

WINTER TWO-ROW BARLEY YIELD RESPONSE TO SOWING DENSITY IN SOUTHEASTERN ROMANIA

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Abstract

*This study evaluated the performance of grain yield and its components of 19 winter two-row barley genotypes under two sowing densities: a reduced density of 350 germinable seeds/m² (D1) and a conventional density of 500 germinable seeds/m² (D2). Field experiments were conducted over three consecutive growing seasons (2020-2022) at the National Agricultural Research and Development Institute Fundulea, Romania, on chernozem soil. Total rainfall varied among years (310.4 mm in 2020, 523.2 mm in 2021, and 316.8 mm in 2022), while mean air temperature was ~2°C above the long-term average. Grain yield was significantly higher at D1, averaging 6426 kg/ha, compared with 6012 kg/ha at D2, a 6.9% increase. Correlation analysis showed strong positive associations between thousand-kernel weight and seed weight per spike ($r = 0.844^{***}$) and a significant negative correlation between yield and spike number ($r = -0.347^{*}$), reflecting compensatory effects among yield components. These results indicate that reduced sowing density enhances individual plant performance and is an effective strategy to increase barley grain yield under Southeastern Romania conditions.*

Key words: winter two-row barley, sowing density, yield components, climate variability.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated cereal crops in the world, with archaeological evidence of domestication dating back over 10,000 years in the Fertile Crescent region of the Middle East (Harlan, 1979). It ranks fourth globally among cereal crops in terms of production, after wheat, maize, and rice (FAO, 2023).

Barley grain is a high-energy feed ingredient for livestock, particularly poultry, swine, and ruminants. Its digestible energy content and moderate protein level (10-12%) make it a cost-effective alternative to maize in feed rations (McDonald et al., 2010).

Two-row and six-row malting varieties are extensively used in the beer and whisky industries. The malting process exploits barley's enzymatic activity (particularly α - and β -amylase) to convert starch into fermentable sugars (MacGregor & Bhatti, 1993).

Barley flour, flakes, and pearl barley are used in bread, soups, and health foods. Barley is notably rich in β -glucan, a soluble dietary fibre shown to reduce LDL cholesterol and improve glycemic

response (Brennan & Cleary, 2005; EFSA, 2011). Lodging is a major concern for barley growers and can reduce cereal yield by reducing grain size and number, or by reducing the amount of crop that can be recovered by the combine harvester (Yadav et al., 2024). There have been reports of yield reductions from this kind of lodging: 31% (Weibel & Pendleton, 1964) to 80% (Easson et al., 1993) for wheat, 28-65% (Sisler & Olsen, 1951; Stanca & Jenkins, 1979; Jedel & Helm, 1991) for barley, and 37% (Pendleton, 1954) for oats.

High plant stands can contribute to lodging risk by intensifying competition for resources such as light, water, and nutrients. High sowing densities promote elongated, weak stems that are more susceptible to bending or breaking (Berry et al., 2003; Spink et al., 2003).

The bulk density of barley grain typically ranges between 560-680 kg/m³ (test weight: 56-68 kg/hl) for commercial and feed grades, while malting barley is generally required to reach higher values, with the European Brewery Convention (EBC) specifying a minimum test weight of 65-75 kg/hl for brewing barley - a key

quality parameter for both trading and storage (Rani et al., 2021).

Field experiments conducted in 2006, 2007 and 2008 at eight locations in western Canada (24 site-years) to evaluate the effects of seeding rate (100, 200, 300, 400 and 500 seeds m⁻²) revealed that yields obtained at densities beyond 350 g.s/m⁻² tended to level off or even decrease (O'Donovan et al., 2012).

For Romanian pedoclimatic conditions, Moga et al. (1996) recommend a seeding density of 400-500 germinating seeds/m⁻², equivalent to approximately 180 to 220 kg/ha depending on the thousand-grain weight (TGW) of the variety used. In conditions of late sowing or drought stress, densities may be increased by 10 to 15% to compensate for reduced tillering capacity (Borleanu et al., 1982).

After a relatively flat and even declining trend between 2015 and 2022 (dipping to a low of 423,500 ha in 2018), barley cultivation area in Romania increased sharply thereafter, reaching 638,04 ha in 2026 (Eurostat, 2026).

