

Article

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

Małgorzata Szczepanek , Rafał Nowak and Karolina Błaszczyk

Department of Agronomy, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Prof. S. Kaliskiego 7, 85-796 Bydgoszcz, Poland; rafnow003@pbs.edu.pl (R.N.); karbla005@pbs.edu.pl (K.B.)

* Correspondence: malgorzata.szczepanek@pbs.edu.pl; Tel.: +48-602502165

Abstract: Black-seeded barley can be a valuable raw material for functional food. However, its restoration to cropping should be preceded by the identification of the characteristics determining productivity. The field study was conducted to identify specific parameters of the black-seeded barley genotypes (*Hordeum vulgare* var. *nigricans* and *H. v.* var. *rimpaui*) and compare them with common barley (*H. vulgare*) under the different hydrothermal conditions of the two growing seasons of 2021 and 2022. Our research has shown that each genotype has a set of specific characteristics that best describe it at a given developmental stage. *H. v. rimpaui* was well characterized by chlorophyll fluorescence parameters such as F_V/F_M , F_V/F_0 , and PI_{ABS} at the seedling stage and *H. v. nigricans* by F_V/F_M and F_V/F_0 at the flag leaf stage. Moreover, *H. v.* var. *rimpaui* was distinguished by a high biomass of shoot (726 g m^{-2}) and straw yield (5.04 t ha^{-1}) but *H. v.* var. *nigricans* by a high number of sterile generative tillers (103 m^{-2} in the dry year 2022). Further research should focus on the response of black-seeded barley genotypes to abiotic stresses, while in agronomic practice, efforts should be made to increase the number of grains per ear and 1000-grain weight.

Keywords: chlorophyll fluorescence; black barley; PCA; LAI; SPAD; grain yield; yield components



Citation: 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* **2023**, *13*, 2033. <https://doi.org/10.3390/agriculture13102033>

Academic Editor: Peng Zhang

Received: 11 September 2023

Revised: 18 October 2023

Accepted: 19 October 2023

Published: 22 October 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Barley, like emmer or einkorn wheat, is one of the earliest domesticated species (12,000 BC). Its native place of origin is the Fertile Crescent region, spanning southwestern Iran, through the Zagros and Taurus Mountains in Iran, Iraq, and Turkey to central Anatolia, northern Syria, and Palestine [1,2]. The genus barley (*Hordeum* spp.) includes over 30 species found in temperate, tropical, and equatorial climate zones. Currently, it is one of the most important field crops. The global sown area of barley in 2021 was 48.9 million hectares, which gives it fifth place after wheat, rice, corn, and soybeans [3]. The most important species of the genus *Hordeum* is common barley (*Hordeum vulgare*). This species occurs in both winter and spring forms. Its genetic diversity is manifested, among others, in rowing (two-, four-, or six-row spike) or hulling (husked or naked grain) [4]. Among the genotypes of barley, there are also forms with awns transformed into hoods (hooded barley). Barley grain can be yellow, blue, purple, and even black [5]. The color of grains results from plant pigments accumulated in the aleurone layer of the grain [6]. The black color of grain is due to the presence of several chemical compounds, including phytochemicals and phenolic compounds [7]. The black barley group includes the species *H. vulgare* var. *nigricans*, also known as *H. distichon* var. *nigricans* (Global Biodiversity Information Facility), which can be found in several regions of the world as a niche local variety, especially in Iraq, Syria, and Turkey [8]. Hooded barley *H. vulgare* var. *rimpaui* may also have black-colored grains.

Barley grain is used for the production of fodder and alcoholic beverages [9]. However, in Asia (mainly Tibet, China), barley is one of the basic food raw materials [10]. The beneficial dietary and health-promoting qualities of barley have influenced the increasing consumption importance of this species in recent years. The high dietary quality of barley grain results, among others, from the content of bioactive substances with antioxidant properties [11,12]. Previous research indicates that some primary barley genotypes (especially those with dark-colored grains) have particularly high antioxidant potential [13–15]. These genotypes are characterized by a higher antioxidant activity and higher concentration of phenolic acids, flavonoids, phytomelanin, lutein, and anthocyanidins than common barley and can be valuable raw materials for the production of functional food [16,17]. The condition for the implementation of these valuable genotypes for cultivation and further consumption use is the recognition of characteristics determining growth, development, and yield in various agroclimatic conditions.

Proper growth and development of plants can be inhibited by biotic and abiotic stresses. Ongoing monitoring of the physiological state of plants and diagnosis of the impact of stresses on plant growth is possible thanks to the use of chlorophyll fluorescence. This phenomenon informs, among other things, the physiological state of plants under abiotic stress conditions (high or low temperature, water shortages or excesses, etc.) at an early stage of their occurrence. The chlorophyll molecule is fluorescent, making it possible to determine the photochemical activity of the plant by detecting photon scattering, which is closely related to changes in electron transport on chloroplast membranes [18]. This disturbance causes a decrease in the photosynthesis activity of the whole plant [19]. By measuring chlorophyll fluorescence, disturbances in the photosynthetic activity of the plant can be detected without disturbing the tissues of the tested plants [18]. The specific features of the parameters describing the fluorescence of chlorophyll, including the most widely used indices F_V/F_M and F_V/F_0 , have made them more and more popular in the diagnosis of the condition of agricultural crops [20]. The F_V/F_M ratio (a parameter that represents the conversion efficiency of primary light energy in the center of PS II) and F_V/F_0 ratio (which represents the photochemical and non-photochemical use of light energy in the reaction center) are the important parameters used as stress indicators in a large number of photosynthetic studies. However, because they depend on the initial (F_0) and maximum (F_M) levels of fluorescence, they may not be sensitive enough to detect variation between samples [21]. In many studies, the performance index (PI_{ABS}) is also presented as a relevant parameter to measure photosynthesis efficiency. The PI is an integrative parameter calculated based on primary photochemistry, the density of the active reaction center per chlorophyll, and the efficiency of electron. As a result, if any of these components is affected by stress, the effect will be visible in the PI [19,21]. For many different plants, the parameters F_V/F_M and F_V/F_0 as measured under non-stress conditions are almost constant and amount to 0.80–0.83 and 4–6, respectively [22]. However, PI does not have a specific value, but it is assumed that for plants subjected to stress, the values of this parameter are much lower than for plants free from abiotic stresses [23,24].

The amount of biomass produced during vegetation and the useful yield of plants also depend on proper nutrition, which can be indirectly assessed by measuring leaf chlorophyll content by SPAD meters using the emission of light of various wavelengths through the plant leaf [25]. The final SPAD value is determined on the basis of the difference between the absorption of red and infrared light waves. The higher the value of this parameter, the more chlorophyll molecules in the plant leaf [26]. Another parameter related to the size of the yield is the leaf area index (LAI), which tells about the leaf area that captures the solar energy that is necessary for plant photosynthesis [27]. It is well known, however, that the final yield of cereal grains is directly influenced by the size and interdependence of structural yielding components such as generative fertile tillers per m^2 , number of grain per spike, and 1000-grain weight [28].

The aim of the research was to identify the physiological and agronomic parameters of the alternative black barley genotypes (*H. v. var. nigricans* and *H. v. var. rimpai*) and

compare them with common barley (*H. vulgare*) under the different hydrothermal conditions of the growing seasons. The results of our study can be used as a theoretical basis for the growing management of black-seeded barley and indicate further research directions.

2. Materials and Methods

2.1. Site Description

Field experiments were conducted in Minikowo, Kuyavian-Pomeranian Voivodeship, central Poland (53°10'2" N 17°44'22" E), over two growing seasons (2021 and 2022). The soil at the experimental site is characterized by an acidic soil pH of 4.7 and has a content of 7.1 mg P₂O₅ 100 g^{−1} soil, 17.1 mg K₂O 100 g^{−1} soil, and 4.5 mg MgO 100 g^{−1} soil.

Weather conditions during the barley-growing period varied in the years of the study. It was noticeably cooler in the period from 21 April to 10 May and warmer in the periods from 1 June to 20 and from 1 July to 20 in 2021 compared to 2022 (Figure 1). In the 2nd and 3rd ten-day periods of April 2021, strong drops in temperature at the ground were observed, reaching −7.0 °C during emergence (Supplementary Materials, Table S2). In 2022, in the same period, the minimum air temperature near the ground oscillated between −2.1 and 6.2 °C.

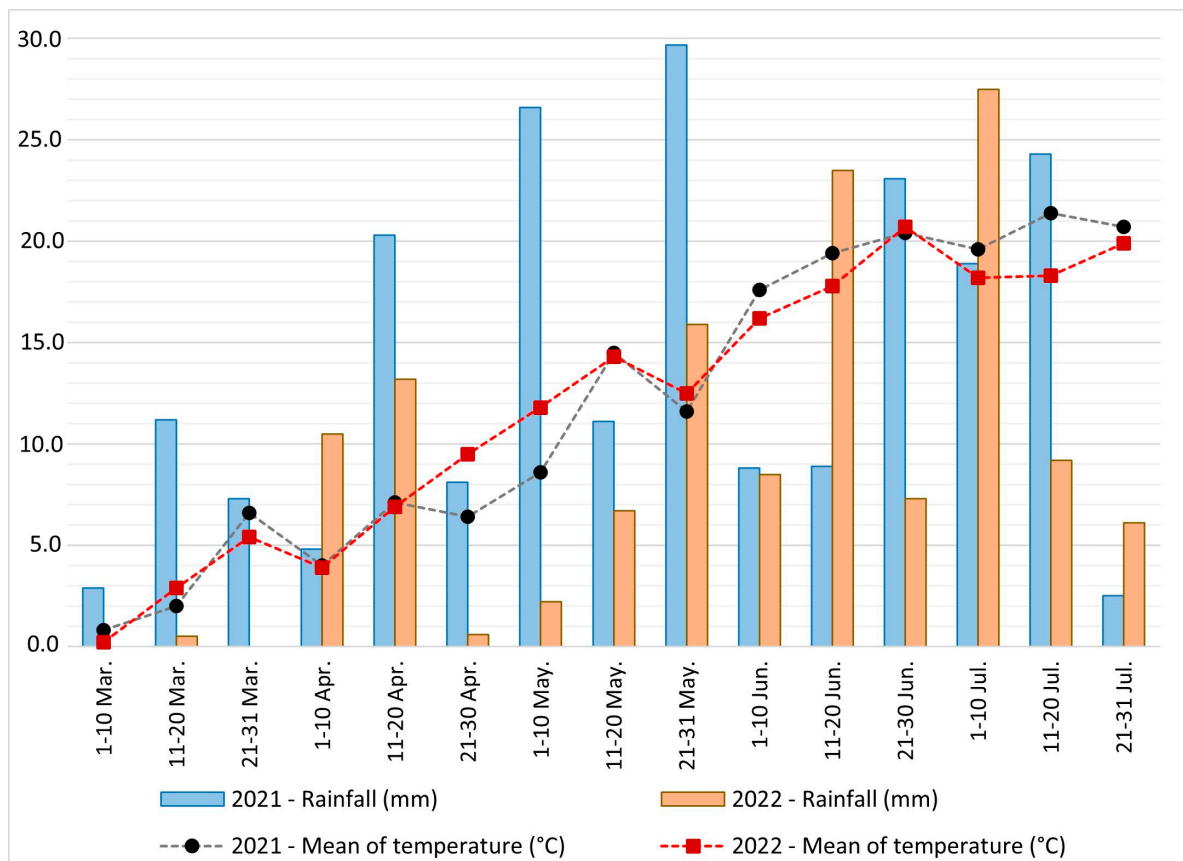


Figure 1. Hydrothermal conditions (air temperature and rainfall) during spring barley-growing seasons, 2021 and 2022.

