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Response of photosynthetic efficiency parameters and leaf area index of alternative barley genotypes to increasing sowing density

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Alternative barley genotypes can be a source of genetic variability for breeding and raw material for functional food production. The reaction of these genotypes to diverse sowing densities is unknown. The study aimed to assess the response in physiological characteristics of alternative barley genotypes *Hordeum vulgare* var. *rimpaui* and *H. v.* var. *nigricans* to increasing sowing density. In a strict field experiment, two barley genotypes and five sowing densities were tested: 250, 300, 350, 400, and 450 grains m^{-2}. Chlorophyll fluorescence indices, relative chlorophyll content (SPAD), and leaf area index (LAI) were assessed. The interaction of the study year, genotype, and sowing density significantly shaped the physiological indices of the canopy. When rainfall was deficient, the plants reduced their leaf area but had higher SPAD and PI$_{abs}$ of the flag leaf. In the year with optimal rainfall, LAI increased with increasing sowing density. In the dry year, PI$_{abs}$ of the flag leaf in *H. v.* var. *rimpaui* was the highest at 250 grains m^{-2} and decreased with increasing density, and in *H. v.* var. *nigricans*, it only reduced at 450 grains m^{-2} A strong negative relationship was observed between LAI and SPAD, as well as between LAI and PI$_{abs}$. Sowing density had a significant effect on grain yield per plant, which was related to the physiological response. However, the genotypes tested responded differently to this factor. The results may by prove for agricultural practice and scientific research, particularly in relation to the optimal sowing density of alternative barley genotypes and the identification of density-tolerant genotypes in response to varying environmental conditions.

Alternative crop plant genotypes are seen as an opportunity to increase food quality and improve biodiversity in the agricultural environment[1]. So far, attention has been focused primarily on various forms of wheat, such as *Triticum spelta* L., *T. sphaeroccocum*, *T. persicum*[2,3]. Recently, there has also been interest in alternative genotypes of other cereals, including barley[4]. Interesting two-row forms of spring barley are the black-seeded genotypes of *Hordeum vulgare* var. *rimpaui* and *Hordeum vulgare* var. *nigricans*distinguished by a high content of antioxidants, including flavonoids, phenolic acids, carotenoids, and phytomelanin[5,6]. According to Glagoleva et al.[7], the increased antioxidant potential and black color of the grains are due to the presence of the Blp gene in this type of cereal genotypes, which also defines the increased tolerance to abiotic and biotic stresses of black-grains cereals. According to these authors, Blp carriers are also characterized by increased synthesis of phytoalexins, suberin and universal stress protein (USP) in lemma and pericarp. These genotypes are also characterized by a satisfactory yield level, justifying their economic usefulness in organic and conventional production systems[6]. However, full use of the yield potential while maintaining a high quality of the usable yield requires the development of appropriate cultivation technology[8].

One of the fundamental agrotechnical factors affecting the growth and development of plants is sowing density[8]. Determining the optimal plant population density is an indispensable element of cultivation technology at the species level and in relation to the cultivar[9]. Barley cultivars may differ in inter- and intraspecific competitiveness[10], morphological structure, and tillering ability[11], meaning they have different requirements

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for the density of plants in the canopy. Sowing density affects the architecture of the canopy[12], which affects the management of water and nutrients and the availability of light for plants, expressed in[12,13]. This factor also influences the pressure from pests and weeds[10,14]and shapes the structure of the plant root system[8]. All these relationships are related to physiological processes occurring in plants, including the functioning of the photosynthetic apparatus and morphological traits such as leaf area[15]. Measuring photochemical processes with Chl a fluorescence gives a picture of the intensity of the stress affecting the plants. Changes occurring in the efficiency of PS II functioning under its influence can be analyzed using the OJIP test. Some parts of the parameters calculated in this test are related to absorption of energy fluxes (ABS), trapping of excitation energy (TR) and conversion of excitation energy to electron transport (ET) per active reaction center (RC) or surface area of the excited sample (CS)[16]. The FV/FM ratio is a parameter representing the efficiency of primary light energy conversion at the center of PS II and indicates the maximum efficiency of PS II. Many studies also evaluate the Performance Index (PIABS) as an important parameter for measuring photosynthetic efficiency. PI is an integrative parameter calculated based on primary photochemistry, the density of the active chlorophyll reaction center and electron yield. As a result, if stress affects any of these components, the effect will be seen in PI[17]. Leaf greenness can be assessed using SPAD meters that use the emission of light at different wavelengths by the plant's leaf[18]. Assimilative surface area, on the other hand, by means of the Leaf area index (LAI), which tells us about the leaf area that captures the solar energy required for plant photosynthesis[19]. The assimilation area's size and the photosynthesis process's efficiency translate into biomass accumulation and the final yield[20].

To date, no studies have been conducted on the subject on the impact of different sowing densities on alternative barley genotypes physiological and morphological parameters. However, such research seems to be very necessary due to the potential economic value of the tested genotypes and the need to develop optimal cultivation technology for them. Alternative genotypes may also be an essential source of genetic variability for breeders, and the assessment of photosynthetic efficiency and leaf area index under conditions of increasing sowing density may provide valuable information about plant adaptation mechanisms.

Materials and methods

Plant material

The research material consisted of two genotypes of *Hordeum vulgare* L. var *nigricans* (Ser.) Körn (*H. v. nigricans*) and *H. vulgare* L. var. *rimpaui* Wittm (*H. v. rimpaui*). These forms produce kernels with dark-coloured husks, fruits, and seed coats, and belong to two-row spring varieties. A characteristic feature of *H. v. rimpaui* is also reduced awns, transformed into hoods (hooded barley). *H. v. nigricans* forms a spike with fully developed awns (Fig. 1). These genotypes are spring barley, two-row forms and have similar growth rates and development dates at different phenological stages.

The seed was derived from the owns multiplication process. The authors' institution is the breeder of the described varieties and holds exclusive rights to them.

Experiment design

The field experiment was conducted in 2021–2022 in Minikowo, Kuyavian-Pomeranian Voivodeship, central Poland (53o1000200 N, 17o4402200 E). The experiment was set up in split-plot design in four replicates, in medium soil with a content of 7.1 mg P_2O_5 100 g^{-1} soil, 17.1 mg K_2O 100 g^{-1} soil, 4.5 mg MgO 100 g^{-1} soil and low mineral nitrogen (21 kg N_{min} ha^{-1} in 2021 and 27 kg N_{min} ha^{-1} in 2022 in the 0–30 cm layer). Seeds were sown in the third decade of March at a row spacing of 12.5 cm, at a depth of 4 cm. Plant emergences in individual years were observed at the end of the second or beginning of the third decade of April. Plants began dispersing together with flowering in the third decade of May or at the beginning of the first decade of June. The first-order factor was two spring barley genotypes: *H. v.* var. *rimpaui* and *H. v.* var. *nigricans*. The second-order factor was the sowing density: 250, 300, 350, 400, and 450 germinating seeds m^{-2}. Variation in seeding density was achieved by sowing a variable seeding rate with the Wintersteiger Plot Drill. Before sowing, plants were fertilised with potassium at a rate of 70 kg ha^{-1} in the form of 60% potassium salt, phosphorus at a rate of 40 kg ha^{-1} in the form of triple superphosphate 46% and nitrogen at a rate of 70 kg ha^{-1} in ammonium sater 34%. Top dressing at the beginning of the shoot stage controlled weeds with a mixture of florasulam and pinoxaden at a dose of 3.75 g s. a. + 40 g s. a. ha^{-1}. Diseases were eradicated twice in development stages BBCH 31 and BBCH 41–49 with prothioconazole at 100 g s.a ha^{-1} and tebuconazole at 100 g s.a ha^{-1}. During the period of flag leaf development, the grain moth larvae were controlled using cypermethrin at a dose of 25 g s. a. ha^{-1}. Chemical growth regulation was also applied using trinexapac-ethyl at a dose of 75 g s. a. ha^{-1}, in stem elongation stage. The size of each plot was 24 m^2 (1.5 m x 16 m).

Selyaninov coefficient

Based on the sum of average daily air temperatures and total precipitation, the Selyaninov coefficient (k)[21] was calculated on a ten-day period basis for the growing seasons of 2021 and 2022.

The hydrothermal coefficient was calculated according to the formula:

$k = (P \cdot 10)/\Sigma t$.

where:

P - total rainfall in mm for the ten days,

Σt - sum of average daily air temperatures > 0 °C for the ten days.

The Selyaninov coefficient determines the mutual ratio of precipitation and evaporation and characterizes the humidity conditions for plant growth. According to Kuklik et al.[17], the periods were classified into 4 groups depending on their values:

Fig. 1. Plants and grains of *H. v.* var. *nigricans* (upper row) and *H. v.* var. *rimpaui* (lower row).

- - extremely dry or very dry - $k \le 0.7$,
- - dry or quite dry – $0.7 < k \le 1.3$,
- - optimal or quite humid – $1.3 < k \le 2.0$,
- - humid, very humid or extremely humid - $k > 2.0$.

Leaf area index (LAI)

During the growing period, leaf area index (LAI) measurements were made twice, in the flag leaf stage (BBCH 39) and in the fully earing stage (BBCH 55), using the SunScan Canopy Analysis System (Delta-T Devices Ltd., Cambridge, UK). The PAR probe was placed in the plant's stalk just above the soil surface. The second sensor measured the direct and scattered radiation above the plant leaf. Radiation measurements were transmitted from the probe and sensor to the PDA terminal, then converted into quantum radiation per leaf area, expressed in m^2 leaf area m^{-2} soil. The measurements were taken on both dates between 12:00 and 14:00, under conditions of clear skies and optimal light.

SPAD value

On the subflag leaf (in stage BBCH 35) and the flag leaf (in stage BBCH 57), the SPAD value (leaf greenness index) was on the 30 plants in each plot, using a N- tester SPAD-502Plus (Konica Minolta, Osaka, Japan). The device provided the middle part of the leaf acting on one side while the photodetector acted on the other side of the leaf. When the clamp was closed, light shone through the connection, and the photodetector provided the availability of light passing through the cable at 650 nm and 940 nm. The difference between the results and the received signal on the access of light absorbed by chlorophyll and other configuration elements. During the measurement, the device converted chlorophyll in the tested number of samples and expressed it in conventional units.

Chlorophyll fluorescence

Direct chlorophyll fluorescence was measured on barley plants once during the growing season, in the second decade of June, in the full earing stage (BBCH 57), on the subflag leaf. Measurements were performed using a Pocket PEA fluorimeter (Pocket Plant Efficiency Analyzer) (Hansatech Instruments, Norfolk, UK). A single measurement was carried out in the middle part of the mature leaf blade, on an area of 4 mm2 of the middle part of the mature leaf blade. To extinguish the light phase of photosynthesis and block the light supply to the test sample, special clips were used for 30 min before the measurement. The measurement was carried out with a light pulse intensity of 3500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a duration of 1 s. In each plot, measures were taken on 5 randomly selected plants.

