same authors, crop yield and quality are not determined by a single ecological factor but rather by several stress factors whose combined effect is more severe than their sum. Given the above, grain protein content is a major malting quality trait of malting barley and the effect of nitrogen fertilisation is directly dependent on soil moisture.

The issue regarding an optimal nitrogen rate remains a topical subject in numerous studies (Malešević et al., 2010; El Metwally et al., 2010; Janković et al., 2011; Magliano et al., 2014; Tanaka and Nakano, 2019). The correlation analysis showed a positive correlation between grain yield and number of spikes per unit area ($r=0.73$, $P<0.01$), plant height ($r=0.60$, $P<0.01$), number of grains per spike ($r=0.58$, $P<0.01$), spike length ($r=0.57$, $P<0.01$) and 1,000-kernel weight ($r=0.11$, $P<0.05$) (Table 4).

The results obtained in this study comply with those of Dyulgerova (2012), Tofiq et al. (2015) and Dorostkar et al. (2015), who determined the highest correlation between grain yield and number of spikes per m^2, and between grain yield and number of grains per spike. Likewise, Deniz et al. (2009) reported a significant positive correlation between grain yield and number of spikes per m^2, and between grain yield and 1,000-kernel weight, but also a significant negative correlation between number of spikes per m^2 and 1,000-kernel weight. An increase in one grain yield component very often leads to a decrease in another, as indicated by the negative correlation between grain number per spike and 1,000-kernel weight ($r=-0.15$, $P<0.01$) and between spike number per m^2 and 1,000-kernel weight ($r=-0.14$, $P<0.01$). Therefore, complex compensatory relationships exist among yield components. The highest number of spikes in 2012 gave the lowest 1,000-kernel weight, whereas the lowest number of spikes and grains per spike in the unfavourable year 2013 stimulated an increase in 1,000-kernel weight. The lower degree of overall and productive tillering in 2014 induced an increase in spike length, number of grains per spike, grain weight per spike and 1,000-kernel weight.

Table 4. Coefficients of correlation between grain yield and its biological components in spring barley

Yield parameters (n = 144)	Plant height	Spike length	Number of grains per spike	1,000 kernel weight	Number of spikes per m^2	Grain yield
Grain protein content	0.19**	0.16**	-0.02ns	0.03ns	0.43**	0.36**
Grain yield	0.60**	0.57**	0.58**	0.11*	0.73**	
Number of spikes per m^2	0.36**	0.11*	0.04ns	-0.14**		
1,000-kernel weight	0.07ns	0.07ns	-0.15**			
Number of grains per spike	0.49**	0.78**				
Spike length	0.51**					

* significant at the 0.05 level; ** significant at the 0.01 level; ^{ns} – non-significant

CONCLUSION

This research on four spring malting barley cultivars showed differences in genotype response to nitrogen fertilisation and environmental conditions, as assessed based on variability in yield, yield components and protein contents.

Nitrogen application rates and weather conditions during the experimental period had different effects on grain yield, yield components and grain protein content in spring barley cultivars.

Significant correlations were observed between grain yield and yield components. Grain yield was positively correlated with number of spikes per m^2, plant height, number of grains per spike, spike length and 1,000-kernel weight.

Increasing the N application rate to 135 kg/ha stimulated grain yield, plant height, number of spikes per unit area, spike length and grain protein content. Number of grains per spike and 1,000-kernel weight increased significantly at N rates up to 105 kg/ha and 75 kg/ha, respectively.

Results showed that the optimum N application rate for spring barley is up to 70 kg/ha for cultivars characterised by short stems and a low degree of grain protein accumulation ('Novosadski 456' and 'Dunavac') and up to 50 kg/ha for cultivars exhibiting higher stems prone to lodging and additional accumulation of protein in the grain ('Novosadski 448' and 'Jadran').

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