The total amount of precipitation in the growing season (March–July) in 2021 amounted to 208.5 mm, while in 2022, it was only 131.7 mm. In March 2021, the total precipitation amounted to 21.4 mm, while in the same month of 2022, it was only 0.5 mm. The period from 21 April to 31 May 2022 was also characterized by a clear rainfall deficit in which the total precipitation amounted to only 25.4 mm. In the same period of 2021, the precipitation was almost three times higher and amounted to 75.5 mm. Before the harvest of barley (21–31 June), rainfall in both years did not exceed 6 mm.

The hydrothermal conditions of the growth season (March–July) were developed on the basis of precipitation sums and air temperature in the years 2021–2022. The sum of average daily air temperatures and the sum of precipitation for individual decades were calculated, which were used to calculate the Sielianinov coefficient (k) [29] for individual years (Supplementary Materials, Table S1) according to the following formula:

$$k = \frac{P \cdot 10}{\Sigma t}$$

where:

P —total precipitation in mm for a decade;

Σt —sum of average daily air temperatures $> 0^{\circ}\text{C}$ for the ten-day period.

The hydrothermal coefficient (Sielianinov coefficient) determines the relationship between precipitation and evaporation and characterizes the moisture conditions for plant growth. Based on the value of the coefficient, the periods were classified in four groups after Kuklik et al. [30]:

- Extremely dry or very dry— $k \leq 0.7$;
- Dry or quite dry— $0.7 < k \leq 1.3$;
- Optimal or quite moist— $1.3 < k \leq 2.0$;
- Humid, very humid, or extremely humid— $k > 2.0$.

2.2. Subject of Research and Agronomic Practice

Two primary barley (*H. vulgare* L. var. *nigricans* (Ser.) Körn (*H. v. nigricans*) and *H. vulgare* L. var. *rimpau* Wittm (*H. v. rimpau*)) and one common barley (*H. vulgare* var. *vulgare* (*H. vulgare*) cultivar ‘Soldo’) were studied. The first two genotypes are two-rows, spring, and are characterized by dark pigmentation of husk, fruit, and seed coat of grain. The common barley ‘Soldo’, being a modern, spring, two-row variety with yellow grains, served as a comparative genotype for two primary forms. *H. v. rimpau* is also distinguished by reduced awns, i.e., the so-called hoods (hooded barley), while *H. nigricans* and *H. vulgare* have spikes with fully developed awns.

The experiment was conducted in a completed randomized block design in three replications in plot areas of 24 m². Barley was sown with a row spacing of 12.5 cm and a density of 350 grains m^{−2} in the 3rd week of March in both years. The fertilizer was applied before sowing at rates of 70 kg N ha^{−1}, 40 kg P₂O₅ ha^{−1}, and 70 kg K₂O ha^{−1}. The weeds were chemically controlled using a mixture of 2,4-D, florasulam, and pinoxaden at rates of 180 g, 3.75 g, and 40 g a.i. ha^{−1} at the end of tillering. Fungicides were applied twice: tiophanate-methyl in a dose of 700 g a.i. ha^{−1} at the beginning of the stem-elongation stage and a mixture of tebuconazole and prothioconazole in doses 125 g a.i. and 125 g a.i. ha^{−1}, respectively, at the flag leaf stage. The cereal leaf beetle was controlled with cypermethrin in a dose of 25 g a.i. ha^{−1} at the flag leaf stage. Grain was harvested in both years between 21st and 31st of July when the grain had 14% of water content.

2.3. Measurement of Physiological, Biometric, and Agronomic Features

2.3.1. Chlorophyll Fluorescence

Measurements of direct fluorescence of chlorophyll in barley plants were carried out at three dates: I—at the second true leaf stage (BBCH 12) in the third decade of April; II—at the flag leaf stage (BBCH 39) in the third week of May; and III—at full heading (BBCH 55) in the second week of June. The tests were performed using the Pocket PEA fluorimeter (Pocket Plant Efficiency Analyzer) (Hansatech Instruments, Norfolk, UK). Measurements of direct chlorophyll fluorescence were carried out on samples with an area of 4 mm² on the middle part of the mature leaf blade; on the youngest fully developed leaf, i.e., the first leaf at the BBCH 12 stage; on the subflag leaf at the BBCH 39 stage; and on the flag leaf at the BBCH 55 stage. Special clips were used to extinguish the light phase of photosynthesis and blocked the light supply to the test sample for 30 min before the

measurement. The measurement was carried out according to the recommendations of the device manufacturer, with a light pulse intensity of $3500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a duration of 1 s. The fluorescent signal was initially sampled at 10 μs intervals for the first 300 μs . Depending on the date, 60–80 measurements were made in a series for each genotype. The following parameters were measured: F_0 —initial fluorescence; F_M —maximum fluorescence; $F_V = F_M - F_0$ —variable fluorescence; F_V/F_M —maximum PSII performance; PI_{ABS} (PI)—PSII functioning index; Area—surface area above the chlorophyll fluorescence induction curve. A detailed analysis of the measured chlorophyll fluorescence signals was also carried out using the OJIP test, which was used to determine phenomenological energy fluxes per excited cross section (CS):

- ABS/CS_0 —energy absorption by excited photosynthesizing sample (CS) at time zero ($t = 0$);
- $\text{TR}_0/\text{CS}_0 = \phi\text{P}_0(\text{ABS}/\text{CS}_X)$ —capacity of absorbed excitation energy by PSII photosynthesizing sample (CS) at time zero ($t = 0$);
- $\text{ET}_0/\text{CS}_0 = \phi\text{E}_0(\text{ABS}/\text{CS}_X)$ —electron transport of PSII of photosynthesizing sample (CS) at time zero ($t = 0$);
- $\text{DI}_0/\text{CS}_0 = (\text{ABS}/\text{CS}_0) - (\text{TR}_0/\text{CS}_0)$ —energy amount dissipated as heat by PSII photosynthesizing sample (CS) at $t = 0$;
- Plabs—an indicator of the functioning of PSII in relation to absorption.

2.3.2. Leaf Area Index (LAI) and Chlorophyll Index (SPAD)

During the growing period, the LAI (leaf area index) was measured twice, at the flag leaf stage (BBCH 39) and at the full heading stage (BBCH 55), using the SunScan Canopy Analysis System device (Delta-T Devices Ltd., Cambridge, UK). The device's 1 m long probe of the device, equipped with 64 PAR sensors, was placed in the plant canopy just above the soil surface. At the same time, a second sensor measuring direct and diffuse radiation was placed above the plant canopy. The radiation measurements were transmitted from the probe and sensor to a PDA terminal, which converted and recorded the radiation quantum values into leaf area, expressed in m^2 of leaf area m^{-2} of soil.

On these dates as well as at the second true leaf stage (BBCH 12), the SPAD index was determined on the 30 youngest leaves in 16 repetitions for each genotype, using the Minolta N-tester (Konica Minolta, Osaka, Japan). The central part of the leaf blade was placed in the clip of the device, which was equipped with a light source on one side and a photodetector on the other side of the leaf blade. When the clip was closed, the light source shone through the leaf, and the amount of light at 650 nm and 940 nm that passed through the leaf blade was read by the photodetector, which indicated the amount of light absorbed by chlorophyll and other components of the system. On this basis, the instrument calculated the amount of chlorophyll in the test sample expressed in conventional units.

2.3.3. Biometric and Agronomic Features

At the flowering stage (BBCH 59), the dry weight of shoots was determined on subsequent plants collected in a row with a length corresponding to an area of 0.5 m^2 in each plot. Then, at the full maturity stage (BBCH 89), an area of 1 m^2 was marked on each experimental plot, and the number of fertile generative spikes (with kernels) and sterile generative spikes (without kernels) was determined. At the same development stage, 30 spikes were randomly collected from each plot, and the number of grains per spike was calculated. Plants from experimental plots with an area of 24 m^2 were harvested using a Winterstaiger Classic plot harvester (Winterstaiger, Ried im Innkreis, Austria). Grain yield and straw yield were determined using the weighing method after harvest, and then, based on these parameters, the above-ground biomass at full maturity stage and the ratio of grain yield to above-ground biomass yield were calculated, presented as the harvest index (HI). The 1000-grain weight was determined on samples taken after achieving constant grain moisture of 12%, based on 500 grains, in two repetitions of each treatment.

2.4. Statistical Analysis

The results were analyzed using a two-way analysis of variance. Differences between means were tested using Tuckey's HSD test at $p = 0.05$. Pearson's simple correlation analysis was also performed within each genotype, as was the principal component analysis. All statistical calculations were performed in the Statistica 13.0 PL statistical package (Statsoft, Kraków, Poland). The radar plot was prepared using the Grapher 21 (Golden Softwear, Golden, CO, USA).

3. Results

3.1. Physiological Features

3.1.1. Chlorophyll Fluorescence

The first measurement of chlorophyll fluorescence carried out on barley seedlings at the BBCH 12 stage indicated significant differences in the efficiency of the photosynthetic apparatus influenced by the interaction of year and genotype ($p \leq 0.0005$; Supplementary Materials, Table S3). In the first season of the study, for most parameters determined at the seedling stage, no significant differences were noted between *H. vulgare* and alternative barley genotypes (*H. v. var. nigricans* and *H. v. var. rimpaii*). Significant differences were observed only in the ET_0/CS_0 index, which was significantly higher in *H. v. var. rimpaii* than in the other genotypes. Additionally, black-seeded genotypes differed from each other in 2021 in terms of ABS/CS_0 and TR_0/CS_0 parameters, which were significantly higher in *H. v. var. rimpaii* than in *H. v. var. nigricans* (Figure 2).

In the second year of the study, much greater variation between genotypes was observed. Higher values of F_V/F_0 , F_V/F_M , and PI_{ABS} indices in 2022 were noted in *H. v. var. rimpaii*. The first two parameters were significantly higher in *H. v. var. rimpaii* than in the other genotypes, while PI_{ABS} was significantly higher in *H. v. var. rimpaii* only in comparison with *H. v. var. nigricans*. In the second year of the study, the conventional barley genotype was characterized by significantly higher area, ET_0/CS_0 , and TR_0/CS_0 than the black-seeded genotypes. *H. v. var. nigricans* in 2022 showed the highest values of the DI_0/CS_0 and ABS/CS_0 indices compared with the other genotypes. The values of these parameters were significantly higher for this genotype by 42.1% and 10.2% compared to *H. vulgare* and 90.3% and 24.5% than in *H. v. var. rimpaii* (Figure 2).