The following parameters were tested

- ABS/RC – energy absorbtion by reaction center (RC) in time zero ($t=0$),
- TRO/RC – capacity of absorbed excitation energy by reaction center (RC), in $t=0$,
- ET_O/RC – electron transport of PSII of reaction center (RC), in $t=0$,
- DI_O/RC – energy amount dissipated as heat by reaction center (RC), in $t=0$,
- PI_{abs} – indices of functioning of PSII concerning absorbtion.
- F_V/F_M – maximum photochemical efficiency of PSII.

Assessment of grain yield per plant

Prior to harvesting, one square 1 m^2 of the canopy area was randomly selected in each plot at the full maturity stage of the barley, and all plants of the genotypes under investigation were taken and counted. Subsequently, the grain was threshed and stored in paper bags to permit the moisture content to stabilise. After that period, the grain collected from a 1 m^2 plot was weighed and then calculation was taken to obtain the yield of grains (g) per plants in the test sample and presented in the graph in the Results section of this study. The detailed yield data for the genotypes tested are described in the article by Nowak et al.[22].

Statistical analysis

The results were subjected to an analysis of variance (ANOVA) to ascertain the influence of multiple factors (interaction between year of study, genotype and sowing density) on the variables under investigation. Differences between treatments were tested using Tukey's test at $p=0.05$. Quadratic polynomial regression analysis was performed for selected variables. Relationships between explanatory variables were analysed using Pearson's simple correlation. All calculations were performed in the Statistica 13.0 PL statistical package (Statsoft, Poland). The Grapher 21 (Golden Software, Golden, CO, USA) prepared the weather plot.

Results

Meteorological data

Air temperature and precipitation during the barley growing season varied in the years of study. In 2021, significantly lower temperatures were observed from April 21 to May 10 than in 2022. However, the first year of the study was warmer than the second in the periods from June 1 to 20 and from July 1 to 20 (Fig. 2). Particularly stressful weather conditions were observed in the last two ten-day periods of April 2021, in which the air temperature at the ground during the emergence period dropped to −7.0 °C (Fig. 2). The same period in 2022 was characterized by temperatures ranging from − 2.1 to 6.2 °C.

The total amount of rainfall from March to July 2021 was 208.5 mm, and in 2022, during the same period, the total amount of rainfall was 131.7 mm. In both years of the study, little rainfall was observed during the emergence period (the total rainfall in March 2021 was 21.4 mm, and in 2022 only 0.5 mm). The period from April 21 to May 31, 2022, was also characterized by a small amount of rainfall (25.4 mm), coinciding with the tillering and the beginning of shooting. In 2021, during this period, the total rainfall was 75.5 mm. Low rainfall in both years of the study was also observed at the end of the ripening stage.

Leaf area index (LAI)

Regardless of sowing density and genotype, the leaf area index (LAI) of barley in the BBCH 35 stage was significantly higher in 2021 than in 2022 and ranged from 3.2 to 4.8. In 2022, the values of this index did not exceed 2.8. In 2021, barley leaf area was significantly differentiated by the interaction of genotype and sowing density (Fig. 3). Increasing the sowing density from 250 grains m^{-2} to 450 grains m^{-2} resulted in a significant increase in the leaf area (LAI) of *H. v.* var. *rimpaui* by 45.5%. Increasing the sowing density from 400 to 450 grains m^{-2} did not result in a further increase in LAI; on the contrary, even a slight downward trend was observed. In the first year of the study, a similar reaction to increasing sowing density was also shown by *H. v.* var. *nigricans*. LAI at the sowing density of 400 and 450 grains m^{-2} was significantly higher than at 250 and 300 grains m^{-2}. In 2022, the tested genotypes did not significantly respond to LAI to increasing sowing density.

In the BBCH 57 stage, the interaction of study year, genotype and sowing density also significantly influenced the leaf area index. In this stage, no large differences between the years of study were observed, as in BBCH 35. *H. v.* var. *rimpaui* in both 2021 and 2022 was characterized by significantly higher LAI values at a sowing density of 300 grains m^{-2} than at a sowing density of 400 grains m^{-2}. In turn, *H. v.* var. *nigricans* in 2022 in the BBCH 57 stage was characterized by significantly higher LAI at a sowing density of 450 grains m^{-2} than at a sowing density of 250 grains m^{-2}. In 2021, sowing density had no significant effect on the leaf area index of *H. v.* var. *nigricans*.

The relationships of experimental treatments are confirmed by the polynomial regression curve, which in *H. v.* var. *rimpaui* in 2021 indicates a significant increase in the LAI value in BBCH 35 when the sowing density is increased from 250 to 350–400 grains m^{-2}, and then its decrease when the sowing density is further increased to 450 grains m^{-2} (Fig. 4). In 2022, no significant relationships were found between sowing density and the LAI value of this genotype. In *H. v.* var. *nigricans*, the LAI value in BBCH 35 increased with increasing sowing density from 250 to 450 grains m^{-2} in both 2021 and 2022.

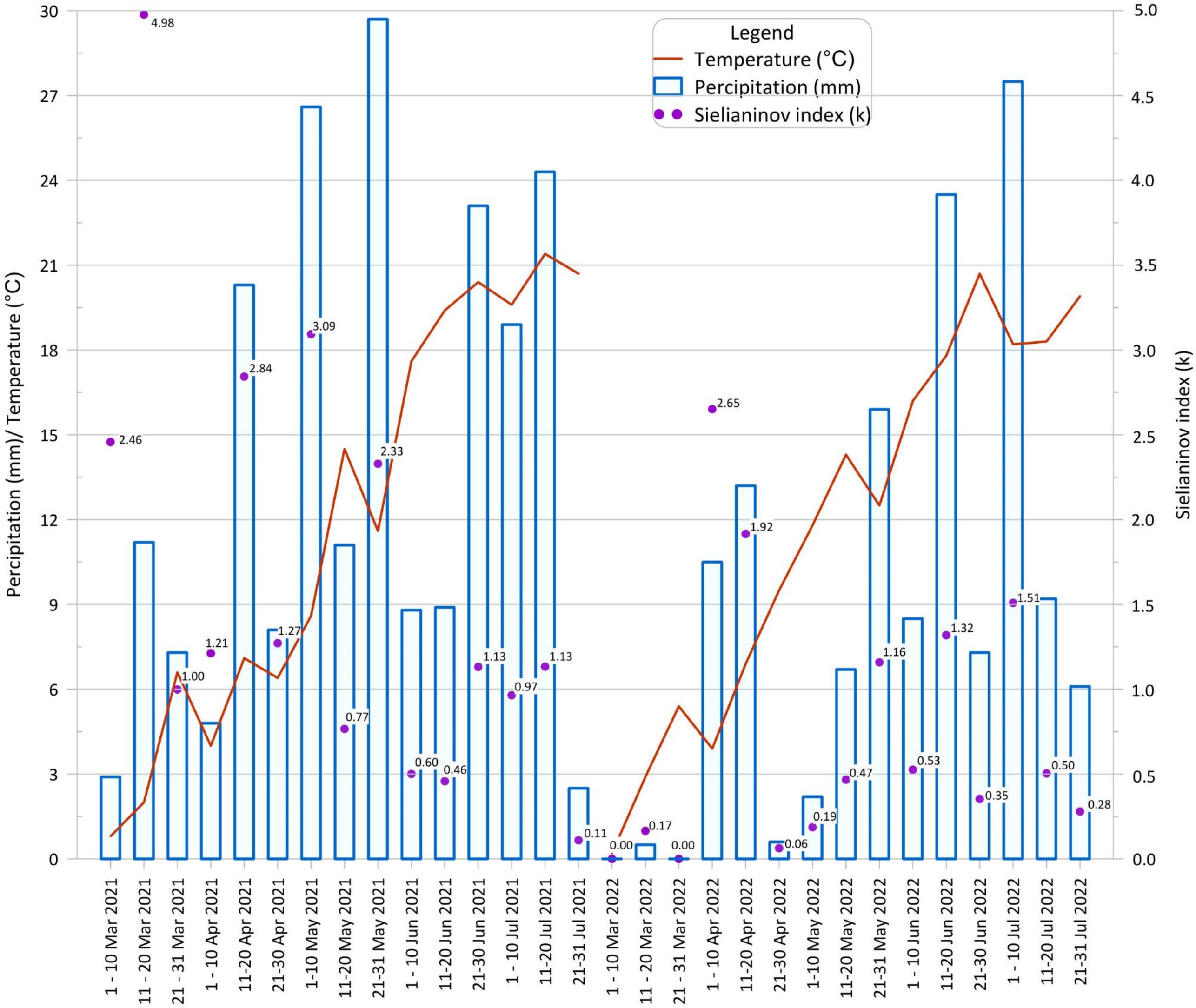

Fig. 2. Atmospheric conditions and the Selyaninov coefficient during the study period.

SPAD value

In 2022, regardless of the sowing density, *H. v.* var. *nigricans* in the BBCH 35 stage was characterized by a significantly higher SPAD value than in 2021, and also compared to *H. v.* var. *rimpaui* in both years of the study In 2022, regardless of the sowing density, *H. v.* var. *nigricans* in BBCH 35 was characterized by a significantly higher SPAD value than in 2021, and also compared to *H. v.* var. *rimpaui* in both years of the study. In no year of the study was there any significant effect of sowing density on the leaf greenness index of the tested genotypes. In turn, the interaction of the year of research, genotype and sowing density significantly impacted a fall in BBCH 57 (Fig. 5). In 2022, increasing the sowing density of *H. v.* var. *nigricans* from 250 to 350 grains m^{-2} significantly reduced the SPAD index in the analyzed development stage. Still, this index increased dramatically with a further increase in the sowing density from 350 to 400 and 450 grains m^{-2}.

In 2021, no significant differences in the mean SPAD value were observed between genotypes. In 2022, at a sowing density of 350 grains m^{-2}*H. v.* var. *rimpaui* was characterized by a significantly higher SPAD compared to *H. v.* var. *nigricans*, while in contrast, at a sowing density of 450 grains m^{-2}*H. v.* var. *nigricans* showed higher SPAD values than *H. v.* var. *rimpaui*.

Regression analysis did not confirm significant relationships between sowing density and SPAD value for *H. v.* var. *rimpaui* nor *H. v.* var. *nigricans* in 2021 (Fig. 6). However, in 2022, a significant non-linear relationship between sowing density and SPAD value in BBCH 57 was observed for both genotypes. In *H. v.* var. *rimpaui* with increasing sowing density from 250 grains m^{-2} to 350 grains m^{-2}, an increase in SPAD value was observed, which remained at a similar level at sowing densities of 350–370 grains m^{-2} and then decreased with the rise in sowing density. In 2022, *H. v.* var. *nigriacans* also showed a significant relationship between sowing density and SPAD value. The regression curve showed a downward trend in this parameter as the sowing density increased from 250 to 300 grains m^{-2}, followed by relatively constant SPAD values at 300–350 grains m^{-2} density. Then, there was an increase as the sowing density increased above 350 grains m^{-2}.