MATERIALS AND METHODS

A field experiment was conducted using conventional tillage production practices in Southeastern Romania. The experimental site was located at the National Agricultural Research and Development Institute Fundulea (44.4547°N, 26.5155°E), as shown in Figure 1.

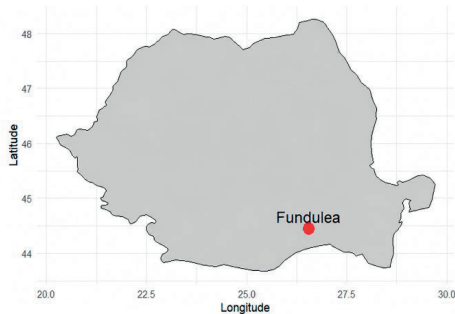


Figure 1. Experimental site over the three consecutive growing seasons

Soil preparation consisted of disc tillage followed by harrowing for seedbed refinement. The study was conducted over three growing seasons (2020, 2021, and 2022). The soil at the experimental site is classified as cambic chernozem, and the climatic conditions recorded

during the experimental period are presented in Table 2.

The experimental design was a randomised split-plot with three replications. Sowing density comprised two treatments: a reduced density of 350 germinable seeds/m⁻² (D1) and a conventional density of 500 germinable seeds/m⁻² (D2).

The comparative trial included 19 two-row winter barley genotypes: five registered varieties and 14 experimental lines developed through the doubled-haploid method. Among the five registered varieties, one produces naked grain (the Ileana variety) (Table 1).

Plot size was 4.5 m² for all genotypes and both density treatments. Sowing depth was 2-3 cm. A standard agronomic management program was applied uniformly across all plots. A complex fertiliser of 200 kg/ha⁻¹ commercial product (18:46, N:P) was applied at sowing in autumn, followed by a rate of 100 kg ha⁻¹ urea (commercial product) in spring. During the growing season, registered herbicides were applied for weed control, and two fungicide treatments were carried out to manage foliar diseases.

Table 1. Studied biological material

No	Variety/line	Registering year	Breeding method
1.	Andreea	1992	Pedigree
2.	Artemis	2021	Pedigree
3.	Gabriela	2017	Pedigree
4.	Diana	2019	Pedigree
5.	Ileana	2021	<i>Bulbosum</i>
6.	DH 381-1	Perspective line	<i>Bulbosum</i>
7.	DH 425-4	Perspective line	<i>Bulbosum</i>
8.	DH 315-10	Perspective line	<i>Bulbosum</i>
9.	F8-114-10	Perspective line	<i>Bulbosum</i>
10.	DH 432-6	Perspective line	<i>Bulbosum</i>
11.	DH 431-1	Perspective line	<i>Bulbosum</i>
12.	DH 425-3	Perspective line	<i>Bulbosum</i>
13.	DH 417-12	Perspective line	<i>Bulbosum</i>
14.	DH 417-13	Perspective line	<i>Bulbosum</i>
15.	DH 437-2	Perspective line	<i>Bulbosum</i>
16.	DH 439-3	Perspective line	<i>Bulbosum</i>
17.	DH 439-8	Perspective line	<i>Bulbosum</i>
18.	DH 403-12	Perspective line	<i>Bulbosum</i>
19.	DH 406-1	Perspective line	<i>Bulbosum</i>

The following yield components and agronomic traits were determined for each sowing density treatment.

Spike number/m⁻² was assessed at full maturity by counting all spikes within a metric frame placed in each plot.

Before harvest, five representative spikes per replicate were sampled from each of the three replicates, threshed individually, and the number of seeds per spike was recorded. The seeds from each spike were then weighed, and the mean value was calculated across the three replicates, yielding the seed weight per spike (g spike⁻¹). Grain yield was determined by harvesting each experimental plot with a Wintersteiger plot combine harvester and was expressed in kg/ha⁻¹ at the standard moisture content of 14%, in accordance with STAS requirements. Thousand-grain weight (TGW) was determined using an automatic seed counter (Contador) and

a precision electronic balance accurate to two decimal places (g). Statistical analyses were performed using SPSS Statistics (version 26.0; IBM Corp., Armonk, NY, USA), while graphical representations were generated in RStudio using the ggplot2 and corplot packages. Analysis of the monthly mean temperatures recorded during the three experimental growing seasons reveals a consistent and notable positive deviation from the 60-year multiannual average of 7.5°C (Table 2). The 2019-2020 growing season recorded the highest seasonal mean temperature of 11.9°C, exceeding the long-term average by 4.4°C - the largest temperature deviation among the three experimental years.