The chlorophyll fluorescence of subflag and flag leaves, described by parameters such as F_V/F_M , F_V/F_0 , or PI_{ABS} , was influenced by the year–genotype interaction ($p \leq 0.0005$; Supplementary Materials, Table S4). In 2021, the F_V/F_M and F_V/F_0 values assessed on the subflag leaf were significantly higher in *H. vulgare* than in alternative barley genotypes (*H. v. var. nigricans* and *H. v. var. rimpaii*) (Table 1). In 2022, only F_V/F_0 had a significantly higher value in *H. vulgare* than in *H. v. var. rimpaii*. In 2021, the F_V/F_M and F_V/F_0 indices of the primary genotypes were similar, while in 2022, *H. v. var. nigricans* was characterized by higher values of these parameters compared to *H. v. var. rimpaii*.

Analysis of the F_V/F_M and F_V/F_0 indices on the flag leaf showed a different ranking of genotypes than previously described for the parameters measured on the subflag leaf (Table 1). In 2021, black-seeded barley genotypes were characterized by higher values of the F_V/F_M and F_V/F_0 parameters than common barley (*H. vulgare*). In 2022, the values of these indices were similar in *H. v. var. nigricans* and *H. vulgare*. In the same year, the value of the F_V/F_0 parameter in hooded barley (*H. v. var. rimpaii*) was the lowest, and the value of the F_V/F_M index was significantly lower only when compared to *H. vulgare* (Table 1).

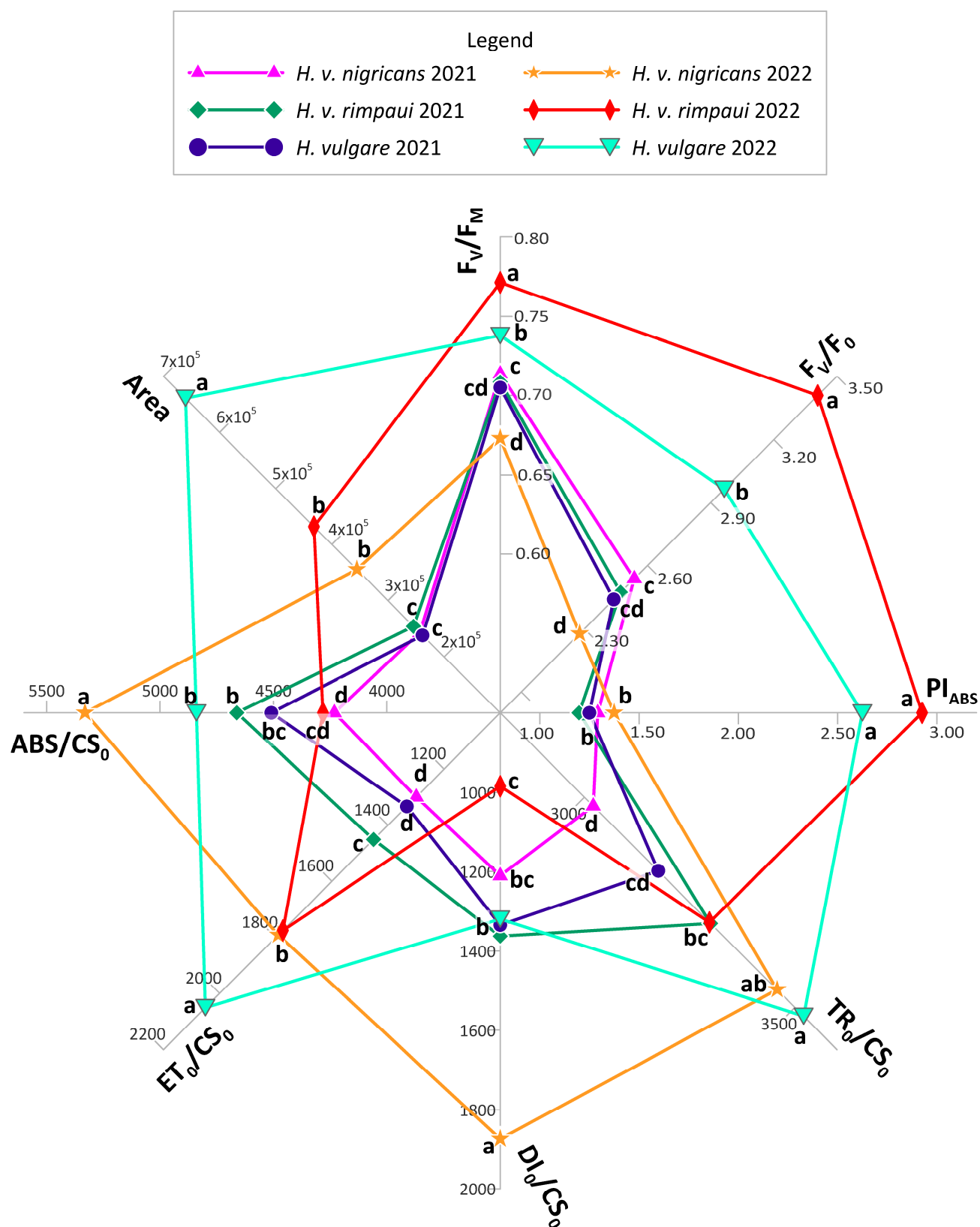


Figure 2. Chlorophyll fluorescence at seedling stage of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpai*, and *H. vulgare*. a–d—the mean values with different letters are significantly different at the significance level $p < 0.05$.

Table 1. Chlorophyll fluorescence on subflag and flag leaf of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpau*, and *H. vulgare*.

Year	Genotype	F _V /F _M Mean ± SD	F _V /F ₀ Mean ± SD	PI _{ABS} Mean ± SD
Subflag leaf				
2021	<i>H. v. nigricans</i>	0.789 ^c ± 0.007	3.78 ^c ± 0.172	2.05 ^d ± 0.163
	<i>H. v. rimpau</i>	0.789 ^c ± 0.013	3.84 ^c ± 0.183	1.93 ^d ± 0.266
	<i>H. vulgare</i>	0.806 ^{ab} ± 0.014	4.25 ^{ab} ± 0.176	3.96 ^c ± 0.518
2022	<i>H. v. nigricans</i>	0.815 ^a ± 0.004	4.42 ^a ± 0.118	5.14 ^b ± 0.635
	<i>H. v. rimpau</i>	0.797 ^{bc} ± 0.013	3.96 ^c ± 0.288	4.04 ^c ± 0.714
	<i>H. vulgare</i>	0.806 ^{ab} ± 0.009	4.18 ^b ± 0.234	5.82 ^a ± 1.28
Flag leaf				
2021	<i>H. v. nigricans</i>	0.835 ^a ± 0.015	4.94 ^a ± 0.076	5.10 ^c ± 0.583
	<i>H. v. rimpau</i>	0.827 ^{ab} ± 0.006	4.79 ^a ± 0.188	5.90 ^{bc} ± 1.09
	<i>H. vulgare</i>	0.805 ^{cd} ± 0.009	4.16 ^c ± 0.234	4.84 ^c ± 0.422
2022	<i>H. v. nigricans</i>	0.808 ^{cd} ± 0.007	4.25 ^{bc} ± 0.220	6.36 ^b ± 1.06
	<i>H. v. rimpau</i>	0.790 ^d ± 0.029	3.71 ^d ± 0.421	5.08 ^c ± 1.44
	<i>H. vulgare</i>	0.811 ^{bc} ± 0.026	4.47 ^b ± 0.250	7.51 ^a ± 1.39

^{a–d}—the mean values in columns with different letters are significantly different at the significance level $p < 0.05$. “±” relates to the standard deviation.

Performance index (PI_{ABS}) measurements on the subflag leaf in 2021 and the subflag and flag leaves in 2022 showed an increased value of this index in common barley (*H. vulgare*) compared to alternative barley genotypes (*H. v. var. nigricans* and *H. v. var. rimpau*) (Table 1). *H. v. rimpau* was characterized significantly by the lowest PI_{ABS} values of the subflag and flag leaves in 2022. There were no differences in the PI_{ABS} parameter between genotypes in the 2021 flag leaf analysis (Table 1).

3.1.2. Leaf Area Index (LAI) and Chlorophyll Index (SPAD)

Our study showed that LAI tested at the BBCH 35 and BBCH 57 stages was on average 71.4 and 19.9% higher, respectively, in 2021 compared to 2022 (Table 2). Thus, we observed the influence of year–genotype interaction for leaf area index (LAI) ($p < 0.0001$; Supplementary Materials, Table S5).

Table 2. Leaf area index (LAI) of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpau*, and *H. vulgare* at subflag leaf stage (BBCH 35) and heading stage (BBCH 57).

Year	Genotype	LAI at BBCH 35 Mean ± SD	LAI at BBCH 57 Mean ± SD
2021	<i>H. v. nigricans</i>	3.49 ^b ± 0.733	3.00 ^b ± 0.515
	<i>H. v. rimpau</i>	4.51 ^a ± 0.516	3.14 ^a ± 0.598
	<i>H. vulgare</i>	2.50 ^c ± 0.539	2.34 ^c ± 0.268
2022	<i>H. v. nigricans</i>	1.76 ^{de} ± 0.261	2.44 ^c ± 0.530
	<i>H. v. rimpau</i>	2.03 ^d ± 0.382	2.55 ^{bc} ± 0.421
	<i>H. vulgare</i>	1.46 ^e ± 0.253	2.08 ^c ± 0.560

^{a–e}—the mean values in columns with different letters are significantly different at the significance level $p < 0.05$. “±” relates to the standard deviation.

In 2021, the LAI of hooded barley (*H. v. var. rimpau*) was higher compared to *H. v. nigricans* and *H. vulgare* at the subflag leaf stage (BBCH 35) as well as at the heading stage (BBCH 57) (Table 2). In 2022, the LAI value of *H. v. var. rimpau* was significantly higher only when compared to ‘Soldo’ *H. vulgare* at the subflag leaf stage (BBCH 35) (Table 2). In 2022, the LAI of the compared black barley genotypes did not differ significantly in both analyzed development stages of barley.

The chlorophyll index (SPAD) measured in 2022 was, on average for genotypes, higher than in 2021 by 10.3% at the seedling stage, 36.1% at the subflag leaf stage, and 38.1% at the heading stage (Table 3).

Table 3. Chlorophyll index (SPAD) at seedling stage (BBCH 12), subflag leaf stage (BBCH 35), and heading stage (BBCH 57) of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpaui*, and *H. vulgare*.

Year	Genotype	SPAD at BBCH 12 Mean $\pm$ SD	SPAD at BBCH 35 Mean $\pm$ SD	SPAD at BBCH 57 Mean $\pm$ SD
2021	<i>H. v. nigricans</i>	345.9 ^b $\pm$ 75.6	310.3 ^c $\pm$ 7.83	457.4 ^b $\pm$ 21.76
	<i>H. v. rimpaui</i>	285.6 ^c $\pm$ 53.13	324.0 ^c $\pm$ 13.4	470.9 ^b $\pm$ 20.7
	<i>H. vulgare</i>	381.4 ^b $\pm$ 52.4	404.4 ^b $\pm$ 13.1	370.8 ^c $\pm$ 85.1
2022	<i>H. v. nigricans</i>	383.1 ^b $\pm$ 37.6	470.7 ^a $\pm$ 25.1	590.9 ^a $\pm$ 59.00
	<i>H. v. rimpaui</i>	352.3 ^b $\pm$ 46.4	456.7 ^a $\pm$ 29.4	571.5 ^a $\pm$ 47.1
	<i>H. vulgare</i>	465.9 ^a $\pm$ 35.9	486.3 ^a $\pm$ 56.3	590.1 ^a $\pm$ 48.9

^{a–c}—the mean values in columns with different letters are significantly different at the significance level $p < 0.05$. “ $\pm$ ” relates to the standard deviation.