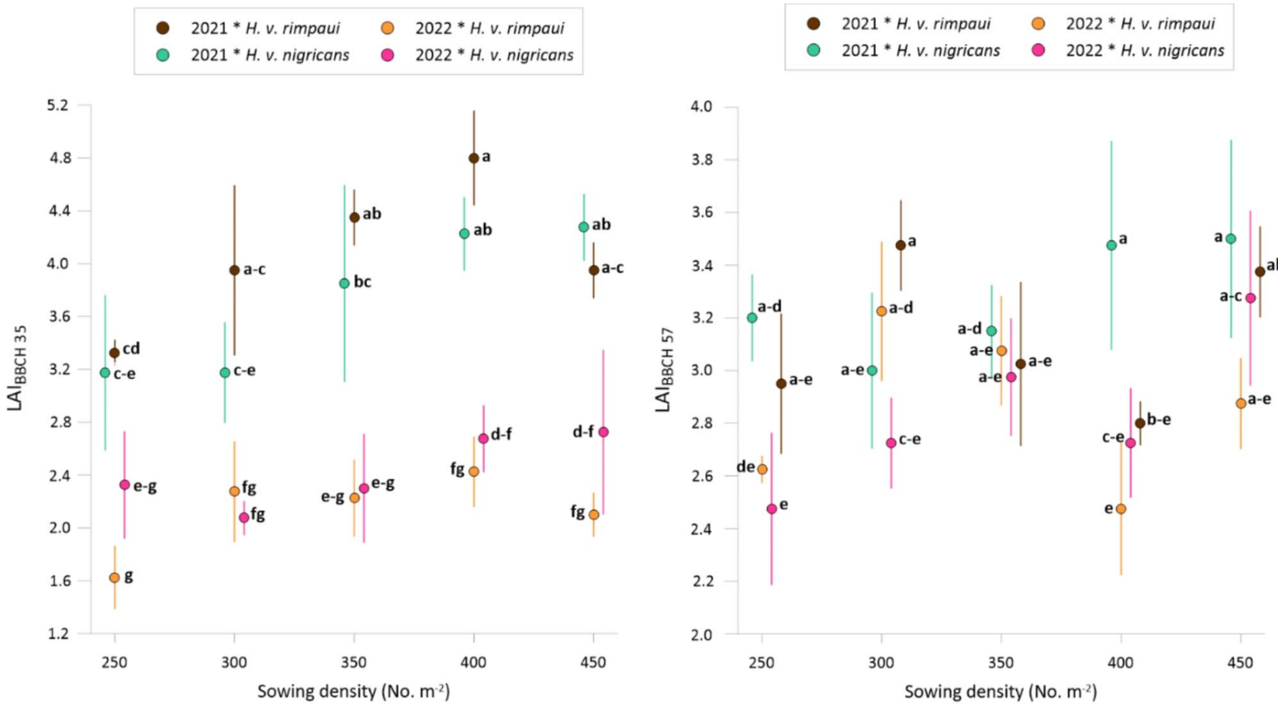

Fig. 3. Leaf Area Index (LAI) of *H. v.* var. *nigricans* and *H. v.* var. *rimpaui* at subflag leaf stage (BBCH 35) and heading stage (BBCH 57). Error bars indicate standard deviation (SD). Letters a-g - mean values followed by different letters indicate significant differences $p < 0.05$.

Chlorophyll fluorescence

ABS/RC

The ABS/RC index in the flag leaf stage, on average for the genotype and sowing density, was significantly higher in 2021 than in 2022. A significant interaction effect of the year of the study, genotype and sowing density on selected chlorophyll fluorescence indices was also observed (Fig. 7). In 2021, the highest ABS/RC value in *H. v.* var. *rimpaui* was observed at a sowing density of 300 grains m^{-2}. It was significantly higher than at densities of 250, 400 and 450 grains m^{-2}. In 2022, this genotype was characterized by significantly higher ABS/RC at sowing densities of 300 and 350 grains m^{-2} than at 250 and 400 grains m^{-2}. However, a further increase in the sowing density from 400 to 450 grains m^{-2} resulted in a sudden rise in ABS/RC to significantly higher than at densities of 250, 300, and 400 grains m^{-2}.

H. v. var. *nigricans* in 2021 with increasing sowing density from 250 to 300, 350 and 400 grains m^{-2} kept ABS/RC at a similar level. However, increasing the sowing density from 400 to 450 grains m^{-2} resulted in a significant decrease in ABS/RC concerning all tested sowing densities. In 2022, expanding the sowing density of *H. v.* var. *nigricans* from 250 grains m^{-2} to 300, 350, and 400 grains m^{-2} resulted in a significant reduction in the ABS/RC index of the flag leaf. However, increasing the sowing density from 400 to 450 grains m^{-2} caused ABS/RC to rise again and was significantly higher than at 400 grains m^{-2}.

Significant differences between genotypes were observed at sowing densities of 250, 350, and 400 grains m^{-2} in 2021 and at 250 grains m^{-2} in 2022, where *H. v.* var. *nigricans* was characterized by higher ABS/RC values than *H. v.* var. *rimpaui*.

TR_o/RC

Regardless of sowing density and genotype, significantly higher TR_o/RC values were observed in 2021 than in 2022. Significant differences between the tested treatments also resulted from the interaction of the study year, genotype, and sowing density.

H. v. var. *rimpaui* in 2021 was characterized by the highest TR_o/RC value at a sowing density of 300 grains m^{-2}. TR_o/RC was significantly higher at this sowing density than at 250 and 450 grains m^{-2}. In 2022, TR_o/RC for *H. v.* var. *rimpaui* increased substantially due to increasing the sowing density from 250 grains m^{-2} to 300, 350, 400, and 450 grains m^{-2}. At the highest sowing density, TRo/RC for *H. v.* var. *rimpaui* also had a significantly higher value than sowing densities of 300, 350, and 400 grains m^{-2}.

In 2021, the TRo/RC in H. v. var. nigricans at a sowing density of 450 grains m-2 was significantly lower than at the other sowing densities tested. In the second year of the study, however, the TRo/RC index in this genotype at the highest of the densities tested was significantly higher when compared with densities of 350 and 400 grains m-2.

In 2021, a significantly higher value of TRo/RC was observed for *H. v.* var. *nigricans* compared to *H. v.* var. *rimpaui* at sowing densities of 250, 300, 350, and 400 grains m^{-2}. In 2022, the same significant relationship was

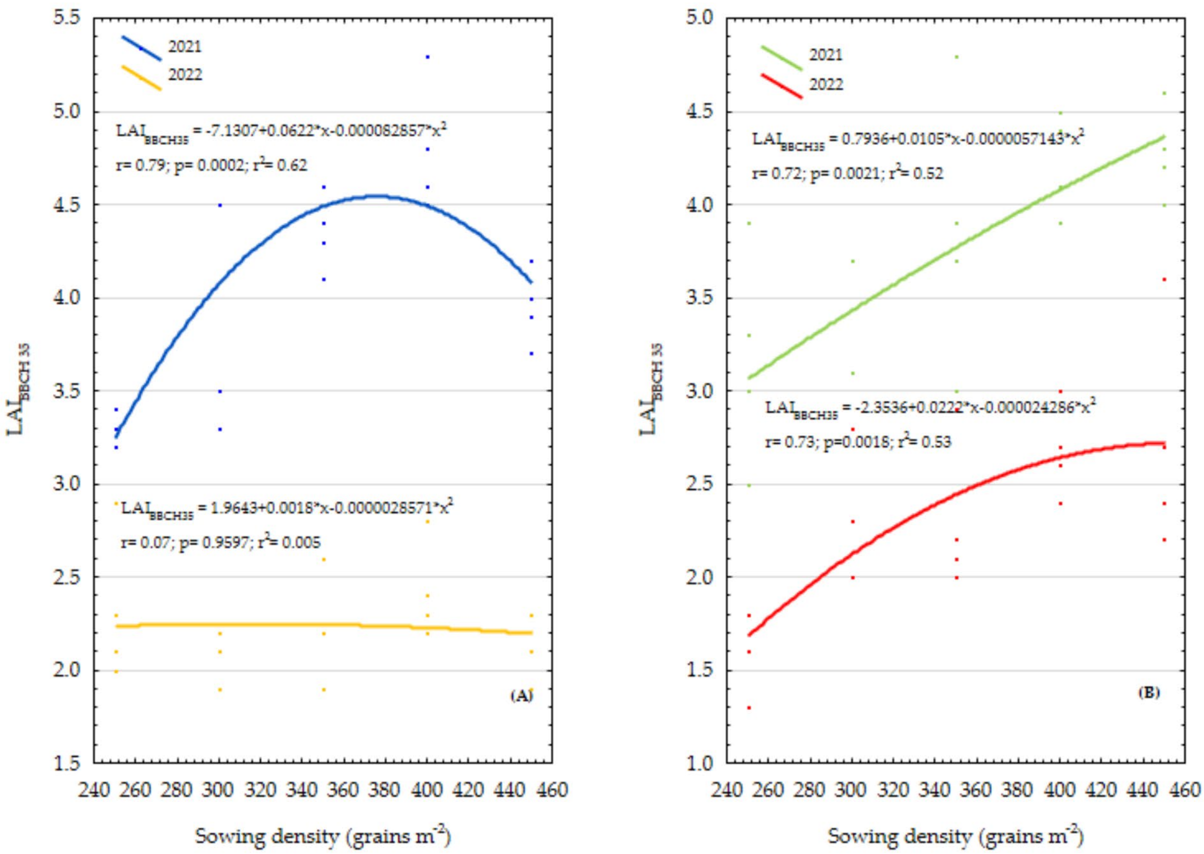

Fig. 4. Relationships between sowing density and leaf area index (LAI) in stage BBCH 35 of *H. v.* var. *rimpaui* (A) and *H. v.* var. *nigricans* (B).

observed only at a density of 250 grains m^{-2}. At the highest tested sowing density of 450 grains m^{-2}, the values of TR_o/RC were equal for the tested genotypes in both years of the study.

DI_o/RC

In 2021, increasing the sowing density of *H. v.* var. *nigricans* from 250 grains m^{-2} to 300, 350, and 400 grains m^{-2} did not cause significant changes in the DI_o/RC value (Fig. 8). However, at a sowing density of 450 grains m^{-2} a substantial decrease in DI_o/RC was observed compared to a sowing density of 400 grains m^{-2}. In 2022, *H. v.* var. *nigricans* also achieved the highest DI_o/RC value at a density of 250 grains m^{-2}. A significant reduction in the DI_o/RC was observed by increasing the sowing density from 250 to 300, 350, 400, and 450 grains m^{-2}.

