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Variability in seed germination of barley cultivars (*Hordeum vulgare* L.) grown under different nitrogen application rates

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Abstract: Variability in the percentage of seed germination was studied in four winter barley cultivars ('Jagodinac', 'Premium', 'NS 489' and 'NS 495'), grown under four nitrogen application rates (control N_0=0, N_1=20, N_2=40 and N_3=60 kg ha^{-1}) during two years of the experiment. The experiment was carried out as a randomised block design in 5m^2 plots and with four replications. In both experimental years, the average seed germination was over 90.0% for all barley cultivars and in each nitrogen fertilisation treatment. The maximum seed germination percentage was 98.0% in 'Premium' in the second year in the treatment N_3=60 kg ha^{-1}, and minimum germination percentage

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(90.1%) was also recorded in 'Premium' 'in the first year of the experiment in the unfertilised control (N_0=0 kg ha^{-1}). The obtained values of seed germination significantly differed among cultivars in the first and second year. Also, the average values of seed germination under four N fertilisation treatments were significantly different in both years. The average values for both years of the experiment were significantly different among cultivars and treatments. The values of seed germination for all cultivars increased with increasing nitrogen rates, suggesting that phenotypic variability in seed germination was affected by nitrogen fertilisation, which indicated a high value (81.87%) of the component of phenotypic variance for seed germination.

Keywords: barley, variability, seed germination, nitrogen rate.

Introduction

The main task of barley breeders is to create cultivars which will achieve high yield, good quality and adaptation to abiotic and biotic stress factors. These plant traits are negatively correlated and influenced by morphological, anatomical, physiological and biochemical characteristics, which are determined by genetic and environmental factors, as well by the genotype/environment interaction (Coventry *et al*., 2003; Knežević *et al*., 2011). The reproduction of barley cultivars requires healthy and quality seeds with high percentage values of germination. Seed germination is a critical stage in the life cycle of plants, which involves proteases and where the proteolytic role has significant economic importance (Diaz-Mendoza *et al*., 2019). Seed germination affects the species abundance distribution of plant communities (Paunović *et al*, 2010). Variations in seed germinability can be related to different barley growing regions and environments due to environmental limitations, such as altitude, topography, soil quality and climate (Ranieri *et al*., 2012). A number of ecological and evolutionary factors can have implications for seed germination. Seed size is an important evolutionary trait which affects the reproductive success of many plant species, and is variable among species (Moles *et al*., 2006) and among cultivars within species (Paunović *et al*., 2010). Germination time, germination percentage and seedling vigour are directly influenced by seed size (Yanlong *et al*., 2007). A high percentage of seed germination affects the distribution and abundance of plant species across agroecological locations (Silveira *et al*., 2012). Research on seed germination in barley cultivars showed a more rapid germination of large seeds and a more rapid field emergence (Chastain *et al*., 1995). Also, seedlings produced from large seeds of winter barley emerged more rapidly and produced higher density stands than small seeds (Paunović *et al*., 2010).

Poor germination can be due to high humidity and high temperatures in the ripening stage, which can cause sprouting. Also, damage to the seed with a moisture content of over 13% during handling (broken seed) and unsafe storage with a risk of fungal and insect infestation can cause a decline in percentage

germination. Postharvest controls have combined chemical, physical and biological approaches, but only limited success has been achieved. Chlorine dioxide is often used to prevent seed infestation with microorganisms, pathogens and viruses. Soaking seeds with moderate ClO_2 did not inhibit the germination of barley seeds and deform chlorophyll in barley leaves. On the contrary, it promoted the growth of barley roots, while treatments with high levels of ClO_2 (1000 and 2000 $mg.L^{-1}$) significantly decreased the germination percentage (Wang *et al*., 2019). An efficient way to preserve seed viability is storing seeds in conditions that cause minimum changes in biological characteristics. The development of morphological traits of plants is influenced by soil fertility, moisture and acidity, temperature, light and precipitation (Kondić *et al*., 2012). Spike traits are important in preserving both sprouting and the germination of seeds after harvest.

The production of vigorous quality seeds requires the use of scientific farming methods and crop control at all stages of plant growth until harvest and storage (Zečević *et al*., 2006). Also, depending on soil type, optimal mineral nutrition should be ensured (Koutna *et al*., 2003). There are differences in nitrogen absorption among cultivars (Kovačević *et al*., 2007; Paunović *et al*., 2007) which are adapted to variable environments. Growing barley cultivars at optimal nitrogen levels is suitable for the production of seeds with a high percentage of germination. Also, during the growing season, nitrogen leads to high seed germination and reduces weed seed emergence and density (Monaco *et al*., 2003). Nitrogen fertilisation increases barley yield and improves yield components (Madić *et al*., 2009; Knežević *et al*., 2015).