In both in 2021 and 2022, *H. vulgare* at the seedling stage (BBCH 12) was characterized by higher values of the SPAD parameter than *H. v. var rimpaui* (Table 3). At the subflag leaf stage (BBCH 35) and the heading stage (BBCH 57), a genotype–year interaction was demonstrated ($p < 0.0001$; Supplementary Materials, Table S5). In 2021, SPAD at the subflag leaf stage (BBCH 35) was significantly higher in common barley (*H. vulgare*) as compared to *H. v. var. nigricans* and *H. v. rimpaui* (Table 3). In turn, measurement in the same year at BBCH 57 indicated significantly lower values of this index in *H. vulgare* as compared to black-seeded barley. SPAD analysis in 2022 at both plant development stages (BBCH 35 and BBCH 57) showed no significant differences between genotypes.

3.2. Biometric and Agronomic Features

In 2021, the amount of dry matter of shoots at the flowering stage of all analyzed genotypes was similar and significantly higher (by 28.5%) compared to 2022. The amount of accumulated shoot mass of alternative black barley genotypes (*H. v. var. nigricans* and *Hordeum v. var. rimpaui*) in 2022 was generally higher than the shoot biomass of *H. vulgare* cv. ‘Soldo’, the difference being statistically significant only for *H. v. rimpaui* (Table 4).

Table 4. Shoot biomass at the flowering stage, number of generative tillers, and sterile generative tillers of *H. vulgare* var. *nigricans*, *H. vulgare* var. *rimpaui*, and *Hordeum vulgare*.

Year	Genotype	Dry Matter of Shoot g m ^{−2}	Generative Tillers No Plant ^{−1}	Sterile Generative Tillers No m ^{−2}
2021	<i>H. v. nigricans</i>	728.2 ^a $\pm$ 98.5	2.05 ^{bc} $\pm$ 0.217	8.06 ^c $\pm$ 8.13
	<i>H. v. rimpaui</i>	776.8 ^a $\pm$ 129.5	2.16 ^{ab} $\pm$ 0.250	2.56 ^c $\pm$ 3.27
	<i>H. vulgare</i>	775.2 ^a $\pm$ 111.0	2.36 ^a $\pm$ 0.192	1.75 ^c $\pm$ 1.57
2022	<i>H. v. nigricans</i>	575.1 ^{bc} $\pm$ 79.4	1.68 ^d $\pm$ 0.208	103.44 ^a $\pm$ 46.32
	<i>H. v. rimpaui</i>	678.2 ^{ab} $\pm$ 145.9	1.87 ^{cd} $\pm$ 0.271	68.88 ^b $\pm$ 31.73
	<i>H. vulgare</i>	558.0 ^c $\pm$ 127.4	1.77 ^d $\pm$ 0.278	75.63 ^b $\pm$ 26.75

^{a–d}—the mean values in columns with different letters are significantly different at the significance level $p < 0.05$. “ $\pm$ ” relates to the standard deviation.

There were more generative tillers from plants of individual genotypes in 2021 than those in 2022 (Table 4). The analysis of the genotype $\times$ year interaction ($p = 0.0152$; Supplementary Materials, Table S6) showed that in 2021, cultivar ‘Soldo’ *H. vulgare* developed significantly more generative tillers per plant compared to *H. v. var. nigricans*, while in 2022, they were similar in the three compared genotypes.

The number of fertile generative tillers per m^2 in individual genotypes was similar in both years of the study and was an average of 684.2 in *H. v. var. nigricans*, 684.9 in *H. v. var. rimpaii*, and 695.3 in *H. vulgare* cv. ‘Soldo’ in the two years of the study (Figure 3). The analysis of the genotype $\times$ year interaction ($p = 0.0194$; Supplementary Materials, Table S6) showed that in 2021, the modern barley cultivar developed significantly more fertile generative tillers per m^{-2} compared to *H. v. var. nigricans*.

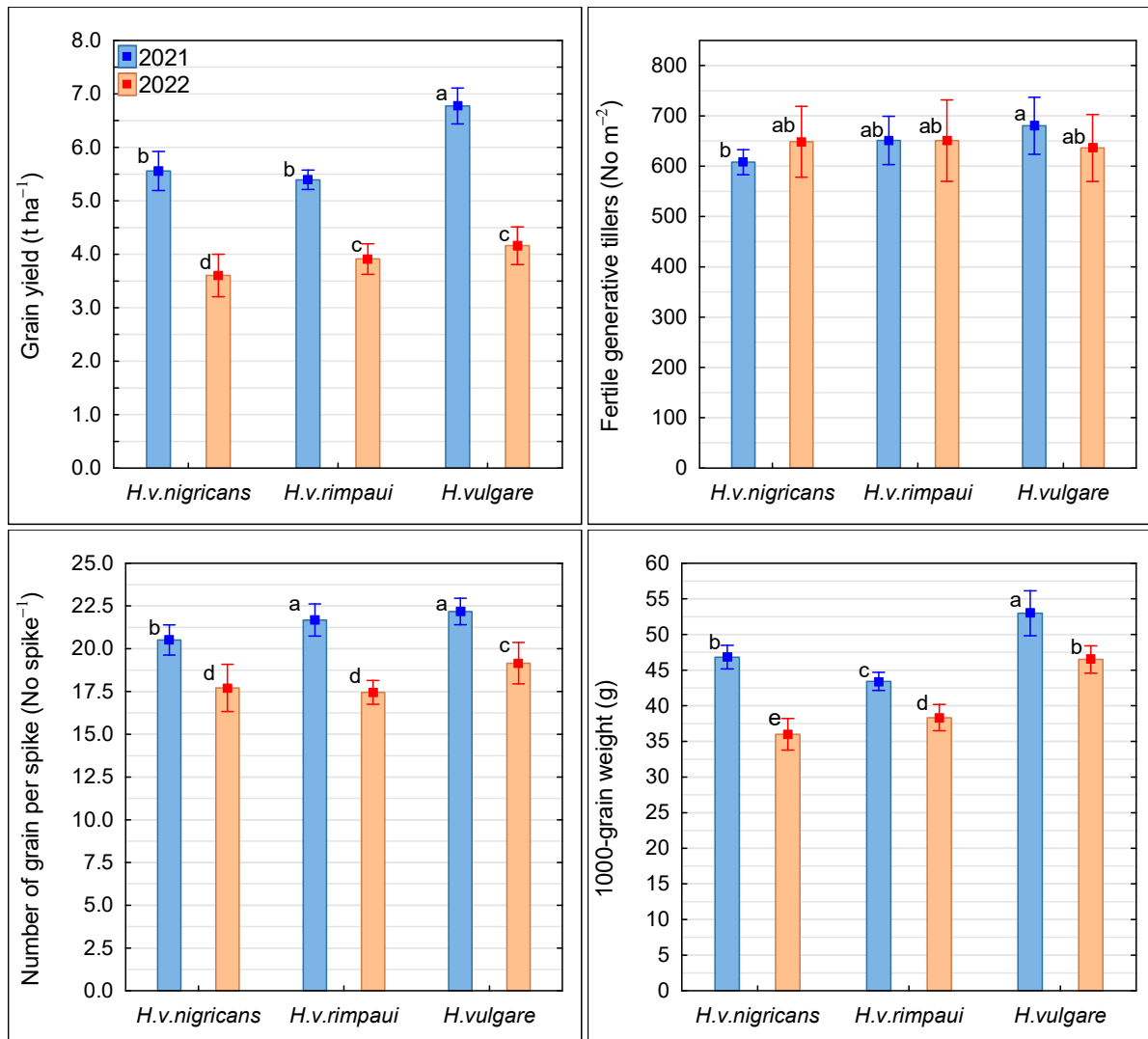


Figure 3. Grain yield and yield components of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpaii*, and *H. vulgare*. Error bars indicate standard deviation. a–e—mean values followed by different letters indicate significant differences $p < 0.05$.

In 2021, almost all generative tillers (99.4% on average) developed spikes with grains. In 2022, some of the generative tillers (11.4% on average) had empty spikes (without grains). An increase in the number of such tillers (sterile generative tillers per m^2) in 2022 compared to 2021 was recorded for each genotype. Primary barley *H. v. var. nigricans* usually developed more such tillers than other genotypes, but the difference was statistically significant only in 2022 (Table 4).

Dry matter of shoot and the number of generative tillers of *H. v. nigricans*, *H. v. rimpaii*, and *H. vulgare* showed lower variability in 2021 than in 2022 (Table 4). The coefficients of variation of the number of sterile generative tillers per m^2 were high, especially in 2021, when plants produced a small number of such tillers. In both years, alternative

barley genotypes were characterized by much greater variability in the number of sterile generative tillers than *H. vulgare*.

The number of grains per spike was significantly lower in 2022 than in 2021 (by 15.6% on average for genotypes). The genotype $\times$ year interaction ($p < 0.0001$; Supplementary Materials, Table S6) indicates that in 2021, the number of grains per spike of *H. v. var. rimpaii* and the modern cultivar ‘Soldo’ *H. vulgare* was similar and significantly higher compared to *H. v. var. nigricans* (Figure 3). However, in 2022, both alternative black barley genotypes (*H. v. var. nigricans* and *H. v. var. rimpaii*) developed significantly fewer grains per spike compared to *H. vulgare*.

The 1000-grain weight was lower for each of the tested genotypes in 2022 compared to that in 2021 (by 15.6% on average). A genotype–year interaction was thus demonstrated ($p < 0.0001$; Supplementary Materials, Table S6). In 2021, the 1000-grain weight of *H. v. var. nigricans* was higher than in *H. v. var. rimpaii*; in contrast, in 2022, the grain of *H. v. var. rimpaii* was characterized by a higher weight. In both years of the study, the 1000-grain weight in the modern cultivar ‘Soldo’ *H. vulgare* was higher than that of the alternative black barley genotypes (*H. v. var. nigricans* and *H. v. var. rimpaii*) (Figure 3).

For each genotype, the grain yield in 2021 was lower (by 34% on average) compared with that in 2022. The genotype $\times$ year interaction ($p < 0.0001$; Supplementary Materials, Table S6) indicated that in 2021, the modern cultivar ‘Soldo’ *H. vulgare* produced a significantly higher grain yield compared to the alternative black barley genotypes. However, in 2022, the grain yields of *H. v. var. rimpaii* and *H. vulgare* were similar and significantly higher than that of *H. v. var. nigricans* (Figure 3).

The straw yield as well as the grain yield of each genotype were lower in 2022 compared to that in 2021 (on average by 28%). In 2021, the straw mass of *H. v. var. rimpaii* was significantly higher than that of *H. v. var. nigricans*; however, in 2022 there were no significant differences in primary genotypes. In each year of the study, the straw yield was the lowest in the modern cultivar ‘Soldo’ *H. vulgare*.