In 2021, *H. v.* var. *rimpaui* did not show significant changes in DI_o/RC under the influence of increasing sowing density, whereas *H. v.* var. *rimpaui* responded to this factor in 2022. A significant increase in DI_o/RC was observed due to the increase in sowing density from 250 grains m^{-2} to 300 and 350 grain m^{-2}. However, saving *H. v.* var. *rimpaui* at a density of 400 grains m^{-2} caused a significant decrease in the DI_o/RC value compared to the density of 350 grains m^{-2}. Then a further increase in the sowing density from 400 to 450 grains m^{-2} caused another increase in DI_o/RC to significantly higher than at densities of 250, 300 and 400 grains m^{-2}.

In both years of the study at a density of 250 grains m^{-2}*H. v.* var. *nigricans* was characterized by significantly higher DI_o/RC than *H. v.* var. *rimpaui*; these differences decreased and were insignificant at higher sowing densities.

ET_o/RC

H. v. var. *nigricans* in 2021 was characterized by a significant reduction in ETo/RC due to the increase in sowing density from 300 to 350 grains m^{-2} to 450 grains m^{-2} (Fig. 8). However, no significant effect of increasing sowing density on ETo/RC was confirmed in *H. v.* var. *rimpaui* in 2021 and 2022 and in *H. v.* var. *nigricans* in 2021. ETo/RC did not differ significantly between genotypes and years of the study. In 2021, at a density of 350 grains m^{-2}, *H. v.* var. *nigricans* was characterized by a significantly higher value of the ETo/RC ratio than *H. v.* var. *rimpaui*.

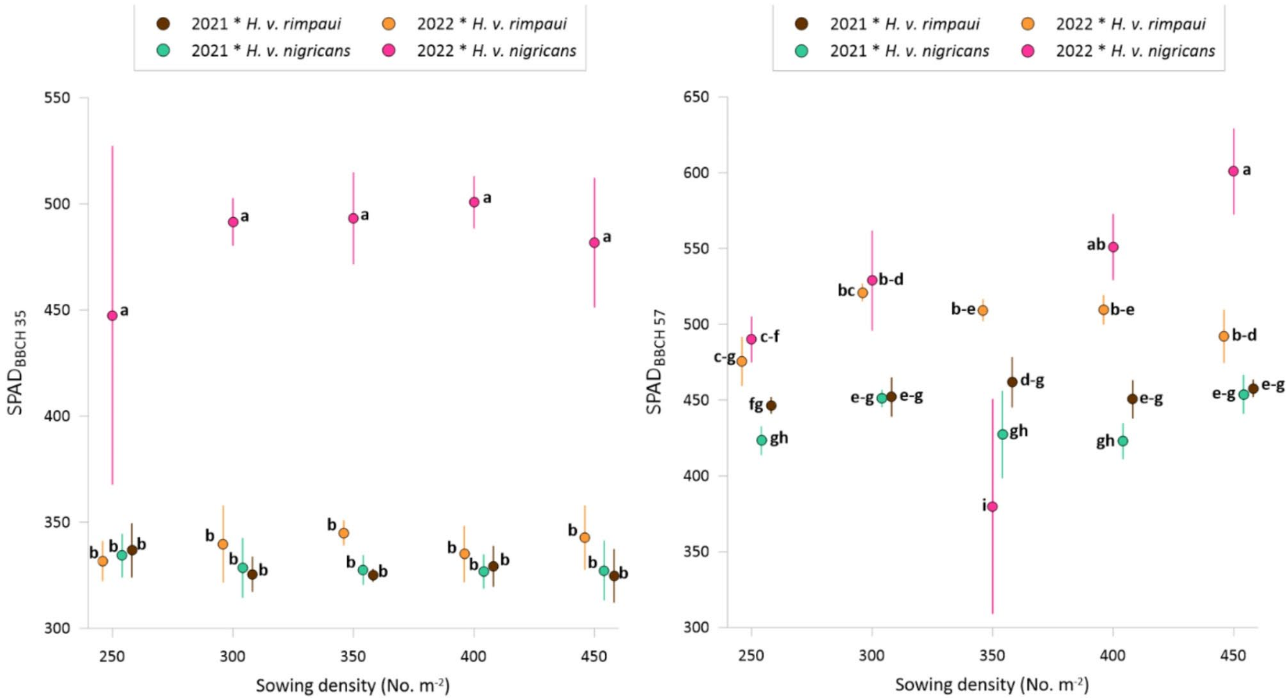

Fig. 5. SPAD value of *H. v.* var. *nigricans* and *H. v.* var. *rimpaui* at subflag leaf stage (BBCH 35) and heading stage (BBCH 57). Error bars indicate standard deviation (SD). Letters a-h - mean values followed by different letters indicate significant differences $p < 0.05$.

F_V/F_M

The F_V/F_M index of the tested barley genotypes was not significantly differentiated by sowing density in 2021 (Fig. 9). In 2022, *H. v.* var. *rimpaui* responded with a significant reduction in F_V/F_M to increase sowing density from 250 grains m^{-2} to 350 grains m^{-2}. However, a further increase in sowing density from 350 to 400 grains m^{-2} resulted in a significant rise in F_V/F_M. At a sowing density of 450 grains m^{-2}, F_V/F_M decreased again and was significantly lower than at 250 and 400 grains m^{-2}. In 2022, at a sowing density of 250 grains m^{-2}, *H. v.* var. *rimpaui* showed significantly higher F_V/F_M than *H. v.* var. *nigricans.* At higher sowing densities, no significant differences in F_V/F_M values were observed between the tested genotypes.

PI_{abs}

In 2022, the tested barley genotypes, on average for sowing density, showed significantly higher PI_{abs} values than in 2021 (Fig. 9). Also, the study year, genotype, and sowing density interaction significantly impacted the PI_{abs} index value. However, the response of the tested genotypes to increasing sowing density was observed only in 2022. *H. v.* var. *rimpaui* had the highest PI_{abs} at a sowing density of 250 grains m^{-2}. Increasing the sowing density to 300, 350, 400, and 450 grains m^{-2} resulted in a significant decrease in the PI_{abs} value compared to a density of 250 grains m^{-2}. *H. v.* var. *rimpaui* sown at a density of 450 grains m^{-2} also showed significantly lower PI_{abs} than 300 and 400 grains m^{-2} densities.

PI_{abs} for *H. v.* var. *nigricans* in 2022 at sowing densities of 250, 300, 350, and 400 grains m^{-2} remained at a similar level. Increasing the sowing density to 450 grains m^{-2} resulted in a significant decrease in PI_{abs} compared to 250, 300, 350 and 400 grains m^{-2} densities.

In 2022, *H. v.* var. *rimpaui* was characterized by significantly higher PI_{abs} compared to *H. v.* var. *nigricans* at a sowing density of 250 grains m^{-2}, at higher sowing densities, the differences between the genotypes decreased and were not significant.

The regression equation (Fig. 10) also indicates no significant response of *H. v.* var. *rimpaui* in terms of PI_{abs} to increasing sowing density in 2021, and a significant decrease in PI_{abs} observed when increasing the sowing density of this genotype in 2022. *H. v.* var. *nigricans*, however, showed a significant relationship between sowing density and PI_{abs} in both 2021 and 2022. In 2021, a small but significant increase in PI_{abs} was observed with increasing sowing density. In 2022, this relationship was different because an increase in PI_{abs} was recorded with an increase in the sowing density from 250 to 300 grains m^{-2}, maximum values for sowing densities of 300–350 grains $m^{-2,}$ and then a decrease in the value of this index with a further increase in sowing density above 350 grains m^{-2}.

Grain yield per plant

A higher grain yield per plant was observed in 2021 compared to 2022, although this depended on the interaction year of study with genotype and sowing density (Fig. 11). In the first year of the study, an increase in the sowing density of *H. v.* var. *rimpaui* from 250 to 350, 400 and 450 grains m^{-2} resulted in a notable reduction in grain yield

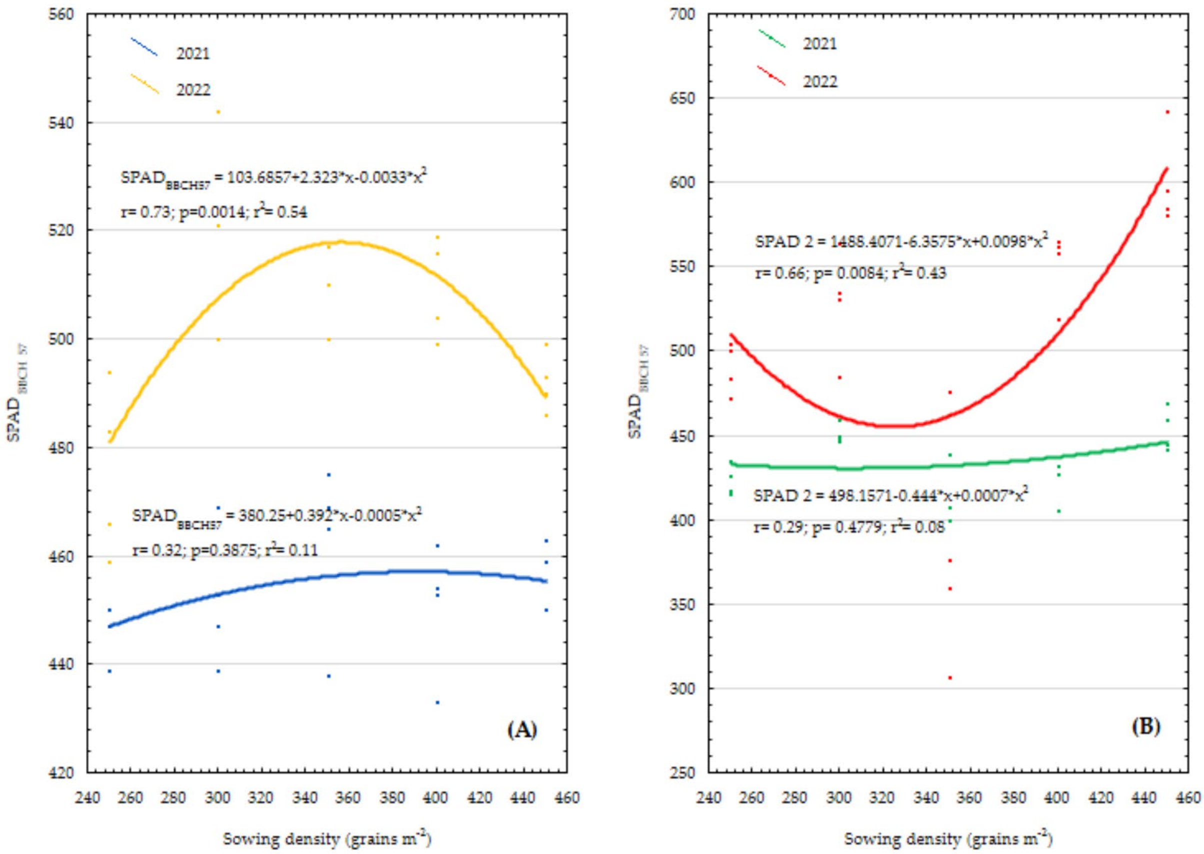

Fig. 6. Relationships between sowing density and leaf chlorophyll index (SPAD) in stage BBCH 57 of *H. v.* var. *rimpaui* (A) and *H. v.* var. *nigricans* (B).

per plant. Furthermore, H. v. var. nigricans exhibited a diminished yield per plant, though this was only evident following an increase in the seed from 250 to 350 grains m^{-2} to 400 and 450 grains m^{-2}. In the range of 250 to 350 grains m^{-2}, the grain yield of *H. v.* var. *nigricans* was found to be similar.