The objective of this research was to evaluate the effect of increasing rates of N, applied during the growing season, on seed germination in four genetically divergent barley cultivars (*Hordeum vulgare* L.).

Materials and methods

Four winter barley cultivars (‘Jagodinac’, ‘Premium’, ‘NS 489’ and ‘NS 495’) were evaluated for seed germination during two years of the experiment which was performed in a randomised block design in $5m^2$ plots and with 4 replications under four mineral fertilisation rates (control N_0=0, N_1=20, N_2=40 and N_3=60 kg ha^{-1}). At full maturity, 80 plants (20 plants per replication) were sampled for analysis. Seed germination analysis was conducted after harvest in rolled paper towels placed in a germinator at 20°C (16 hours) and then at 30°C (8 hours) according to the rules of the International Seed Testing Association (2010). The germinator was set to provide light during the high-temperature cycle (8 hours) and to remain dark during the low-temperature cycle (16 hours). An initial count of the percentage of germination was after four days and the final score was obtained on the seventh day of the experiment .

The average value (x), variance (σ^2), and analysis of variance were computed. The analysis of variance was performed according to a randomised block design with two factors, allowing the calculation of the components of variance (σ^2_g - genetic, σ^2_{gl}-interaction; σ^2_E-environment; σ^2_f-phenotypic), Falconer (1981). The significant differences among the average values were estimated according to the least significant difference (LSD) Hadživuković (1991).

Weather conditions during the growing seasons

During the study period, the values of temperature and precipitation were different between the two years of the experiment. The registered values of climatic parameters per experimental year were compared to the computed average values for the previous ten years (Table 1).

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

Temp & Precip	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
^{0}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
^{0}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
^{0}C	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
(mm)	2000/2010	64.3	57.4	48.5	42.8	44.7	52.5	66.6	74.9	92.2	60.4	543.8

In the first year, the average temperature (8.74^{o}C) was similar to the ten-year average (9.08 ^{o}C), and in the second year, the average temperature (8.12 ^{o}C) was on average slightly lower than in the first year and than the ten year period. In the first year 2010/11, the amount of precipitation (520.5mm) was higher than in the second year 2011/12 (474.7 mm), but precipitation amounts in the first and second year were lower than the average amount of precipitation during the ten-year period (543.8 mm). The amount of precipitation in the first year was higher and favourable until the plant ripening stage in June. In the second year, the amount of precipitation during plant emergence (November) was extremely low, the distribution of precipitation from December to June was favourable at each stage of plant development, and precipitation amounts were low in June (17.8 mm). These precipitation amounts were suitable for seed maturity, and were lower by 52.9 mm than in the first year and by 74.4 mm than the ten-year average.

Results and discussion

The seed germination values of the barley cultivars were dependent on experimental year and nitrogen fertilisation treatment applied during the growing season. The obtained data indicate that all cultivars had higher values of seed germination in the second year than in the first year (Table 2). In the first year of the experiment, the highest average values of seed germination were found in the barley cultivar 'NS 589' (93.65%), which had the highest average value of seed germination in all fertilisation treatments (N_0=0, N_1=20, N_2=40 and N_3=60 kg ha^{-1}) except the control (without N), in which the highest value of germination was found in the cultivar 'Jagodinac' (92.7%). The lowest average seed germination was recorded in 'Premium' (92.16%), which was the lowest value of seed germination in the control and in all treatments with different N application rates in the first experimental year.

However, 'Premium' had the highest average percentage of seed germination (96.64%) in the second year of the investigation in all N fertilisation treatments and in the control. Also, this cultivar had the highest value of germination (98.0%), which was found in N_3 treatment during the two years of the experiment. The cultivar 'Jagodinac' had the lowest average percentage of seed germination (94.70%) in the second year of the experiment as well in all N fertilisation treatments. In the control, the percentage of seed germination was lowest in 'NS-589' (93.8%), which was slightly lower than in 'Jagodinac' (93.85%).

All barley cultivars in both experimental years had the highest average percentage of seed germination (94.7%) in the first year and (97.21%) in the second year at the highest rate of nitrogen (60 kg ha^{-1}), while in the control treatment (without nitrogen) the lowest average seed germination was recorded in all cultivars – 91.5% in the first year and 94.15% in the second year (Table 2).

The average values of seed germination significantly differed among barley cultivars in the first and second experimental year (Tables 2 and 3). Also, the four cultivars showed significant differences in seed germination percentage between the first and the second year, and across treatments, indicating that weather conditions in the second experimental year favoured nitrogen uptake efficiency and that the rate of N 60 kg ha^{-1} was most effective in promoting seed germination. However, seed germination increased on average with increasing N application rate in all barley cultivars in both years, indicating that nitrogen effect on seed germination is dependent on N application rate i.e. the effect of the environment (81.87%) on the expression of germination percentage is greater than that of the genotype (0.82%) (Table 3).