A genotype–year interaction was demonstrated ($p = 0.0027$; Supplementary Materials, Table S6) as affecting the harvest index value. In 2021, it was significantly higher in comparison with 2022 in *H. vulgare* cv. ‘Soldo’ and *H. v. var. nigricans* (Table 5). In both years, the harvest index value in *H. vulgare* cv. ‘Soldo’ was significantly higher than in the primary genotypes. Only in 2021 was it significantly higher in *H. v. var. nigricans* than in *H. v. rimpaii*. There were no major genotypic differences in the coefficient of variation of straw yield or harvest index.

Table 5. Straw yield and harvest index of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpaii*, and *H. vulgare*.

Year	Genotype	Straw Yield t ha ^{−1}	Harvest Index
2021	<i>H. v. nigricans</i>	5.35 ^b $\pm$ 0.549	0.510 ^c $\pm$ 0.028
	<i>H. v. rimpaii</i>	5.87 ^a $\pm$ 0.415	0.478 ^d $\pm$ 0.015
	<i>H. vulgare</i>	4.69 ^c $\pm$ 0.511	0.591 ^a $\pm$ 0.028
2022	<i>H. v. nigricans</i>	3.84 ^d $\pm$ 0.268	0.484 ^d $\pm$ 0.032
	<i>H. v. rimpaii</i>	4.22 ^d $\pm$ 0.357	0.481 ^d $\pm$ 0.018
	<i>H. vulgare</i>	3.35 ^e $\pm$ 0.334	0.555 ^b $\pm$ 0.013

^{a–e}—the mean values in columns with different letters are significantly different at the significance level $p < 0.05$. “ $\pm$ ” relates to the standard deviation.

3.3. Relationship of Physiological, Biometric, and Agronomic Features

In our research, the correlations (Pearson’s coefficient) between chlorophyll fluorescence parameters (F_V/F_M , F_V/F_0 , and PI_{ABS}) and chlorophyll index (SPAD) were calculated (Table 6). The correlation coefficient for the SPAD and the PI_{ABS} parameter, both at the BBCH 35 and BBCH 57 stages, for all three genotypes was positive except for *H. v. rimpaii* at the BBCH 57 stage, for which the correlation was not significant.

Table 6. Relationship between chlorophyll index (SPAD) at subflag leaf stage (BBCH 35) and at heading stage (BBCH 57) and chlorophyll fluorescence parameters (F_V/F_M , F_V/F_0 , and PI_{ABS}) for *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpaui*, and *H. vulgare* (Pearson's correlation coefficients).

Traits		<i>H. v. nigricans</i>	<i>H. v. rimpaui</i>	<i>H. vulgare</i>
SPAD				
BBCH 35	F_V/F_M	0.902 *	0.292	−0.007
	F_V/F_0	0.884 *	0.261	−0.275
	PI_{ABS}	0.925 *	0.853 *	0.381 *
SPAD				
BBCH 57	F_V/F_M	−0.585 *	−0.620 *	0.240
	F_V/F_0	−0.738 *	−0.752 *	0.615 *
	PI_{ABS}	0.488 *	−0.314	0.677 *

*—significant at the level of $p < 0.05$.

The relationship between the F_V/F_M and F_V/F_0 parameters and SPAD varied among genotypes. In both black-seeded barley genotypes at the BBCH 57 stage, the correlation for F_V/F_M and F_V/F_0 and SPAD was negative (Table 6). However, measurement at the earlier stage (BBCH 35) indicated a significant, positive correlation between these indices only in *H. v. nigricans*.

Correlation coefficients were also calculated for the leaf area index (LAI), biometric and agronomic features, and grain yield of each genotype (Table 7). The LAI parameter, determined at the subflag leaf stage (BBCH 35), showed a significantly strong, positive correlation with the grain yield of all analyzed genotypes. However, at the later stage of plant development (BBCH 57), this correlation was significant only for alternative barley genotypes.

Table 7. Relationship between grain yield and leaf area index (LAI) and biometric and agronomic features of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpaui*, and *H. vulgare* (Pearson's correlation coefficients).

Traits	<i>H. v. var. nigricans</i>	<i>H. v. var. rimpaui</i>	<i>H. vulgare</i>
LAI at BBCH 35	0.779 *	0.917 *	0.737 *
LAI at BBCH 57	0.469 *	0.561 *	0.221
Shoot biomass at the flowering stage (g m^{-2})	0.667 *	0.378 *	0.711 *
Fertile generative tillers (No m^{-2})	−0.248	−0.011	0.364 *
1000-grain weight (g)	0.917 *	0.852 *	0.827 *
Number of grains per spike (No spike^{-1})	0.834 *	0.722 *	0.915 *

*—significant at the level of $p < 0.05$.

A positive correlation between the shoot biomass at the flowering stage and the final grain yield was shown for both black-seeded barley and *H. vulgare*, although these relationships were moderately strong (Table 7). Calculated correlation coefficients for the main yield components, such as 1000-grain weight and the number of grains per spike for *H. v. var. nigricans*, *H. v. var. rimpaui*, and *H. vulgare* show a significantly strong or very strong positive relationship between these parameters and grain yield. The number of fertile generative tillers per m^2 , for which the only significant, weak correlation with grain yield was shown for common barley, is distinctive among the yield structure components (Table 7).

3.4. Principal Component Analysis (PCA)

In order to evaluate the correlations between genotypes and analyzed parameters such as chlorophyll fluorescence, LAI, SPAD, or agronomic and biometric parameters, a principal component analysis (PCA) was conducted. For the seedling stage (BBCH 12) analysis, the biplot PCA explained 76.32% of the first principal component (PC1) and 23.68% of the second principal component (PC2) from 100% of total variance. For the analysis at subflag leaf stage (BBCH 35) and heading stage (BBCH 57) from the 100% of total variance, the PC1 defined 74.66% and PC2 25.34% (Figure 4A,B). The variances obtained for PC2, both in the seedling stage and in BBCH 35 or BBCH 57, were influenced to the greatest extent by the PI parameter (Figure 4A,B). During the analyses of plants in the seedling stage, the area parameter had the greatest impact on the PC1 variance, while in the later measurements, the parameters F_V/F_M (BBCH 57) and F_V/F_0 (BBCH 57) had the greatest contribution to the variance for PC1 (Figure 4A,B).

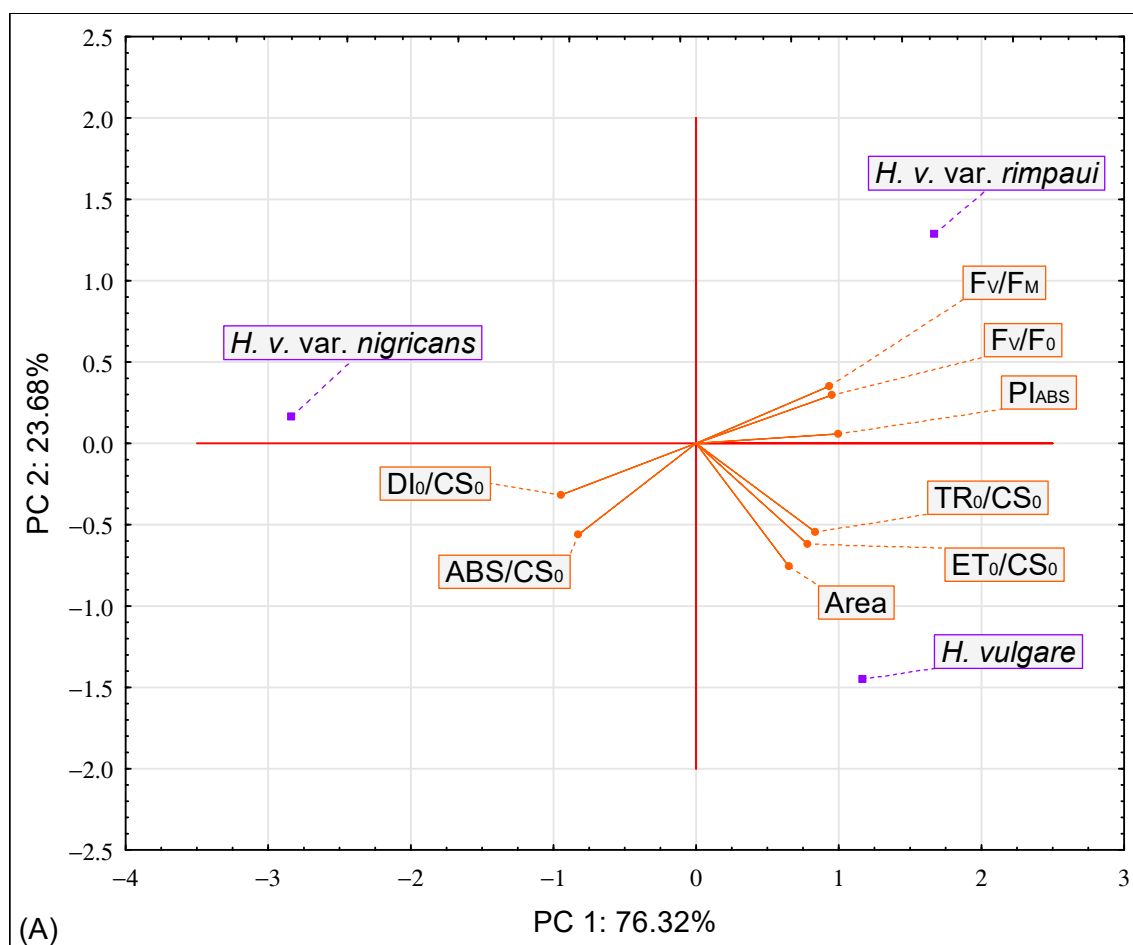


Figure 4. Cont.