Table 2. Monthly average temperature (°C) and registered rainfall (mm) during the barley growing period (October–June) at Fundulea Meteorological Station (2020–2022)

AVERAGE TEMPERATURE (°C)											
Growing Season	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total / Mean
Multiannual average (60 years)	11.3	5.4	0.0	-2.4	-0.4	4.9	11.3	17.0	20.8	22.7	7.5
2019-2020	12.8 (+1.5)	10.2 (+4.8)	4.0 (+4.0)	0.9 (+3.3)	5.2 (+5.6)	8.3 (+3.4)	12.3 (+1.0)	17.0	21.7 (+0.9)	25.1 (+2.4)	11.9 (+4.4)
2020-2021	14.7 (+3.4)	6.1 (+0.7)	3.9 (+3.9)	1.6 (+4.0)	3.2 (+3.6)	5.1 (+0.2)	9.7 (-1.6)	17.2 (+0.2)	21.1 (+0.3)	25.3 (+2.6)	10.7 (+3.2)
2021-2022	10.2 (-1.1)	7.7 (+2.3)	2.6 (+2.6)	2.1 (+4.5)	4.7 (+5.1)	4.4 (-0.5)	12.1 (+0.8)	17.9 (+0.9)	22.6 (+1.8)	25 (+2.3)	11.1 (+3.6)
RAINFALL (mm)											
Growing Season	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total / Mean
Multiannual average (60 years)	43.0	42.0	43.7	35.1	32.0	37.4	45.1	62.5	74.9	71.1	486.1
2019-2020	38.0 (-5.0)	33.2 (-8.8)	16.2 (-27.5)	2.0 (-33.1)	16.6 (-15.4)	29.8 (-7.6)	14.0 (-31.1)	58.0 (-4.5)	68.4 (-6.5)	34.2 (-36.9)	310.4 (-175.7)
2020-2021	28.6 (-14.4)	20.0 (-22.0)	77.6 (+33.9)	77.0 (+41.9)	16.2 (-15.8)	59.0 (+21.6)	31.0 (-14.1)	57.6 (-4.9)	135.0 (+60.1)	21.2 (-49.9)	523.2 (+37.1)
2021-2022	56.4 (+13.4)	33.8 (-8.2)	37.6 (-6.1)	4.8 (-30.3)	5.4 (-26.6)	12.3 (-25.1)	47.6 (+2.5)	30.1 (-32.4)	59.6 (-15.3)	29.2 (-41.9)	316.8 (-169.3)

*Values in parentheses indicate deviation from the multiannual average (1960-2019).

The 2020-2021 season recorded a mean of 10.7°C (+3.2°C), with a more uniform distribution of positive deviations throughout the season; the only below-average month was April (-1.6°C), which coincides with a critical phase of tillering and stem elongation in two-row barley. The 2021-2022 season also exhibited a mean temperature of 11.1°C (+3.6°C above the multiannual average). Overall, all three experimental seasons were characterised by mild winters and warm growing periods, which may have influenced both crop development rates and winter hardiness. The above-average temperatures recorded throughout the experimental period must be considered a significant environmental

factor when interpreting the agronomic results obtained. The rainfall regime during the experimental period was highly variable between seasons, contrasting markedly with the 60-year multiannual total of 486.1 mm (Table 2). The 2019-2020 growing season was characterised by a severe rainfall deficit, with a total of only 310.4 mm, representing a shortfall of 175.7 mm. In contrast, the 2020-2021 season was the only year to exceed the multiannual rainfall average, recording a total of 523.2 mm (+37.1 mm). The 2021-2022 season returned to conditions of severe water deficit, with a seasonal total of 316.8 mm (-169.3 mm). Consequently, two of

the three experimental years were affected by pronounced drought conditions.