In 2022, no significant response was observed in the tested genotypes to sowing density (Fig. 11).

Analysis of relationships between traits

Pearson's linear correlation analysis showed a significant positive correlation between LAI at BBCH 35 and BBCH 57 and the F_v/F_m, ABS/RC and TR_o/RC indices, and a negative correlation with PI_{abs} (Table 1). A significant positive correlation was also observed between LAI at BBCH 35 and BBCH 57 and DI_o/RC in *H. v.* var. *rimpaui*. This correlation was not confirmed in *H. v.* var. *nigricans*. The LAI and chlorophyll indexes (SPAD) were negatively correlated in both tested genotypes. *H. v.* var. *rimpaui* showed a robust significant interaction between LAI in BBCH 35 and SPAD in BBCH 57, while *H. v.* var. *nigricans* was characterized by a strong correlation between LAI in BBCH 35 and SPAD in BBCH 35. The strength of these interactions was significantly higher at the BBCH 35 stage, while it decreased at the BBCH 57 stage. A significant positive correlation was observed between grain yield per plant and the indices F_v/F_m, ABS/RC. Furthermore, a strong positive correlation was evident between grain yield per plant and TR/RC. Additionally, a significant negative correlation was identified between grain yield and SPAD and PI_{abs}.

Discussion

The physiological reactions of plants can be evaluated, among others, based on chlorophyll content and fluorescence indices, which are used in assessing plant productivity[23]. In agronomic research, indices providing information on the assimilation area and photosynthetic efficiency are also used to indicate the effects of agricultural practices and assess the crop condition[24]. In this study, plants also showed a response to changing hydrothermal conditions and increasing sowing density over the study years.

The interaction of the year of the study, genotype, and sowing density was a significant determinant of the assimilation area of plants. The LAI index in BBCH 35 in 2021 for *H. v.* var. *nigricans* increased due to the

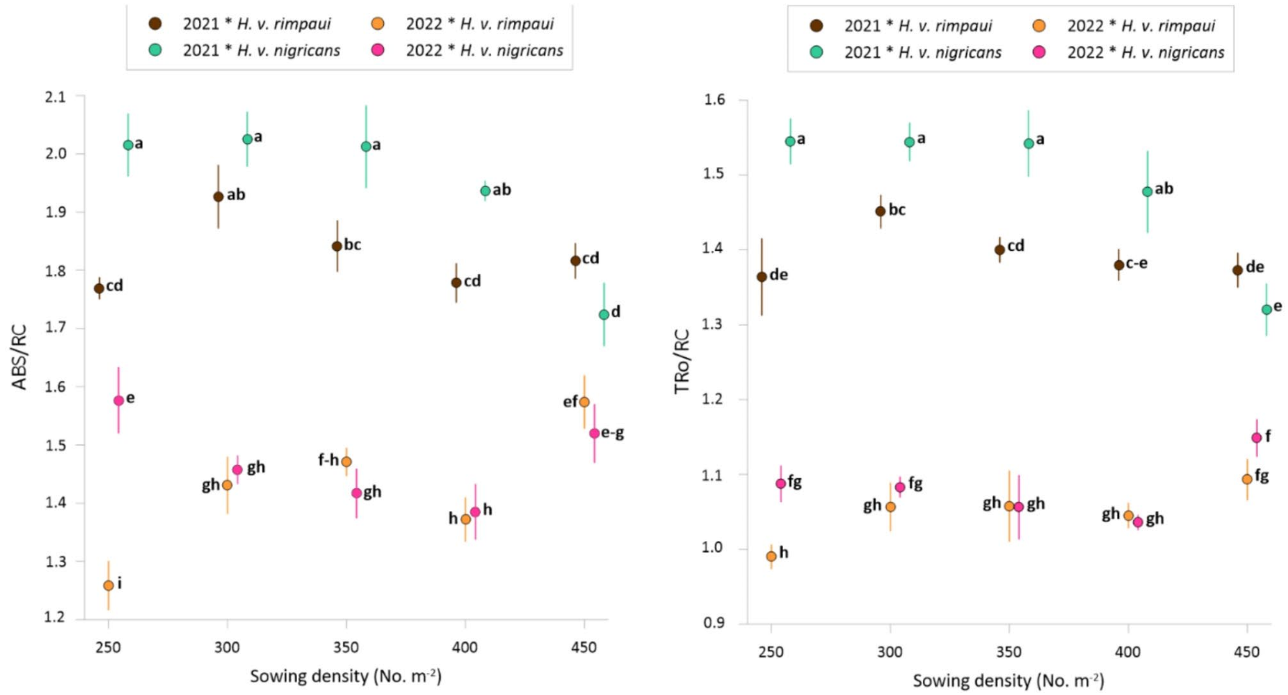

Fig. 7. ABS/RC and TRo/RC indicators of *H. v.* var. *nigricans* and *H. v.* var. *rimpaui* at heading stage (BBCH 57). Error bars indicate standard deviation (SD). Letters a-h - mean values followed by different letters indicate significant differences $p < 0.05$.

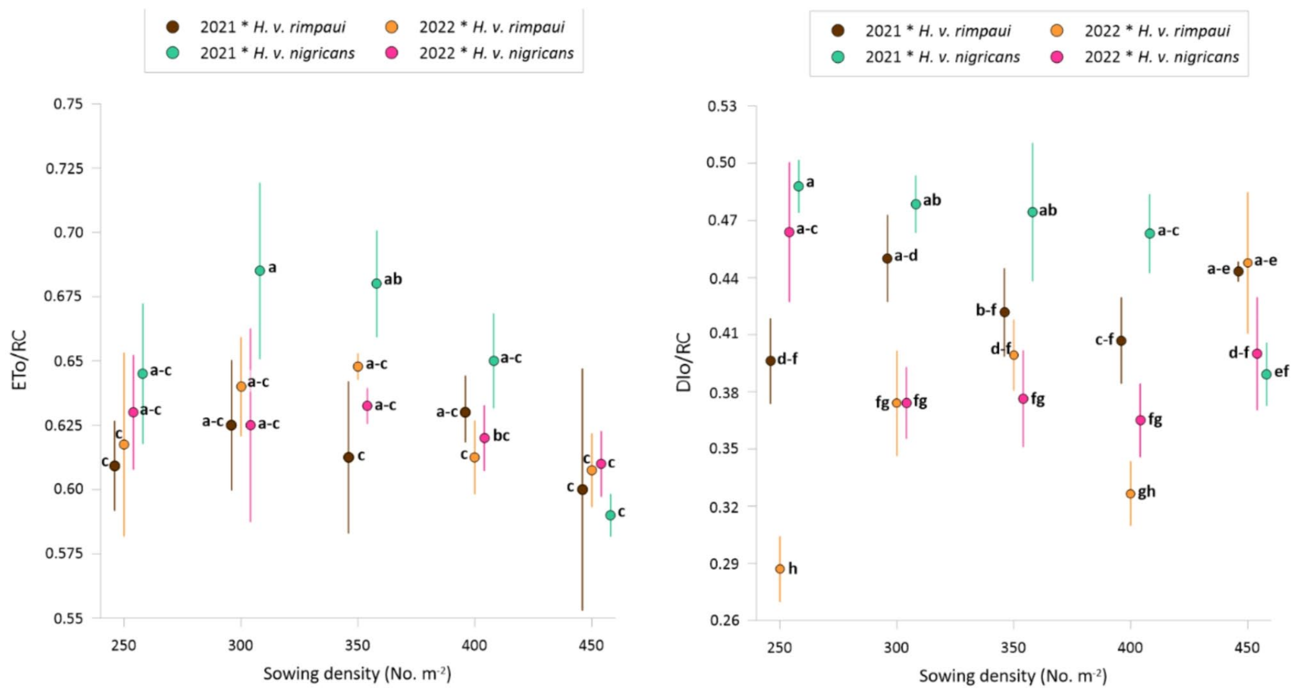

Fig. 8. Eto/RC and Dio/RC indicators of *H. v.* var. *nigricans* and *H. v.* var. *rimpaui* at heading stage (BBCH 57). Error bars indicate standard deviation (SD). Letters a-h - mean values followed by different letters indicate significant differences $p < 0.05$.

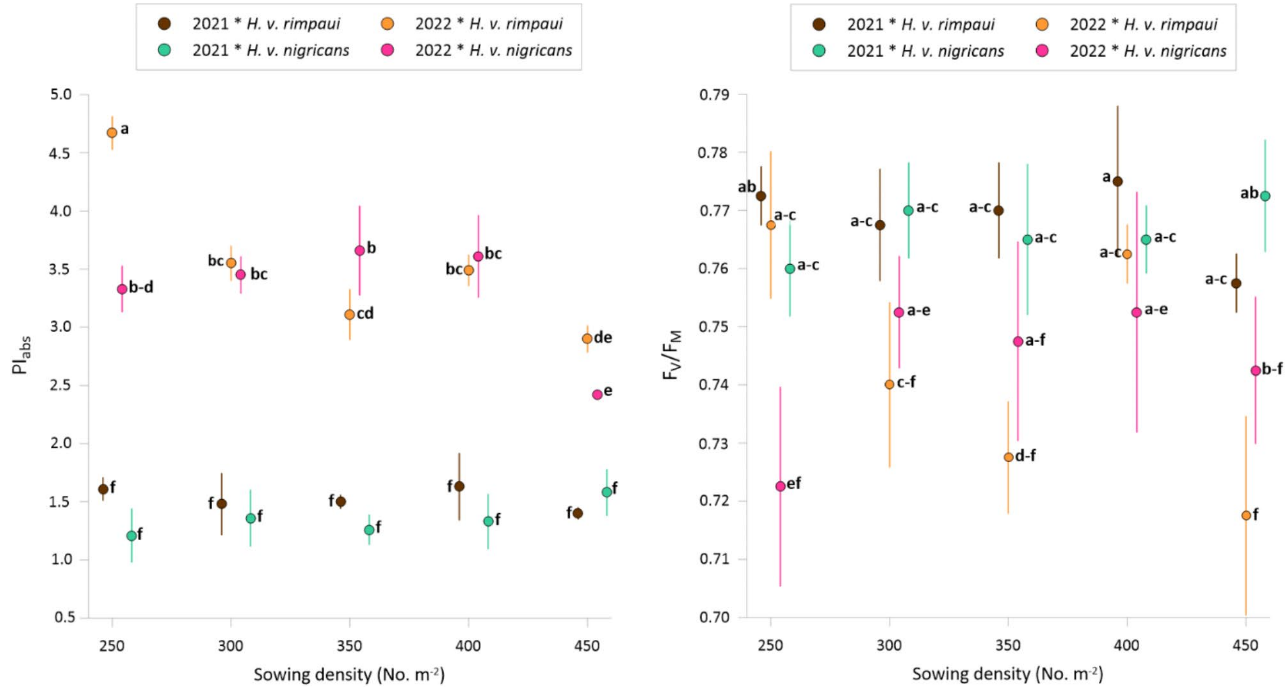

Fig. 9. F_V/F_M and PI_{abs} indicators of *H. v.* var. *nigricans* and *H. v.* var. *rimpaui* at heading stage (BBCH 57). Error bars indicate standard deviation (SD). Letters a-f - mean values followed by different letters indicate significant differences $p < 0.05$.