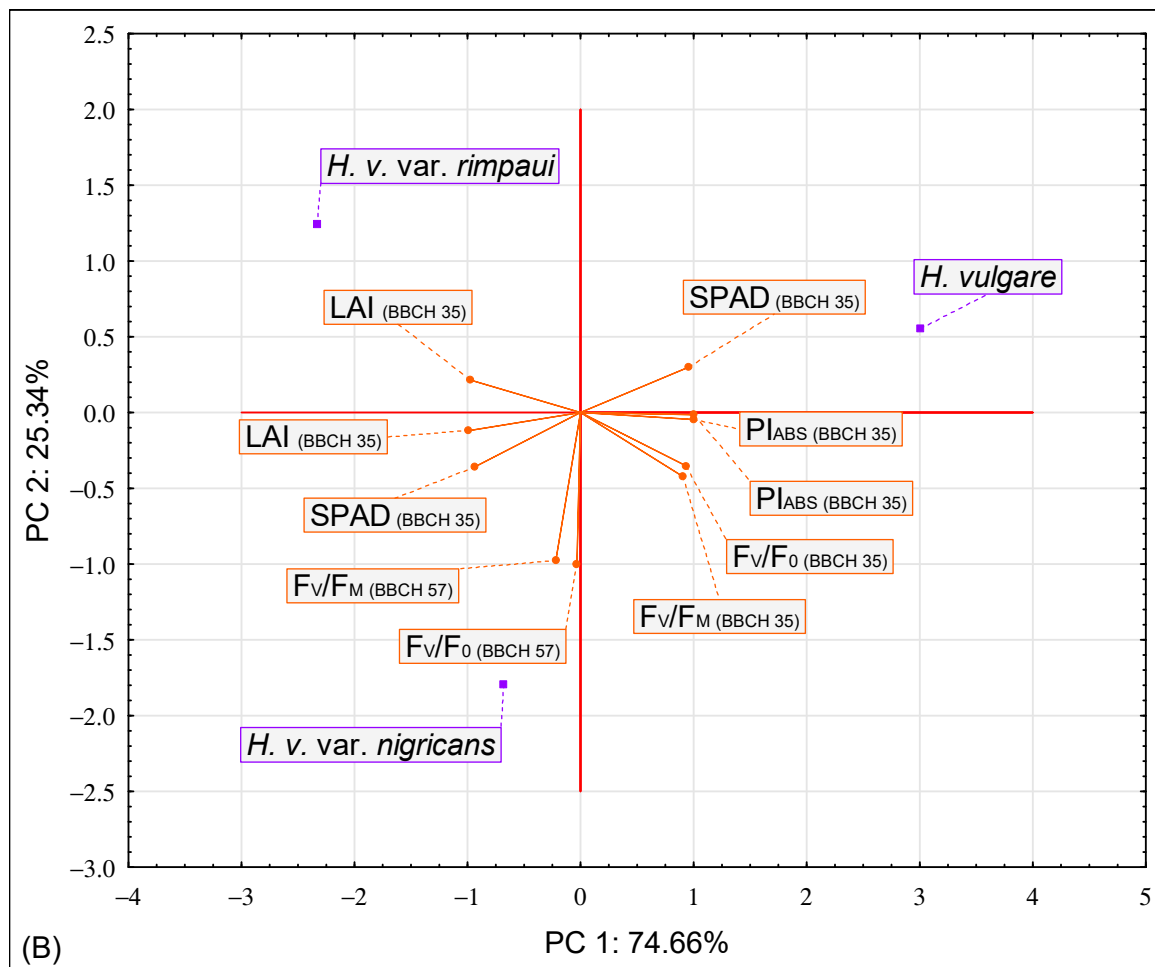


Figure 4. Biplot principal component analysis (PCA) of fluorescence parameters LAI and SPAD of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpai*, and *H. vulgare* at (A) seedling stage (BBCH 12), (B) subflag leaf stage BBCH (35), and heading stage BBCH (57).

In both biplots, the angles between F_V/F_M and F_V/F_0 at each measurement time are very narrow, which indicates their strong correlation (Figure 4A,B). In Figure 4A, narrow angles can also be observed between TR_0/CS_0 , ET_0/CS_0 , and area, but in Figure 4B a strong correlation appears between PI_{ABS} measured in BBCH 35 and PI_{ABS} in BBCH 57. The other directions and wide angles between DI_0/CS_0 and ABS/CS_0 or $LAI_{(BBCH\ 35)}$ and $SPAD_{(BBCH\ 35)}$ suggest they are not correlated with the rest of the dependents.

In the PCA analysis, most parameters (F_V/F_M , F_V/F_0 , PI_{ABS} , TR_0/CS_0 , ET_0/CS_0 , and area) at the seedling stage (BBCH 12) and $SPAD_{(BBCH\ 35)}$, F_V/F_M , F_V/F_0 , and PI_{ABS} all in both growing stages (BBCH 35 and BBCH 57) are better described by PC1. On the contrary, DI_0/CS_0 and ABS/CS_0 at BBCH 12 as well as $LAI_{(BBCH\ 35)}$, $LAI_{(BBCH\ 57)}$, and $SPAD_{(BBCH\ 57)}$ are better represented by PC2.

At the seedling stage, *H. v. var. rimpai* was characterized well by F_V/F_M , F_V/F_0 , and PI_{ABS} and the common barley by TR_0/CS_0 , ET_0/CS_0 , and area. On the other hand, at subsequent measurement dates, the biplot had a different layout. Hooded barley was distinguished only by LAI at BBCH 35. Common barley was characterized well by SPAD at the subflag leaf stage and slightly by PI_{ABS} at the subflag and flag leaf stages; however, *H. v. nigricans* was mainly described by F_V/F_M , F_V/F_0 , and SPAD at the flag leaf stage (BBCH 57).

The total variance of PCA for agronomic and biometric parameters was described 100% by PC1 (67.11%) and PC2 (32.89%). The variance obtained for PC2 was influenced to the greatest extent by the parameter of number of grains per spike and the variance for

PC1 by dry matter of shoot (Figure 5). Very narrow angles between grain yield, 1000-grain weight, and harvest index of the fertile generative tillers and generative tillers per plant indicated their high correlation (Figure 5).

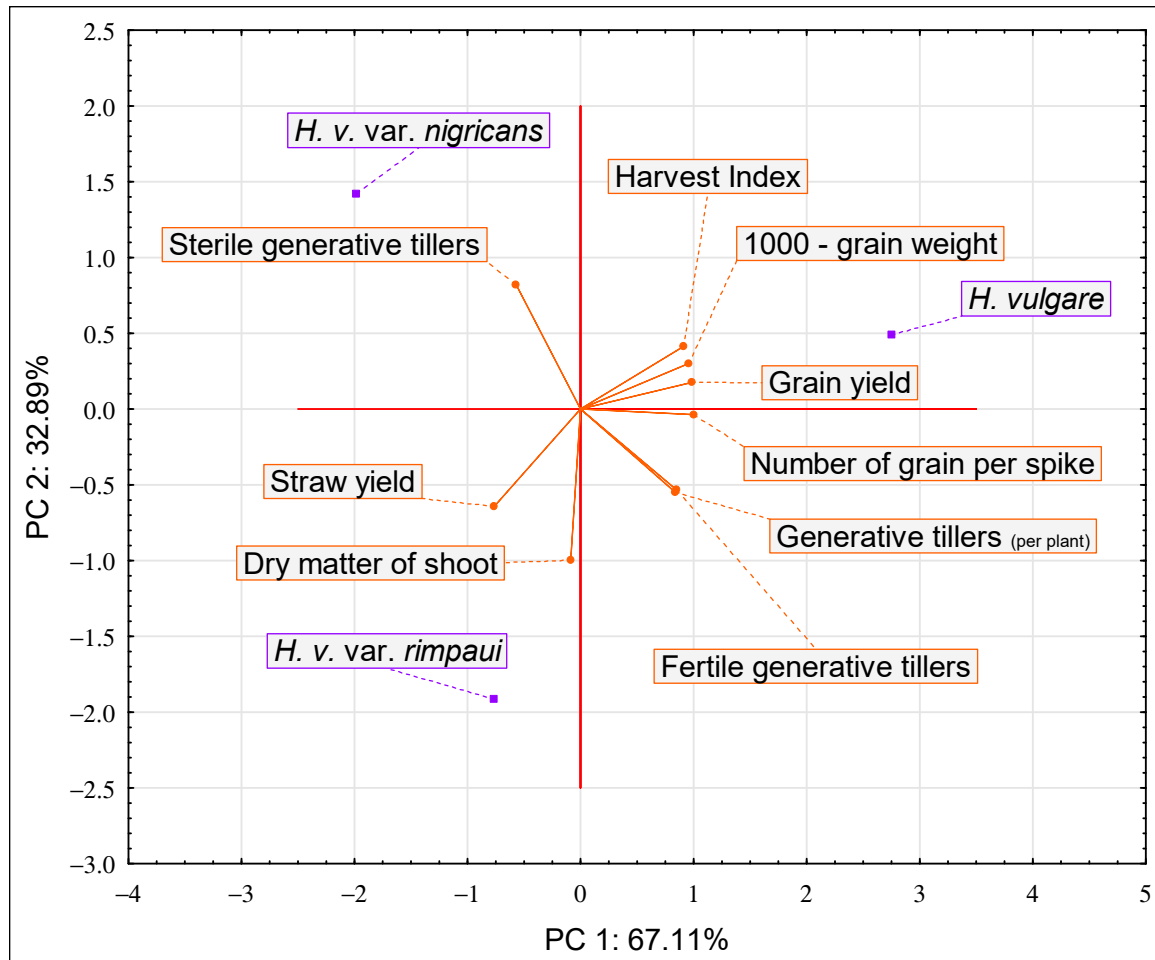


Figure 5. Biplot principal component analysis (PCA) of agronomic and biometric parameters of *Hordeum vulgare* var. *nigricans*, *H. vulgare* var. *rimpai*, and *H. vulgare*.

The wide angles and other directions between straw yield and dry matter of shoot or sterile generative tillers suggested they had no relationship with other dependents (Figure 5). Straw yield and sterile generative tillers were the only dependents described better by PC1; the rest of the analyzed parameters were described mainly by PC2. In the PCA, it was shown that *H. vulgare* was characterized by the high grain yield, 1000-grain weight, and harvest index. However, *H. v. var. nigricans* was distinguished by high dry matter of shoot and straw yield and *H. v. var. rimpai* by the increased number of sterile generative tillers.

4. Discussion

Our research showed a diverse response of chlorophyll fluorescence indicators to the hydrothermal conditions of the growing seasons. The assessment of these parameters at the seedling stage indicated the lower-efficiency photosynthesis of barley in 2021. This could have resulted from negative temperatures during germination and emergence (Supplementary Materials, Table S2). All parameters related to the performance of photosystem II (PS II), including F_V/F_M , F_V/F_0 , and PI_{ABS} , were indicative of plant stress. There were no significant differences between genotypes in these parameters. According to Elakhdar et al. [31], young barley plants exposed to negative temperatures exhibited significantly

reduced PS II performance, which was manifested by lower F_V/F_0 and F_V/F_M values. However, our research showed that in 2021, *H. v. var. rimpai* was characterized by higher values of ET_0/CS_0 , TR_0/CS_0 , and ABS/CS_0 (indices related to photosynthetic electron transport and absorption) than *H. v. var. nigricans*. This might indicate a slightly higher tolerance of this genotype to stress associated with the occurrence of negative temperatures. Efficient electron transport may reduce the risk of damage from the overexcitation of centers and due to a lower amount of antenna chlorophyll [32].

In 2022, during the period of measurements on seedlings (BBCH 12), air temperatures were not as low as in 2021, which excluded the occurrence of strong thermal stress in plants. However, barley seedlings could have been exposed to water stress associated with extremely low rainfall (Figure 1). The occurrence of drought in this period was also indicated by the very low hydro-thermal coefficient of Sielianinow in April (Supplementary Materials, Table S1). Hydrothermal conditions in 2022 did not disturb the functioning of PS II as strongly as those in 2021, which is indicated by the much higher values of most chlorophyll fluorescence parameters. However, the response of the plants depended significantly on the genotype. Parameters showing the function of PS II indicated that *H. v. rimpai* showed greater tolerance to water deficiency at the seedling stage than the other genotypes. This genotype was characterized by significantly higher values of the F_V/F_0 , F_V/F_M , and PI_{ABS} parameters. Similar results in terms of PS II efficiency in barley seedlings subjected to drought stress were obtained by Oukarroum et al. [33]. According to that study, the PI_{ABS} index was differentiated by changing humidity conditions, as drought could affect various stages of the photosynthesis process.