RESULTS AND DISCUSSIONS

The results of the three-way analysis of variance (ANOVA) revealed highly significant effects of the main factors - genotype (G), year (Y), and sowing density (D) - on grain yield and the main yield components analysed (Table 3). All three main factors exerted a statistically significant influence ($p \leq 0.05$) on grain yield (kg/ha^{-1}), number of spikes per square meter, seeds per spike, and seed weight per spike, indicating that genetic differences between

genotypes, environmental variation across growing seasons, and sowing density all played a decisive role in the yield performance of two-row barley. Regarding thousand-grain weight (TGW), the genotype and year factors were both highly significant ($p = .000$), confirming that grain size is strongly determined by genotype characteristics and by seasonal environmental conditions. However, sowing density did not significantly affect TGW ($p = .066$, ns), suggesting that, within the range of densities tested, plant competition had no substantial impact on individual grain filling.

Table 3. Three-way ANOVA for studied parameters

Source	Grain yield (kg ha^{-1})	TGW (g)	No. of spikes/ m^2	Seeds/spike	Seed weight (g/spike)
Genotype (G)	.000*	.000*	.000*	.000*	.000*
Year (Y)	.000*	.000*	.000*	.000*	.000*
Density (D)	.000*	.066 ns	.000*	.000*	.000*
Genotype $\times$ Year (G $\times$ Y)	.000*	.000*	.001*	.000*	.000*
Genotype $\times$ Density (G $\times$ D)	.889 ns	.000*	.061 ns	.000*	.001*
Year $\times$ Density (Y $\times$ D)	.000*	.000*	.000*	.011 ns	.000*
Genotype $\times$ Year $\times$ Density (G $\times$ Y $\times$ D)	.998 ns	.000*	.151 ns	.000*	.000*

* = significant; ns = non-significant

Among the two-way interactions, the genotype and year interaction (G $\times$ Y) was statistically significant for all five traits studied ($p \leq 0.05$), demonstrating that genotypes responded differently to the environmental conditions of each growing season - a finding that underlines the importance of multi-year evaluation in variety testing. The genotype and density interaction (G $\times$ D) was significant for TGW, seeds per spike, and seed weight per spike, but non-significant for grain yield ($p = .889$, ns) and number of spikes per m^2 ($p = .061$, ns), indicating that the yield response to sowing density was largely consistent across genotypes. The year and density interaction (Y $\times$ D) was significant for grain yield, TGW, number of spikes per m^2 , and seed weight per spike, but not for seeds per spike ($p = .011$, ns), reflecting the fact that the optimal density response varied with seasonal conditions. The three-way interaction (G $\times$ Y $\times$ D) was statistically significant for TGW, seeds per spike, and seed weight per spike, indicating complex interdependencies between genotype,

year, and sowing density for these components. In contrast, grain yield ($p = .998$, ns) and number of spikes per m^2 ($p = .151$, ns) were not significantly influenced by the three-way interaction, suggesting that the combined effect of all three factors did not produce differential responses beyond those already captured by the other interactions. Overall, the analysis of variance confirms that both genetic and agronomic factors, as well as their interactions with climatic conditions, must be taken into account when optimising the productivity of two-row barley genotypes. Substantial variation in mean grain yield was observed among growing seasons, confirming the dominant effect of the year factor identified in the ANOVA (Table 3). The lowest yields were recorded in the 2020-2021 growing season, with means of 5011 kg/ha^{-1} at D1 (reduced density) and 5225 kg ha^{-1} at D2 (conventional density), while the 2019-2020 season produced intermediate yields of 5387 kg ha^{-1} and 4735 kg ha^{-1} at the lower and higher densities, respectively (Figure 2).

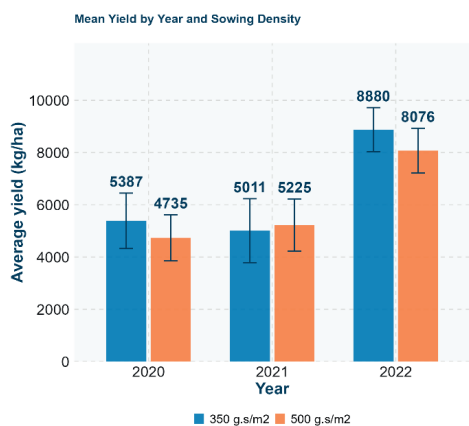


Figure 2. Mean grain yield by growing season and sowing density

The highest yield was recorded in the 2021-2022 season, with mean yields of 8880 kg ha⁻¹ at 350 g.s/m² and 8076 kg/ha⁻¹ at 500 g.s/m², a difference of 804 kg ha⁻¹. This variation between studied years is attributable to the contrasting climatic conditions described earlier, particularly the severe rainfall deficits recorded in 2019-2020 and 2021-2022.