increase in sowing density from 250 to 400 and 450 grains m^{-2}, while for *H. v.* var. *rimpaui* it peaked at 400 grains m^{-2}. Regression analysis also confirmed a significant relationship between sowing density and LAI in 2021. In the second year of the study, no effect of sowing density on the leaf area index was observed in BBCH 35. The year 2021 was characterized by a much more tremendous amount of rainfall during the growing season, approximately 77 mm, which resulted in a higher value of the Selyaninov hydrothermal coefficient than in 2022, especially in the initial plant growing season. In 2022, for most of the barley growing season, the coefficient value remained below 0.7, indicating unfavourable hydrothermal conditions for plant growth[25]. Unfavourable in terms of rainfall, 2022 resulted in a significant reduction in the assimilation area of the barley canopy in the sub-flag leaf stage compared to 2021. In 2022, the Selyaninov coefficient in the stem elongation stage was at 0.53–1.16, indicating unfavourable hydrothermal conditions. In the study by Fukai et al.[26], barley sowing density also did not affect the amount of aboveground plant biomass at low rainfall during the growing season, but the authors noted significant differences in years with optimal humidity conditions. This is because the LAI value depends on genotypic traits, weather conditions, and the availability of nutrients[27]. The study by Ju et al.[28] and Zhang et al.[29] confirm that sowing density significantly shapes the leaf area index of cereals, and the response to variable sowing density is characteristic of the genotype and depends on the weather conditions during the growing period and the nitrogen supply of the plants.

The response of plants to weather conditions was also observed in the LAI index distribution in the BBCH 57 stage. In 2021, leaf area index was reduced between stages BBCH 35 and BBCH 57. In 2022, LAI in BBCH 57 was similar to or slightly higher than in BBCH 35. A higher amount of precipitation characterized the first year of the study during the growing season. Still, unfavorable hydrothermal conditions occurred during the flag leaf and ear development, expressed by a low Selyaninov coefficient (first and second ten-day periods of June). In the second year of the study, more favorable humidity conditions were observed just during the development of the flag leaf and ear. Under conditions of lower water availability, plants form the smaller leaf area, which allows for limited evapotranspiration[30]. In the study by Sepp et al.[31], barley plants growing in controlled conditions, subjected to drought in the initial period of growth, significantly reduced the leaf area by 26% compared to the control. LAI reduction between BBCH 35 and BBCH 57 phases in 2021 was observed primarily in H. v. var. rimpaui, which was distinguished by very high values of this index. It should be noted that the plants reduced the leaf area expressed by the LAI index especially from LAI values of 4.0–4.8 to LAI values of about 2.8–3.5. During the course of the study, it was observed that too much leaf area causes shading of the lower leaves of these plants in alternative barley genotypes, resulting in loss of their vigor and green color, which is probably the result of chlorophyll decomposition under insufficient sunlight. As a result, the lower, older leaves undergo accelerated aging and premature dying, especially under conditions of limited soil moisture and high temperatures. Also, Neumann et al.[32] confirmed the negative effect of water shortage on barley biomass; however, they report that the amount of losses caused by drought varies depending on the genotype and significantly depends on the regenerative abilities of the cultivar. Neumann et al.[32] claim that barley plants can partially regenerate losses resulting from water shortage, among others, through the rapid development of new leaves, and the dynamics

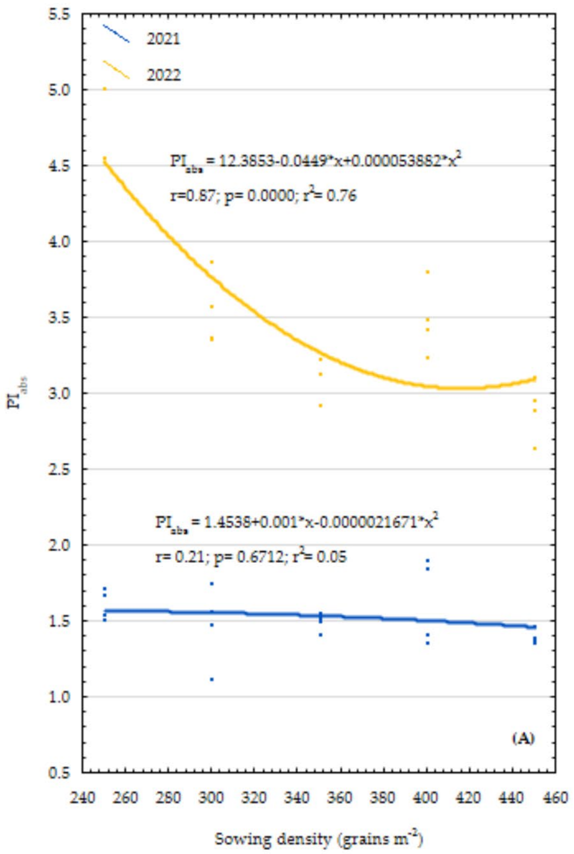

Fig. 10. Relationships between sowing density and PI_{abs} of *H. v.* var. *rimpaui* (A) and *H. v.* var. *nigricans* (B).

of leaf mass development translates into the amount of losses resulting from stress. The growth of new leaves in the shoot of the stem elongation phase after the apply of irrigation they observed also Hasanuzzaman et al.[33]. In the research of Hura et al.[34] rehydration in this growth stage had the effect of photosynthesis of the flag leaf.

LAI value in BBCH 57 in 2021 and in 2022 in *H. v.* var. *rimpaui* was the highest at a sowing density of 300 grains m^{-2} and significantly exceeded the values obtained at 400 grains m^{-2}. *H. v.* var. *nigricans* showed a significant response to increasing sowing density only in 2022 and had a significantly higher LAI in BBCH 57 at a density of 450 grains m^{-2} than at 250 grains m^{-2}. According to Hecht et al.[35], an increase in barley sowing density results in intraspecific competition for solar radiation, which increases the growth of shoots and thus increases aboveground biomass. However, this happens at the expense of the root system, and the optimal sowing density depends on the varietal characteristics of barley. According to Parker[36], LAI is not a linearly increasing indicator and is almost always asymptotic. The LAI value reaches a different maximum value for different plants and then stabilises or collapses. This is due to the absorption and reflection of light by the leaf surface, which can deprive the lower parts of the plant of the light needed for development. LAI also affects the microclimate of the canopy and thus the course of vegetation. An LAI of around 3.0 is considered optimal for most plants.

Different sowing densities and variable weather conditions also significantly affected the chlorophyll fluorescence indices of the tested barley genotypes determined on the flag leaf in BBCH 57. The ABS/RC, TR_o/RC, and DI_o/RC indices, regardless of the genotype and sowing density, were significantly higher in 2021 than in 2022. However, no significant variation in the ET_o/RC index was observed over the years of the study in *H. v.* var. *rimpaui*. The significant slowing of electron transport (Eto/RC) in *H. v.* var. *nigricans* when sown at a density of 450 grains m^{-2} compared with lower densities of 300 and 350 grains m^{-2} could be due to the reduced availability of water and nutrients required at the emergence stage, caused by a higher number of plants per unit area. The PI_{abs} index was significantly higher for both tested genotypes in 2022, regardless of the sowing density. In the first year of the study, which was characterized by a more considerable amount of rainfall during the barley growing season, in the first and second ten-day periods of June, i.e., during the period of flag leaf development and earing, the Selyaninov hydrothermal coefficient had values of 0.60 and 0.46, indicating arid conditions. In 2022, the second ten-day period of June was characterized by optimal humidity conditions, which improved after a long dry period in the previous ten-day periods of this growing season. Thus, weather conditions explain the higher PSII functioning index (PI_{abs}) in 2022 and higher ABS/RC, TR_o/RC and DI_o/RC values with unchanged ET_o/

Fig. 11. Grain yield per plant of *H. v.* var. *nigricans* and *H. v.* var. *rimpaui*. Error bars indicate standard deviation (SD). Letters a-e - mean values followed by different letters indicate significant differences $p < 0.05$.

RC. In the study by Kalaji[37], barley plants exposed to various stress factors, such as low and high PAR, water shortage, low and high temperature, and nutrient deficiency, after seven days of stress showed a significant reduction in PI_{abs} and an increase in ABS/RC, TR_o/RC, and DI_o/RC with a simultaneous decrease or lack of ET_o/RC response. Reducing some fluorescence indices, such as PI_{abs} under dry conditions, maybe a specific defence reaction of the tested genotypes. Compared to modern ones, older cereal genotypes have stomata that are more sensitive to water deficiency, but the reduced activity of the PSII reaction centre helps them avoid damage caused by photoinhibition[38].