In our research, values of the F_V/F_M and F_V/F_0 for the subflag and flag leaves significantly differed depending on the genotype and year of the study (Table 1). However, it was noticed that all the results oscillated around the values considered appropriate for non-stressed plants [24]. When analyzing the PI_{ABS} of the subflag and flag leaves (Table 1), higher values were usually observed in 2022 than in 2021, although 2022 was less favorable for the growth and yield of barley (Figures 1 and 3; Supplementary Materials, Table S1). Higher PI_{ABS} values in 2022 were most likely due to the hydrothermal conditions at the dates of the measurements. In 2022 (Supplementary Materials, Table S1), at the first and second measurement dates (3rd week of May for subflag leaf and 2nd of June for flag leaf), the weather conditions were optimal or close to optimal for plants (Sielianinow coefficient 1.16 and 1.32, respectively). However, in 2021, the Sielianinow coefficients at both measurement dates (1st week of June for subflag leaf and 2nd of June for flag leaf) indicated the occurrence of water stress (Sielianinow coefficient 0.50 and 0.46, respectively) [30].

In our study, the chlorophyll index (SPAD value) depended mainly on the development phase of plants and the hydrothermal conditions of the growing season. Measurements taken at the seedling stage, when the young leaves were starting to develop, showed a lower value than measurements taken on the flag leaf (Table 3). This parameter varied depending on the genotype, but the year of study seemed to have a greater impact on the SPAD value, which was higher in 2022 than in 2021. In 2022, where the weather conditions during the growing season were less favorable, plants produced less leaf area (Table 2) and total biomass (Table 4), which in turn contributed to a higher concentration of chlorophyll molecules in leaves and higher SPAD values. A similar view was presented by Hasanuzzama et al. [34] in their research on barley and drought stress.

In our study, an analysis of the correlation between SPAD and chlorophyll fluorescence parameters was presented (Table 6), which is a novel approach and not commonly presented in the literature so far. Generally, a significant, positive relationship was found between the PI_{ABS} index and the SPAD value for the three analyzed genotypes in both measurement growing stages (BBCH 35 and BBCH 57). The relationship allowed the conclusion that with an increase in the concentration of chlorophyll molecules on the leaf surface, the unit efficiency of photosynthesis increases, which is described by the PI_{ABS} parameter, a commonly used indicator during abiotic stresses [35].

In years with favorable weather conditions for plant productivity (in our study, 2021) (Figure 1), the chlorophyll content may be lower; however, it is usually compensated by a larger leaf area, which allows to maintain high productivity [36]. Our results are in line with this view, as we observed a higher leaf area ratio (LAI) in 2021 than in 2022 for the genotypes analyzed (Table 2).

In 2021, at both stages of development (BBCH 37 and 57), common barley cv. ‘Soldo’ had a lower LAI than *H. v. var. rimpaii* and *H. v. var. nigricans* (Table 2). This finding corroborated with Alqudah et al.’s [37] findings that lower LAI values in modern cereal varieties were due to plant breeding. One of the goals of these processes was to grow plants that produce shorter and narrower leaves for better adaptation to different environmental conditions. *H. v. rimpaii* and *H. v. nigricans*, as representatives of ancient cereals, have not undergone any improvement and are characterized by a higher LAI, especially hooded barley. However, under less favorable weather conditions (as in 2022 in our study), black-seeded barley does not develop a significantly larger leaf area (LAI), which in drought stress allows for reducing evapotranspiration [38].

The leaf area index (LAI), which is the area of green leaves per unit area, on the agronomic level, is analyzed primarily in terms of its impact on the final yield [39]. Our study showed a significant strong correlation between the leaf area index and grain yield for all three barley genotypes tested (Table 7), mainly at the stage of intensive development of photosynthetic organs and accumulation of aboveground biomass (BBCH 35). On the other hand, the lack of a relationship between grain yield and LAI in the later stages of common barley development may also be explained by the breeding process. One of the biggest milestones in cereal breeding was the introgression of *Rht* genes, which are responsible for shortening the height of the straw [40]. In previous studies, it was observed that cereals with active *Rht* genes (the majority of modern wheat and barley genotypes) have smaller leaf areas, but the photosynthetic capacity is even higher than among wild grains [41]. The implementation of these genes into plants also affected other traits. One of the primary effects was a reduction in straw yield and shoot biomass, which agreed with our results (Tables 4 and 5). It is also believed that *Rht* genes have an influence on kernels by increasing their weight, which in turn increases the final grain yield. Besides the higher grain yield and lower straw yield, the harvest index (HI) is also a parameter that benefited from the shortening process [42], which in our research was significantly higher for *H. vulgare* than for alternative genotypes of barley (Table 5). In addition, some studies indicated that the *Rht* genes affected the number of grains per spike by reducing the demand for assimilates by shoots and by increasing the transport of assimilates from shoots to developing spikes, resulting in the higher fertility of florets [43]. However, in 2021, there was no significant difference in the number of grains per spike between common barley and *H. v. var. rimpaii* (the primary form of barley) (Figure 3). This indicated the ability of some primary genotypes to produce a similar number of grains per spike as the modern barley.

Common barley to a greater extent has genetically determined production potential in favorable weather conditions (2021), mainly due to the increase in 1000-grain weight compared to both primary genotypes as well as the number of grain per spike and fertile generative tillers per m² compared to *H. v. var. nigricans* (Figure 5). In turn, no differences in grain yield in 2022 between *H. v. var. rimpaii* and *H. vulgare* were found, which might be related to the number of fertile generative tillers per m² and indirectly through generative tillers per plant (Table 4, Figures 4 and 5). It is worth noticing that in unfavorable weather conditions (2022), no differences were found between any of the analyzed genotypes (primary and modern) in the number of fertile generative tillers per m². This is in line with Yessen et al. [44], who claimed that black barley growing under low soil matric potential (which points to low water availability for plants) produced even more tillers than *H. vulgare*.

In our research, some generative tillers developed spikes without kernels (sterile generative tillers) (Table 4). The number of such tillers was closely related to environmental

conditions. The largest number of generative tillers remained unproductive under the rainfall deficit in 2022. This is in line with previous research [45], which showed an increase in the number of sterile generative tillers in spring wheat under drought during the growing season. In the current study, *H. v. var. nigricans* was observed to have the largest number of spikes without kernels (Table 4, Figure 5). According to Pecio et al. [46], spring barley genotypes differed in their responses to water deficiency, resulting in a different number of spikes with fully developed grains.

5. Conclusions

At the seedling stage, *H. v. var. rimpai* was characterized by higher chlorophyll fluorescence parameters such as ET_0/CS_0 , TR_0/CS_0 , and ABS/CS_0 than *H. v. var. nigricans*. This may indicate greater tolerance to stress associated with negative temperatures. Moreover, seedlings of this barley genotype had increased parameters, showing the functioning of PS II (F_V/F_0 , F_V/F_M), which might indicate increased tolerance to water deficiency. In turn, in the flag leaf stage, under conditions of periodic water shortages, higher abiotic stress indicators (performance index (PI_{ABS}) and F_V/F_0) were shown in *H. v. var. nigricans* compared to *H. v. var. rimpai*.

Common barley made greater use of its genetically determined production potential in favorable weather conditions (2021), mainly due to the increase in 1000-grain weight compared to both black barley genotypes as well as the number of grain per spike and fertile generative tillers per m^2 compared to *H. v. var. nigricans*.

Further studies should focus on the response of black barley genotypes to induced abiotic stresses at the seedling stage of *H. v. var. rimpai* and at the flag leaf stage of *H. v. var. nigricans*. However, in agronomic practice, the number of grains per spike and 1000-grain weight should be increased.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture13102033/s1>, Table S1: Sielaninov index (hydrothermal coefficient) from April to June 2021 and 2022 (recorded at the agrometeorological station in Minikowo, Poland); Table S2: Meteorological data from 11 to 30 of April 2021 and 2022; Table S3: Analysis of variance ANOVA for chlorophyll fluorescence at seedling stage of barley genotypes (*H. vulgare* var. *nigricans*, *H. vulgare* var. *rimpai*, and *H. vulgare*), growing in 2021 and 2022; Table S4: Analysis of variance ANOVA for chlorophyll fluorescence at subflag leaf stage (BBCH 35) and heading stage (BBCH 57) of barley genotypes (*H. vulgare* var. *nigricans*, *H. vulgare* var. *rimpai*, and *H. vulgare*), growing in 2021 and 2022; Table S5: Analysis of variance (ANOVA) for SPAD and LAI at seedling stage (BBCH 12), at subflag leaf stage (BBCH 35), and heading stage (BBCH 57) of barley genotypes (*H. vulgare* var. *nigricans*, *H. vulgare* var. *rimpai*, and *H. vulgare*), growing in 2021 and 2022; Table S6: Analysis of variance (ANOVA) for grain yield, straw yield, harvest index, shoot biomass at the flowering stage, number of grains per spike, 1000-grain weight, generative tillers ($No\ Plant^{-1}$), fertile generative tillers ($No\ m^{-2}$), and sterile generative tillers ($No\ m^{-2}$) of barley genotypes (*H. vulgare* var. *nigricans*, *H. vulgare* var. *rimpai*, and *H. vulgare*), growing in 2021 and 2022.