The distribution of grain yield (kg ha⁻¹) across the two sowing densities, averaged over all tested genotypes and growing seasons, is illustrated in Figure 3 as violin plots overlaid with box plots. The diamond symbol represents the mean, and the median is indicated by the horizontal line within the interquartile-range box.

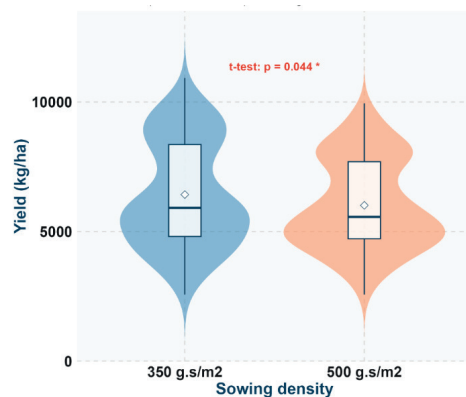


Figure 3. Effect of sowing density on grain yield distribution

An independent-samples t-test revealed a statistically significant difference in mean grain yield between the two sowing densities ($p = 0.044$). The lower-density treatment (350 g.s. m⁻²) registered a mean of 6426 kg/ha⁻¹ compared with 6012 kg/ha⁻¹ (conventional density) as a mean for 3 studied years, suggesting that, on average, the reduced plant competition at 350 g.s/m² was more favourable for overall grain productivity. Both treatments exhibited a wide overall yield range (per replication), from approximately 2568 kg ha⁻¹ in 2020 to 10928 kg ha⁻¹ in 2022, reflecting the strong influence of genotype and year on productivity, as confirmed by the ANOVA (Table 3). The distributions were broadly similar in shape and spread, although a small number of low outliers were recorded at the higher density.

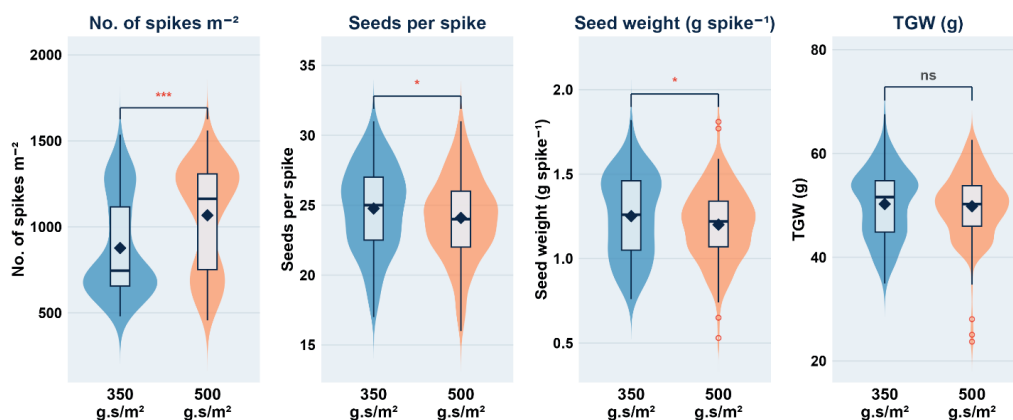


Figure 4. Yield key components under the two sowing densities tested

The number of spikes/m² increased with density, from a mean of 876.8 at D1 to 1067.4 at D2 confirming the expected direct relationship between sowing density and the number of plants. Genotype DH 431-1 recorded the lowest spike density at both sowing rates (754.3 and 910.4 spikes m⁻², respectively), while the highest values were obtained by Andreea (1002.7 spikes m⁻²) at 350 g.s. m⁻² (D1) and by DH 432-6 (1164.3 spikes m⁻²) at 500 g.s. m⁻² (D2).

In contrast, grain weight showed a slight decreasing trend with increasing density, from a mean of 1.25 g at D1 to 1.20 g at D2, reflecting the typical trade-off between spike number and individual grain filling under higher plant competition. DH 384-1 consistently produced the heaviest grains at both densities (1.44 g and 1.38 g), while the lowest values were recorded

by Andreea (1.03 g) at D1 and DH 439-3 (1.07 g) at D2.