In 2021, sowing density had no significant impact on PI_{abs} in *H. v.* var. *nigricans*, similarly, H. v. var. rimpaui showed little change, as indicated by the regression curve. The situation was different in Dry 2022, where a clear trend of decreasing PIabs values was observed in both genotypes tested as a result of increasing sowing density. This indicates a stronger negative effect of the growing conditions on the functioning of PSII at high plant

Traits	H. v. var. rimpaui			H. v. var. nigricans		
	LAI $_{BBCH\ 35}$	LAI $_{BBCH\ 57}$	Grain yield per plant (g)	LAI $_{BBCH\ 35}$	LAI $_{BBCH\ 57}$	Grain yield per plant (g)
SPAD $_{BBCH\ 35}$	−0.4163*	−0.1235	−0.2536	−0.6642*	−0.4979*	−0.8028*
SPAD $_{BBCH\ 57}$	−0.7526*	−0.2184	−0.7127*	−0.2898	−0.1833	−0.4987*
Fv/Fm	0.5941*	0.5286*	0.5037*	0.6473*	0.3947*	0.4391*
ABS/RC	0.7969*	0.5549*	0.6568*	0.5892*	0.3965*	0.8788*
DIo/RC	0.4171*	0.5549*	0.2979	0.2359	0.1778	0.6973*
TRo/RC	0.8660*	0.4423*	0.7309*	0.6534*	0.4758*	0.8723*
ETo/RC	−0.1823	−0.0148	−0.0392	0.0767	−0.0738	0.5084*
PI abs	−0.7878*	−0.4606*	−0.6487*	−0.7290*	−0.5985*	−0.8281*

Table 1. Correlations of physiological indicators with leaf area index. * - significant correlations, $p = 0.05$.

densities. Also a substantial reduction in ABS/RC, TR_o/RC, and DI_o/RC was observed at a sowing density of 450 grains m^{-2} compared to other sowing densities. *H. v.* var. *Rimpaui*, in turn, in 2021 was characterized by the highest ABS/RC, TR_o/RC, and DI_o/RC values at a sowing density of 300 grains m^{-2}. ET_o/RC, similarly to PI_{abs}, did not vary considerably under the influence of variable sowing density of *H. v.* var. *rimpaui*. In 2022, the PIabs index was the highest for this genotype at a sowing density of 250 grains m^{-2}. It decreased significantly with an increase in the sowing density to 450 grains m^{-2}, contrary to ABS/RC, TR_o/RC, and DI_o/RC, which increased with increasing the sowing density. In the second year of the study H. v. var. nigricans was characterized by lower PIabs under the influence of 450 grains m-2. The highest electron absorption per reaction center (ABS/RC) by the lowest ETo/RC, indicated electron transport, resulting in increased energy losses in heat (DIo/RC). Different tolerances to plant density in the canopy are also confirmed by the maximum photochemical efficiency index PSII (F_V/F_M). In 2022, F_V/F_M of plants sown at a density of 250 grains m^{-2} was significantly higher in *H. v.* var. *rimpaui*, while at a sowing density of 450 grains m^{-2}, a considerably higher F_V/F_M was found in *H. v.* var. *nigricans*.

Sowing density affects the supply of nutrients to plants and the availability of light, which affects the efficiency of the photosynthesis process[39]. In the study by Zhang et al.[29], plants grown at higher densities required higher nitrogen fertilization rates. A larger number of plants results in increased competition between plants for water and nutrients; plants at high density also reduce the biomass of the root system, which may result in poorer plant nutrition and less efficient photosynthesis[35]. In the present study, increasing sowing density resulted in a decrease in indices showing the efficiency of photosynthesis (PI_{abs}, F_V/F_M), especially in *H. v.* var. *rimpaui*, which may have resulted from lower availability of water, light, and nutrients relating to the creation of a larger assimilation area. Plants could use a larger amount of nutrients and water to produce a larger leaf area and, thus, a larger plant biomass, which resulted in poorer nutrition of the flag leaf and less effective photosynthesis. The dependence of the maximum photosynthetic functional efficiency (F_V/F_M) on LAI is well illustrated by comparing Figs. 3 and 9 in the section for *H. v.* var. *rimpaui* in 2022. They show how, at a sowing density of 400 grains m^{-2}, the LAI index at BBCH 57 reaches the lowest value among the combinations tested, deviating significantly from densities of 350 and 450 grains m^{-2}. A significant increase in F_V/F_M can be observed at the same site.

In the present study, the SPAD value in BBCH 35 did not differ significantly depending on the tested sowing densities. The genotype and year of cultivation had a greater impact on the leaf greenness index. In 2022, *H. v.* var. *nigricans* was characterized by a higher SPAD index than the other tested combinations of the study year and genotype. However, in BBCH 57, the interaction of experimental factors resulted in significant differentiation. The effect of the genotype on the SPAD index value was marked because both in 2021 and 2022 this genotype significantly exceeded *H. v.* var. *rimpaui*regarding leaf greenness, regardless of sowing density. SPAD value is a commonly used indicator of the nutritional status of plants, especially concerning nitrogen, and is also related to the chlorophyll content in leaves[40]. Also, in the study by Janušauskaite et al.[41], barley plants responded significantly regarding chlorophyll fluorescence and SPAD indices to different sowing densities. This reaction was also ambiguous in the present study and depended on the interaction of the tested genotype, sowing density, and year of cultivation. The study observed a significant negative relationship between SPAD in BBCH 57 and LAI in BBCH 35. By creating a larger leaf surface in the flag leaf stage, plants probably increased their food and water needs and thus could negatively impact the flag leaf's nutrition and greenness. A negative correlation between SPAD and LAI was also observed in previous studies on *H. v.* var. *nigricans* and *H. v.* var. *rimpaui*[42]. Furthermore, it is noteworthy that there is a discrepancy in SPAD values at 350 grains m^{-2} in *H. v.* var. *nigricans* in 2022 when compared to the LAI index and the alterations that have occurred between growth stages. The SPAD index for the combination exhibited a pronounced decline in comparison to the other densities that were tested. Concomitantly, the highest LAI value was observed at BBCH 57 in *H. v.* var. *nigricans* in 2022, precisely at 350 grains m^{-2}. Furthermore, the expansion in leaf area between LAI at BBCH 35 and BBCH 57 was markedly more pronounced at this sowing density than at the other combinations (approximately 0.8 greater). Consequently, a decline in SPAD was observed between the subflag and flag leaf.

A significant negative correlation was also observed between LAI in BBCH 35 and PI_{abs} in BBCH 57, and a positive correlation between LAI in BBCH 35 and BBCH 57 and Fv/Fm, ABS/RC, DI_o/RC, and TR_o/RC. According to Kalaji[37], PI_{abs} indicate the functioning of PSII concerning absorption and is closely related to the chlorophyll content in leaves. ABS/RC and the related DI_o/RC and TR_o/RC indicate the absorption and dissipation of excitation energy per reaction centre. Pearson's linear correlation analysis may therefore lead to the conclusion that an increase in the assimilation surface of plants in BBCH 35 resulted in reduced chlorophyll content in the flag leaf, expressed in SPAD units, which in turn translated into a smaller number of reaction centres in the system and an increase in absorption per one reaction center (ABS/RC), which also increased the amount of energy absorbed (TR_o/RC), but also lost (DI_o/RC). The positive relationship between Fv/Fm may be due to the fact that Fv/Fm is a very sensitive indicator that indicates the plant is experiencing stress[34]. The larger leaf area at BBCH 35 caused the plant to be stronger at later developmental stages (BBCH 57) and this translated into a higher Fv/Fm ratio. In the other direction, too, a higher Fv/Fm ratio would indicate that the plant grew under more comfortable conditions, and this contributed to the production and maintenance of a larger leaf area. The higher values of the correlation index at BBCH 35 may be due to the importance of this phase for the growth and yield of the barley genotypes in question. According to our previous research on alternative barley genotypes[38], the subflag leaf determines the further growth and yield of these plants to the greatest extent. The surface area of the flag leaf, although important, is of lesser importance. A significant reduction in grain yield per barley plant was observed when sowing density was increased, with genotype influencing the extent of this response. The highest yield was achieved by *H. v.* var. *rimpaui*, at 250 grains m^{-2}. However, this decreased significantly with increasing density. *H. v.* var. *nigricans* exhibited no discernible yield differences within the range of 250 to 350 grains m^{-2}. These differences were only discernible in the first year of the study, which was characterised by optimal hydrothermal conditions. It can thus be hypothesised that the reduction in yield per plant was attributable to the LAI and its elevated values in 2021, which may have limited light access and increased nutrient requirements. In conditions of optimal hydrothermal stress, plants that develop a large leaf area may shade and compete with one another, potentially leading to a reduction in yield. *H. v.* var. *nigricans* exhibits a slightly smaller leaf area than *H. v.* var. *rimpaui*, which results in its reduced ability to compete, including within its own species[22]. The results of the correlation analysis indicated a strong positive correlation between grain yield per plant and the TR_o/RC. This suggests that effective electron capture plays an important role in determining the yield of alternative black-grain barley genotypes.

Conclusions

The interaction of the study year, genotype, and sowing density significantly affected photosynthetic efficiency indices and the LAI index. In 2021, with optimal rainfall, the plants were characterized by higher LAI in BBCH 35 and chlorophyll fluorescence indices ABS/RC and TR_o/RC, as well as lower PI_{abs}, in BBCH 57 than in the dry year 2022 as a result of a strong negative relationship between LAI and SPAD value and PI_{abs} and a strong positive relationship between LAI and ABS/RC, TR_o/RC and DI_o/RC. In 2021, favourable for plant development, LAI of *H. v.* var. *nigricans* in BBCH 35 increased significantly with increasing sowing density, while in *H. v.* var. *rimpaui*, it increased significantly as the sowing density increased to 350 grains m^{-2} but decreased at a density above 400 grains m^{-2}. In dry 2022, increasing sowing density harmed PI_{abs} and F_V/F_M, while boosting ABS/RC and DI_o/RC, which may indicate a reduction in photosynthetic efficiency due to stress. This effect was already seen in *H. v.* var. *rimpaui* at a density of 300 grains m^{-2}. In *H. v.* var. *nigricans*, the efficiency of photosynthesis decreased only at a sowing density of 450 grains m^{-2}. In summary, the genotypes studied create a lush assimilative surface under optimal hydrothermal conditions, albeit with lower greenness and photosynthetic efficiency. In a dry year, the assimilative surface area is much lower, but the efficiency of PSII functioning increases. *H. v.* var. *rimpaui* responds by reducing LAI or PSII functioning efficiency at a density of 450 grains m^{-2}, while *H. v.* var. *nigricans* maintains these rates. The grain yield per single plant is observed to decrease with increasing sowing density. However, *H. v.* var. *nigricans* has been found to demonstrate less sensitivity to this factor. The grain yield per plant is correlated with photosynthetic parameters, particularly TR_o/RC. Further research should focus on explaining the conditions influencing the tolerance of genotypes to increase sowing density in unfavourable environmental conditions. An increased number of plants in a canopy while maintaining high photosynthetic efficiency may be vital to improving plant productivity.

Data availability

The datasets used and analysed during the current study available from the corresponding author on resonable request.