Author Contributions: Conceptualization, M.S.; methodology, M.S. and R.N.; software, K.B.; validation, M.S., R.N. and K.B.; formal analysis, M.S.; investigation, R.N. and M.S.; resources, M.S.; data curation, R.N.; writing—original draft preparation, M.S., K.B. and R.N.; writing—review and editing, M.S., K.B. and R.N.; visualization, K.B.; supervision, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Dai, F.; Nevo, E.; Wu, D.; Comadran, J.; Zhou, M.; Qiu, L.; Chen, Z.; Beiles, A.; Chen, G.; Zhang, G. Tibet is one of the centers of domestication of cultivated barley. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 16969–16973. [\[CrossRef\]](#)
- Haas, M.; Schreiber, M.; Mascher, M. Domestication and crop evolution of wheat and barley: Genes, genomics, and future directions. *J. Integr. Plant Biol.* **2019**, *61*, 204–225. [\[CrossRef\]](#)
- Food and Agriculture Organization of the United Nation. FAOSTAT: Crops and Livestock Products. Available online: <https://www.fao.org/faostat/en/#data/QCL> (accessed on 1 June 2022).
- Gong, L. Barley. In *Bioactive Factors and Processing Technology for Cereal Foods*; Wang, J., San, B., Tsao, R., Eds.; Springer: Berlin, Germany, 2019.
- Gordeeva, E.I.; Glagoleva, A.Y.; Kukoeva, T.V.; Khlestkina, E.K.; Shoeva, O.Y. Purple-grained barley (*Hordeum vulgare* L.): Marker-assisted development of NILs for investigating peculiarities of the anthocyanin biosynthesis regulatory network. *BMC Plant Biol.* **2019**, *19*, 49–57. [\[CrossRef\]](#)
- Strygina, K.V.; Börner, A.; Khlestkina, E.K. Identification and characterization of regulatory network components for anthocyanin synthesis in barley aleurone. *BMC Plant Biol.* **2017**, *17*, 184. [\[CrossRef\]](#)
- Shoeva, O.Y.; Mock, H.-P.; Kukoeva, T.V.; Börner, A.; Khlestkina, E.K. Regulation of the Flavonoid Biosynthesis Pathway Genes in Purple and Black Grains of *Hordeum vulgare*. *PLoS ONE* **2016**, *11*, e0163782. [\[CrossRef\]](#) [\[PubMed\]](#)
- Özberk, F.; Özberk, I.; Bayhan, M.; Odabaşoğlu, C. Black Barley Marketing Prices and Profitability vs. White from 2005 to 2015 in South-East Anatolia. *Transylv. Rev.* **2015**, *XXIV*, 1–10.
- Idehen, E.; Tang, Y.; Sang, S. Bioactive phytochemicals in barley. *J. Food Drug Anal.* **2017**, *25*, 148–161. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sakellariou, M.; Mylona, P.V. New Uses for Traditional Crops: The Case of Barley Biofortification. *Agronomy* **2020**, *10*, 1964. [\[CrossRef\]](#)
- Horvat, D.; Šimić, G.; Drezner, G.; Lalić, A.; Ledencan, T.; Tucak, M.; Plavšić, H.; Andrić, L.; Zdunić, Z. Phenolic acid profiles and antioxidants activity of major cereal crops. *Antioxidants* **2020**, *9*, 527. [\[CrossRef\]](#)
- Panizo-Casado, M.; Déniz-Expósito, P.; Rodríguez-Galdón, B.; Afonso-Morales, D.; Ríos-Mesa, D.; Díaz-Romero, C.; Rodríguez-Rodríguez, E.M. The chemical composition of barley grain (*Hordeum vulgare* L.) landraces from the Canary Islands. *J. Food Sci.* **2020**, *85*, 1725–1734. [\[CrossRef\]](#)
- Kim, M.J.; Hyun, J.N.; Kim, J.A.E.; Park, J.C.; Kim, M.Y.; Kim, J.G.; Lee, S.J.; Chun, S.C.; Chung, I.M. Relationship between Phenolic Compounds, Anthocyanins Content and Antioxidant Activity in Colored Barley Germplasm. *J. Agric. Food Chem.* **2007**, *55*, 4802–4809. [\[CrossRef\]](#)
- Abdel-Aal, E.S.M.; Choo, T.M.; Dhillon, S.; Rabalski, I. Free and bound phenolic acids and total phenolics in black, blue, and yellow barley and their contribution to free radical scavenging capacity. *Cereal Chem.* **2012**, *89*, 198–204. [\[CrossRef\]](#)
- Yang, X.J.; Dang, B.; Fan, M.T. Free and bound phenolic compound content and antioxidant activity of different cultivated blue highland barley varieties from the Qinghai-Tibet Plateau. *Molecules* **2018**, *23*, 879. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nowak, R.; Szczepanek, M.; Kobus-Cisowska, J.; Stuper-Szablewska, K.; Dziedziński, M.; Błaszczuk, K. Profile of phenolic compounds and antioxidant activity of organically and conventionally grown black-grain barley genotypes treated with biostimulant. *PLoS ONE* **2023**, *18*, e0288428. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nowak, R.; Szczepanek, M.; Błaszczuk, K.; Kobus-Cisowska, J.; Przybylska-Balcerek, A.; Stuper-Szablewska, K.; Pobereżny, J.; Hassanpouraghdam, M.B.; Rasouli, F. 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* **2023**, *13*, 1852. [\[CrossRef\]](#)
- Sánchez-Reinoso, A.D.; Ligarreto-Moreno, G.A.; Restrepo-Díaz, H. Chlorophyll α Fluorescence Parameters as an Indicator to Identify Drought Susceptibility in Common Bush Bean. *Agronomy* **2019**, *9*, 526. [\[CrossRef\]](#)
- Faseela, P.; Sinisha, A.K.; Brestić, M.; Puthur, J.T. Chlorophyll α fluorescence parameters as indicators of a particular abiotic stress in rice. *Photosynthetica* **2019**, *57*, 108–115. [\[CrossRef\]](#)
- Roostaeia, M.; Mohammadi, S.A.; Amri, A.; Majidi, E.; Nachit, M.; Haghparast, R. Chlorophyll Fluorescence Parameters and Drought Tolerance in a Mapping Population of Winter Bread Wheat in the Highlands of Iran. *Russ. J. Plant Physiol.* **2011**, *58*, 351–358. [\[CrossRef\]](#)
- Singh, H.; Kumar, D.; Soni, V. Performance of chlorophyll α fluorescence parameters in Lemna minor under heavy metal stress induced by various concentration of copper. *Sci. Rep.* **2022**, *12*, 10620. [\[CrossRef\]](#)
- Roháček, K. Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica* **2002**, *40*, 13–29. [\[CrossRef\]](#)
- He, L.; Yu, L.; Li, B.; Du, N.; Guo, S. The effect of exogenous calcium on cucumber fruit quality, photosynthesis, chlorophyll fluorescence, and fast chlorophyll fluorescence during the fruiting period under hypoxic stress. *BMC Plant Biol.* **2018**, *18*, 180. [\[CrossRef\]](#)
- Luo, H.; Merope, T.; Zhang, Y.; Zhang, W. Combining gas exchange and chlorophyll α fluorescence measurements to analyze the photosynthetic activity of drip-irrigated cotton under different soil water deficits. *J. Integr. Agric.* **2016**, *15*, 1256–1266. [\[CrossRef\]](#)
- Zhang, R.; Yang, P.; Liu, S.; Wang, C.; Liu, J. Evaluation of the Methods for Estimating Leaf Chlorophyll Content with SPAD Chlorophyll Meters. *Remote Sens.* **2022**, *14*, 5144. [\[CrossRef\]](#)
- 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.* **2012**, *95*, 83–89.

27. Fang, H.; Baret, F.; Plummer, S.; Schaepman-Strub, G. An overview of global leaf area index (LAI): Methods, products, validation, and applications. *Rev. Geophys.* **2019**, *57*, 739–799. [\[CrossRef\]](#)
28. Gozdowski, D.; Mądry, W.; Wyszynski, Z.; Kalinowska-Zdun, M. Characteristics and empirical comparison of simple and complex path analysis in grain yield determination by yield—Related traits. Part II. Example on spring barley. *Biul. Inst. Hod. I Aklim. Roślin* **2008**, *249*, 125–132.
29. Selyaninov, G.T. On the agricultural estimation of climate. *Tr. Po Sel'skokhozyaistvennoi Meteorol.* **1928**, *20*, 165–177.
30. Kuklik, M.; Baryła, R.; Czarnecki, Z.; Bochniak, A. Warunki hydrotermiczne w centralnej części rejonu kanału Wieprz-Krzna w 50-leciu (1966–2015). *Ann. UMCS Sect. E Agric.* **2016**, *LXXI*, 1–12.
31. Elakhdar, A.; Slaski, J.; Kubo, T.; Hamwieh, A.; Hernandez-Ramirez, G.; Beattie, A.D.; Capo-Chichi, L.J.A. Genome—Wide association analysis provides insights into the genetic basis of photosynthetic responses to low-temperature stress in spring barley. *Front. Plant Sci.* **2023**, *14*, 1159016. [\[CrossRef\]](#)
32. Watanabe, N.; Naruse, J.; Austin, B.; Morgan, C.L. Variation in thylakoid proteins and photosynthesis in Syrian landraces of barley. *Euphytica* **1995**, *82*, 213–220. [\[CrossRef\]](#)
33. Oukarroum, A.; El Madidi, S.; Schansker, G.; Strasser, R.J. Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering. *Environ. Exp. Bot.* **2007**, *60*, 438–446. [\[CrossRef\]](#)
34. Hasanuzzaman, M.; Shabala, L.; Brodribb, T.J.; Zhou, M.; Shabala, S. Understanding the Role of Physiological and Agronomical Traits during Drought Recovery as a Determinant of Differential Drought Stress Tolerance in Barley. *Agronomy* **2022**, *12*, 2136. [\[CrossRef\]](#)
35. Arab, M.M.; Askari, H.; Aliniaefard, S.; Mokhtassi-Bidgoli, A.; Estaji, A.; Sadat-Hosseini, M.; Sohrabi, S.S.; Mesgaran, M.B.; Leslie, C.A.; Brown, P.J.; et al. Natural variation in photosynthesis and water use efficiency of locally adapted Persian walnut populations under drought stress and recovery. *Plant Physiol. Biochem.* **2023**, *201*, 107859. [\[CrossRef\]](#)
36. Eliş, S.; Yıldırım, M. Yield and quality changes in barley genotypes during high temperature stress caused by late sowing. *Rom. Agric. Res.* **2023**, *40*, 139–150. [\[CrossRef\]](#)
37. Alqudah, A.M.; Schnurbusch, T. Barley Leaf Area and Leaf Growth Rates Are Maximized during the Pre-Anthesis Phase. *Agronomy* **2015**, *5*, 107–129. [\[CrossRef\]](#)
38. Jia, Q.; Wang, Y.-P. Relationships between Leaf Area Index and Evapotranspiration and Crop Coefficient of Hilly Apple Orchard in the Loess Plateau. *Water* **2021**, *13*, 1957. [\[CrossRef\]](#)
39. Liu, X.-J.; Qiang, C.; Yuan, Z.-F.; Xia, L.; Wang, X.-L.; Tian, Y.-C.; Cao, W.-X.; Yan, Z. Leaf area index based nitrogen diagnosis in irrigated lowland rice. *J. Integr. Agric.* **2018**, *17*, 111–121. [\[CrossRef\]](#)
40. Wu, K.; Wang, S.; Song, W.; Zhang, J.; Wang, Y.; Liu, Q.; Yu, J.; Ye, Y.; Li, S.; Chen, J.; et al. Enhanced sustainable green revolution yield via nitrogen-responsive chromatin modulation in rice. *Science* **2020**, *367*, eaaz2046. [\[CrossRef\]](#)
41. Moregan, J.A.; LeCain, D.R.; Wells, R. Semi-dwarfing genes concentrate photosynthetic machinery and affect leaf gas exchange of wheat. *Crop Sci.* **1990**, *30*, 602–608. [\[CrossRef\]](#)
42. Ukozehasi, C.; Ober, E.S.; Griffiths, H. The Other Mechanisms by Which the Rht Genes Improve the Harvest Index of Wheat. *Plants* **2022**, *11*, 2837. [\[CrossRef\]](#)
43. Youssefian, S.; Kirby, E.J.M.; Gale, M.D. Pleiotropic effects of the GA insensitive Rht dwarfing genes in wheat. II. Effects on leaf, stem, ear, and floret growth. *Field Crops Res.* **1992**, *28*, 191–210. [\[CrossRef\]](#)
44. Yasseen, B.T.; Al-Maamari, B.K.S. Further Evaluation of the Resistance of Black Barley to Water Stress: Preliminary Assessment for Selecting Drought Resistant Barley. *J. Agron. Crop Sci.* **1995**, *174*, 9–19. [\[CrossRef\]](#)
45. Szczepanek, M.; Lemańczyk, G.; Nowak, R.; Graczyk, R. Response of Indian Dwarf Wheat and Persian Wheat to Sowing Density and Hydrothermal Conditions of the Growing Seasons. *Agriculture* **2022**, *12*, 205. [\[CrossRef\]](#)
46. Pecio, A.; Wach, D. Grain yield and yield components of spring barley genotypes as the indicators of their tolerance to temporal drought stress. *Pol. J. Agron.* **2015**, *21*, 19–27.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.