The number of grains per spike remained relatively stable across densities, with means of 24.76 and 24.09 at D1 and D2, respectively. The maximum value (26.11 grains/spike) was reached by two different genotypes depending on density: DH 315-10 at D1 and DH 403-12 at D2, while the minimum was recorded by the closely related genotypes DH 425-3 (21.89) and DH 425-4 (22.33).

Overall, a higher sowing density (500 seeds/m² instead of 350) led to more spikes per square meter, but slightly lower yield and grain weight. The non-significant response of TGW to sowing density is consistent with the ANOVA results (Table 3) and suggests that individual grain-filling capacity was largely maintained regardless of plant density.

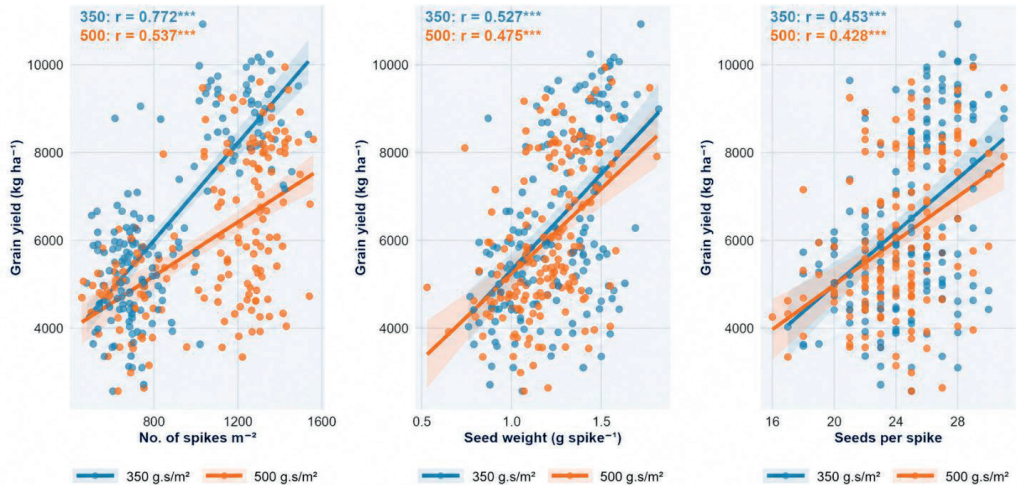


Figure 5. Correlations between yield components and grain yield across sowing densities

Pearson correlation coefficients (*r*) between grain yield (kg/ha⁻¹) and three key yield components - number of spikes/m², seed weight/spike (g/spike⁻¹), and seeds/ spike - are shown in Figure 5, analysed separately for the two sowing densities (350 and 500 g.s/m²). All correlations were statistically highly significant (*p* ≤ 0.001) across both density treatments, confirming that each of the three components contributed meaningfully to grain yield variation under the experimental conditions.

The strongest association with grain yield was found for the number of spikes per m² (left

panel), with correlation coefficients of *r* = 0.772 at 350 g.s./m² and *r* = 0.537 at 500 g.s./m². Both values were the highest recorded among the three components, indicating that spike density was the primary determinant of grain yield in two-row barley under the tested conditions. Notably, the regression slope was markedly steeper at the lower density (350 g.s./m²), suggesting that individual spikes contribute more to yield when plant competition is reduced. Seed weight per spike (central panel) showed moderate positive correlations with grain yield at both densities (*r* = 0.527 at 350 g.s./m²; *r* =

0.475 at 500 g.s. m⁻²). The two regression lines were nearly parallel, with the reduced-density treatment showing a slightly higher slope, indicating that heavier spikes translated more efficiently into yield at the lower density. Seeds per spike (right panel) showed the weakest, though still highly significant, correlations with grain yield among the three components evaluated ($r = 0.453$ at 350 g.s/m²; $r = 0.428$ at 500 g.s/m²). The regression lines for the two densities were nearly coincident, suggesting that the relationship between seeds per spike and yield was largely consistent regardless of plant density. The wide horizontal spread of observations, particularly at higher seed numbers per spike, indicates considerable variability in this trait across genotypes and growing seasons. Overall, the correlation analysis confirms that spike number per unit area was the dominant yield-trigger component in this experiment, followed by seed weight per spike and seeds per spike.