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References

1. Dogra, D. & Awasthi, C. P. Comparative nutritional evaluation of common buckwheat genotypes with major cereal and pseudocereals crops. *Agricultural Sci. Digest - Res. J.* **35** (1), 36. https://doi.org/10.5958/0976-0547.2015.00007.5 (2015).
2. Mencin, M., Jamnik, P., Mikulič Petkovšek, M., Veberič, R. & Terpinc, P. Enzymatic treatments of raw, germinated and fermented spelt (Triticum spelta L.) seeds improve the accessibility and antioxidant activity of their phenolics. *LWT* 169. https://doi.org/10.1016/j.lwt.2022.114046 (2022).
3. Szczepanek, M. et al. Ancient wheat species (Triticum sphaerococcum Perc. And T. Persicum Vav.) In organic farming: influence of sowing density on agronomic traits, pests and diseases occurrence, and weed infestation. *Agriculture* **10** (11), 1–21. https://doi.org/10.3390/agriculture10110556 (2020).
4. Zhang, J. et al. Enhancement of polyphenols and antioxidant activity in Germinated Black Highland Barley by Ultrasonication. *Molecules* **28** (9). https://doi.org/10.3390/molecules28093679 (2023).
5. Nowak, R. et al. Profile of phenolic compounds and antioxidant activity of organically and conventionally grown black-grain barley genotypes treated with biostimulant. *PLoS ONE*. **18** (7). https://doi.org/10.1371/journal.pone.0288428 (2023).
6. Nowak, R. et al. Impact of the Farming system and amino-acid biostimulants on the content of carotenoids, fatty acids, and polyphenols in alternative and common barley genotypes. *Agronomy* **13** (7), 1852. https://doi.org/10.3390/agronomy13071852 (2023b).

7. Glagoleva, A. Y. et al. Metabolic pathways and genes identified by RNA-seq analysis of barley near-isogenic lines differing by allelic state of the black lemma and pericarp (blp) gene. *BMC Plant. Biol.* **17** (Suppl 1), 182. https://doi.org/10.1186/s12870-017-1124-1 (2017).
8. Naeem, M. et al. The impact of different crop sequences on weed infestation and productivity of barley (Hordeum vulgare L.) under different tillage systems. *Crop Prot.* 149. https://doi.org/10.1016/j.cropro.2021.105759 (2021).
9. Janušauskaite, D., Auškalniene, O. & Pšibišauskiene, G. Photosynthetic responses of spring barley varieties to different stand densities under field conditions. *Acta Agriculturae Scand. Sect. B: Soil. Plant. Sci.* **62** (5), 441–448. https://doi.org/10.1080/09064710.2011.641029 (2012).
10. Pilipavicius, V., Romaneckiene, R. & Romaneckas, K. Crop stand density enhances competitive ability of spring barley (Hordeum vulgare L). *Acta Agriculturae Scand. Sect. B: Soil. Plant. Sci.* **61** (7), 648–660. https://doi.org/10.1080/09064710.2010.539574 (2011).
11. Hudzenko, V. M. et al. Elucidation of gene action and combining ability for productive tillering in spring barley. *Regul. Mech. Biosystems*. **13** (2), 197–206. https://doi.org/10.15421/022225 (2022).
12. Gao, Y. et al. Increasing seeding density under limited irrigation improves crop yield and water productivity of winter wheat by constructing a reasonable population architecture. *Agric. Water Manage.* 253. https://doi.org/10.1016/j.agwat.2021.106951 (2021).
13. Huang, G. et al. Plant Architecture influences the Population Transpiration and Canopy temperature in Winter Wheat genotypes. *Agronomy* **13** (3). https://doi.org/10.3390/agronomy13030742 (2023).
14. Robert, C. et al. Does canopy architecture play a role in the effect of plant density and sowing date on epidemics of Septoria Tritici in wheat crops? Comparative Biochemistry & Physiology Part. *Mol. Integr. Physiol.* **153** (2), S224–S225. https://doi.org/10.1016/j.cbpa.2009.04.558 (2009).
15. Song, Q., Zhang, G. & Zhu, X. G. Optimal crop canopy architecture to maximise canopy photosynthetic CO 2 uptake under elevated CO 2 - a theoretical study using a mechanistic model of canopy photosynthesis. *Funct. Plant Biol.* **40** (2), 3. https://doi.org/10.1071/FP12056 (2013).
16. FASEELA, P., BRESTIČ, S. I. N. I. S. H. A. A. K., PUTHUR, J. T. & M., & Special issue in honour of Prof. Reto J. Strasser – Chlorophyll a fluorescence parameters as indicators of a particular abiotic stress in rice. *Photosynthetica* **58** (SPECIAL ISSUE), 293–300. https://doi.org/10.32615/ps.2019.147 (2020).
17. Singh, H., Kumar, D. & Soni, V. Performance of chlorophyll a fluorescence parameters in Lemna minor under heavy metal stress induced by various concentration of copper. *Sci. Rep.* **12**, 10620. https://doi.org/10.1038/s41598-022-14985-2 (2022).
18. Nyi, N., Sridokchan, W., Chai-arree, W. & Srinives, P. Nondestructive measurement of photosynthetic pigments and nitrogen status in Jatropha (Jatropha curcas L.) by chlorophyll meter. *Phillipp Agric. Sci.* **95**, 83–89 (2012). [Google Scholar].
19. Fang, H., Baret, F., Plummer, S. & Schaepman-Strub, G. An overview of global leaf area index (LAI): methods, products, validation, and applications. *Rev. Geophys.* **57**, 739–799 (2019).
20. Márquez-Prieto, A. K. et al. Impact of the foliar application of potassium nanofertilizer on biomass, yield, nitrogen assimilation and photosynthetic activity in green beans. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. **50** (1), 1–12. https://doi.org/10.15835/nbha50112569 (2022).
21. Selyaninov, G. T. On the agricultural estimation of climate. *Trudy Po Sel'Skokhozyaistvennoi Meteorologii*. **20**, 165–177 (1928).
22. Nowak, R., Szczepanek, M. & Błaszczyk, K. Productivity of Alternative Barley genotypes under Variable Intraspecific Competition resulting from increasing sowing density. *Agronomy* **14**, 2275. https://doi.org/10.3390/agronomy14102275 (2024).
23. Kuklik, M., Baryła, R., Czarnecki, Z. & Bochniak, A. Warunki hydrotermiczne w centralnej części rejonu kanału wieprz-krzna w 50-leciu (1966–2015). ANNALES UMCS: SECTIO E AGRICULTURA 2016, LXXI (3).
24. Holland, J. E. et al. Proximal fluorescence sensing of potassium responsive crops to develop improved predictions of biomass, yield and grain quality of wheat and barley. *Precision Agric.* **20** (2), 379–397. https://doi.org/10.1007/s11119-018-09629-3 (2019).
25. Han, L. et al. Modeling maize above-ground biomass based on machine learning approaches using UAV remote-sensing data. *Plant. Methods.* **15** (1). https://doi.org/10.1186/s13007-019-0394-z (2019).
26. Fukai, S., Searle, C., Baiquni, H., Choenthong, S. & Kywe, M. Growth and grain-yield of contrasting barley cultivars under different plant densities. *Field Crops Res.* **23** (3), 239–254. https://doi.org/10.1016/0378-4290(90)90057-I (1990).
27. Tie-mei, L. et al. Simulation model of barley leaf area index. *Yingyong Shengtai Xuebao*. **21** (1), 121–128 (2010). PMID: 20387433.
28. Ju, Z., Liu, K., Zhao, G., Ma, X. & Jia, Z. Nitrogen Fertilizer and Sowing Density Affect Flag Leaf photosynthetic characteristics, Grain Yield, and Yield Components of Oat in a Semiarid Region of Northwest China. *Agronomy* **12** (9). https://doi.org/10.3390/agronomy12092108 (2022).
29. Zhang, F. et al. Effect of planting density on Canopy structure, Microenvironment, and yields of uniformly sown Winter Wheat. *Agronomy* **13** (3). https://doi.org/10.3390/agronomy13030870 (2023).
30. Jia, Q. & Wang, Y. P. Relationships between Leaf Area Index and Evapotranspiration and Crop Coefficient of Hilly Apple Orchard in the Loess Plateau. *Water* **13**, 1957. https://doi.org/10.3390/w13141957 (2021).
31. Sepp, S. S., Tamm, Ü. & Loit, E. Is Estonian barley ready to tackle climate change- induced water regimes? Agraarteadus. *J. Agric. Sci.* **32** (Issue 2), 314–318. https://doi.org/10.15159/jas.21.29 (2021).
32. Neumann, K. et al. Dissecting spatiotemporal biomass accumulation in barley under different water regimes using high-throughput image analysis. *Plant. Cell. Environ.* **38** (10), 1980–1996. https://doi.org/10.1111/pce.12516 (2015).
33. Hasanuzzaman, A. C. et al. Assessing the suitability of various screening methods as a proxy for drought tolerance in barley. *Funct. Plant Biol.* **44**(2) 253–266. https://doi.org/10.1071/FP16263
34. Hura, T. et al. Rapid plant rehydration initiates permanent and adverse changes in the photosynthetic apparatus of triticale. *Plant. Soil.* **397**, 127–145. https://doi.org/10.1007/s11104-015-2607-1 (2015).
35. Hecht, V. L., Temperton, V. M., Nagel, K. A., Rascher, U. & Postma, J. A. Sowing density: a neglected factor fundamentally affecting root distribution and biomass allocation of field grown spring barley (Hordeum Vulgare L). *Front. Plant Sci.* 7. https://doi.org/10.3389/fpls.2016.00944 (2016).
36. Parker, G. G. Tamm review: Leaf Area Index (LAI) is both a determinant and a consequence of important processes in vegetation canopies. *For. Ecol. Manage.* **477** (1), 118496. https://doi.org/10.1016/j.foreco.2020.118496 (2020).
37. Kalaji, H. M. *Treatises and Monographs: Influence of Abiotic Stress Factors on Chlorophyll Fluorescence in Selected Varieties of Barley (Hordeum vulgare L.) Plants* (Warsaw University of Life Sciences Press, Warsaw, Poland, 2011).
38. Guan, X. K., Song, L., Tongchao, W., Turner, N., Li, F. M. & & & & Effect of Drought on the Gas Exchange, Chlorophyll fluorescence and yield of six different-era spring wheat cultivars. *J. Agron. Crop. Sci.* 201. https://doi.org/10.1111/jac.12103 (2014).
39. Tobiasz-Salach, R., Jańczak-Pieniążek, M. & Augustyńska-Prejsnar, A. Effect of different row spacing and sowing density on selected photosynthesis indices, yield, and quality of White Lupine seeds. *Agriculture* **13** (9). https://doi.org/10.3390/agriculture13091845 (2023).
40. Xiong, D. et al. SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. *Sci. Rep.* 5. https://doi.org/10.1038/srep13389 (2015).
41. Hecht, V. L. et al. Plant density modifies root system architecture in spring barley (Hordeum vulgare L.) through a change in nodal root number. *Plant. Soil.* **439** (1–2), 179–200. https://doi.org/10.1007/s11104-018-3764-9 (2019).
42. Szczepanek, M., Nowak, R. & Błaszczyk, K. Physiological and Agronomic Characteristics of Alternative Black Barley Genotypes (Hordeum vulgare var. Nigricans and H. v. var. Rimpaui) under different hydrothermal conditions of the growing Seasons. *Agriculture* **13** (10), 2033. https://doi.org/10.3390/agriculture13102033 (2023).

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Declarations

Competing interests
The authors declare no competing interests.

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