Table 2. Average percentage of seed germination in barley cultivars

Cultivars	Nitrogen kg ha^{-1}	Years		Two-year average
		2010/11	2011/12	
Jagodinac	0	92.7efg*	93.8f	93.3fg
	20	92.9def	94.5ef	93.7ef
	40	93.7cde	94.9de	94.4de
	60	94.4abc	95.5d	95.0cd
	$\bar{x}$	**93.4**	**94.7**	**94.1**
Premijum	0	90.1i	95.1de	92.6g
	20	91.6gh	96.6c	94.1e
	40	93.0cd	96.8bc	94.9cd
	60	93.9cd	98.0a	96.0ab
	$\bar{x}$	**92.2**	**96.6**	**94.4**
NS-589	0	91.9fgh	93.8f	92.8g
	20	91.9fgh	95.0de	94.0ef
	40	94.4abc	96.4c	95.4bc
	60	95.4a	97.5ab	96.4a
	$\bar{x}$	**93.6**	**95.7**	**94.7**
NS-595	0	91.3h	93.9f	92.6g
	20	92.8def	95.0de	93.9ef
	40	94.2bc	96.3c	95.3bc
	60	95.2ab	97.9a	96.5a
	$\bar{x}$	**93.4**	**95.8**	**94.6**
Average for nitrogen level				
	0	**91.5**	**94.2**	**92.8**
	20	**92.5**	**95.3**	**93.9**
	40	**93.8**	**96.1**	**95.0**
	60	**94.7**	**97.2**	**96.0**
Total average		**93.2**	**95.7**	**94.4**

∗ Values within columns with different superscripts are significantly different ($p \leq 0.05$)

Table 3. Components of phenotypic variance for seed germination in barley cultivars

Source of variance	DF	MS	F-test	LSD		Components of variance	
				0.05	0.01	σ^2	%
Repetitions (R)	3	0.618	2.5949	-	-	-	-
Genotypes (G)	3	1.090	4.5788**	0.5489	1.007	0.018	0.82
Nitrogen (N)	3	29.488	123.8657**	0.5489	1.007	1.793	81.87
Interaction(GxN)	9	0.803	3.3716**	0.7804	1.121	0.141	6.44
Error	45	0.238	-	-	-	0.238	10.87
Total	63	-	-	-	-	2.190	100.00

** Significant at $p \leq 0.01$

The analysis of variance showed significant differences in the percentage of seed germination across barley cultivars and N application rates in both years (Table 3). It also detected highly significant differences across genotypes (G), as well as between experimental years (Y), and in the genotype x year (GxY) interactions for the seed germination trait.

Seed germination is controlled by plant hormones, including abscisic acid (ABA), ethylene, gibberellins, auxin (IAA), cytokinins etc., which are involved in many physiological and biochemical processes in the plant. The balance between gibberellins and ABA determines seed germination ability or the pathways necessary for seed maturation (Miransari and Smith, 2014; Majidi *et al*., 2016). Reduced biosynthesis of gibberellins during seed germination is related to stress response (Colebrook *et al*., 2014). The functioning of connection gene hormones is related to germination response, which can be reduced under stress conditions (salt, drought) in sensitive barley cultivars (Emre *et al*., 2011; Thabet *et al*., 2018), and the physiological damage to the seed caused by stress can negatively affect seed viability and vigour, depending on genotype (Sun *et al*., 2007). In addition, the dynamic nature of root tip properties could have a significant impact on drought tolerance (Carter *et al*., 2019). Also, under salinity stress conditions, barley seed treatment with tryptophan and ascorbic acid as magneto-priming can improve seed germination (Hozayn and Ahmed, 2019).

Conclusions

Highly significant differences in seed germination were determined among barley cultivars, and the effect of mineral N fertilisation on the expression of the trait was established. For all cultivars, in both years, the lowest average values of seed germination were found in the control treatment (without N). In treatments with increasing rates of nitrogen, the percentage of germinated seeds increased in all cultivars. Averaged across cultivars, the highest percentage of germinated seeds was obtained at the highest nitrogen application rate in both years of the experiment . The barley cultivar ‘Premium’ had the lowest percentage of seed germination in the first year and the highest in the second year. The analysis of variance showed that nitrogen rate had a significant influence on the expression of percentage seed germination. The effect of nitrogen varies depending on environmental conditions in experimental years and on the genetic diversity of the investigated barley cultivars. In the expression of the percentage of germinated seeds, the effect of genetic factors was very low and the impact of environmental factors was highly significant. Interactions between genotypes, nitrogen application rates and years were also low. Germination percentage can be used as a marker for determining the capacity of barley plants to tolerate environmental stress and for predicting the adaptability of cultivars during germination and early seedling growth.

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