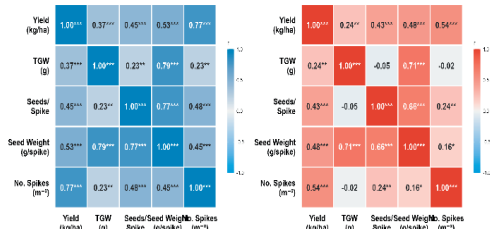


Figure 6. Pearson correlation matrix between grain yield and its key components, by sowing density

At the lower sowing density (blue panel), all correlations between grain yield and the four yield components were positive and statistically significant (Figure 6). The strongest association was observed between yield and number of spikes per m² ($r = 0.77***$), confirming spike density as the primary yield driver at this density level. Grain yield was also strongly correlated with seed weight per spike ($r = 0.53***$) and seeds per spike ($r = 0.45***$), while the correlation with TGW was moderate ($r = 0.37***$). Among the yield components themselves, seed weight per spike showed the strongest inter-component correlations, particularly with seeds per spike ($r = 0.77***$) and number of spikes ($r = 0.45***$), suggesting

that at lower densities, spike-level productivity traits are narrowly co-regulated. At the higher sowing density (D2), the correlation structure changed considerably. While the association between yield and number of spikes per m² remained significant and positive ($r = 0.54***$), several other correlations shifted in sign or lost significance. Most notably, TGW showed negative, though largely non-significant, correlations with yield ($r = -0.05$, ns), seeds per spike ($r = -0.95***$), and number of spikes per m² ($r = -0.02$, ns). The strongly negative correlation between TGW and seeds per spike ($r = -0.95***$) at D2 is particularly noteworthy, indicating a pronounced trade-off between grain size and seed number per spike under conditions of increased intraspecific competition. The mean yield difference (kg) between the two sowing densities shown in Figure 7 highlights that 18 out of 19 two-row barley genotypes evaluated across the three experimental growing seasons had a positive effect on sowing density. Positive values (blue bars) indicate a yield advantage at the lower density (D1) while negative values (orange bars) indicate superior performance at the higher density (D2).

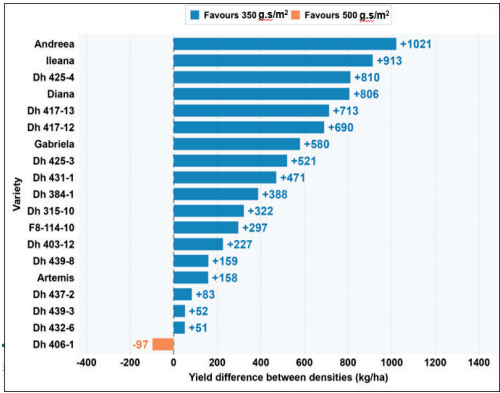


Figure 7. Genotype-specific yield response to sowing density

Yield advantages ranged from 51 kg/ha⁻¹ (DH 432-6) to 1021 kg/ha⁻¹ (Andreaea). The variety Andreaea exhibited the strongest density response, followed by Ileana (913/kg ha⁻¹), DH 425-4 (810 kg/ha⁻¹), and Diana (806 kg/ha⁻¹), all showing yield differences exceeding 800 kg/ha⁻¹ in favour of the lower density.

CONCLUSIONS

Sowing density significantly influenced yield components across all varieties: the lower density of 350 germinating seeds/m² consistently produced higher grain yields than 500 seeds/m², with an average gain of 414 kg/ha over three growing seasons.

Climatic variation caused by year was the dominant factor affecting barley yield, accounting for yield differences of 68% between the least favourable (2020: 5061 kg/ha⁻¹) and most favourable (2022: 8478 kg/ha⁻¹) season, outweighing both variety and sowing density effects.

From 19 evaluated genotypes, we have identified varieties Gabriela, Artemis, and perspective line DH 384-1 that demonstrated the highest and most stable yield performance across both sowing densities and all three experimental years, confirming their adaptability to the pedoclimatic conditions of the study area.

RECOMMENDATIONS

A sowing density of 350 germinating seeds/m² is recommended as the optimal density for winter two-row barley tested genotypes in Southeastern Romania. The classical density of 500 g.s/m² did not improve yield and led to increased competition for water, light, and nutrients between plants.

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