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Impact of Selected PSII Parameters on Barley DH Lines Biomass and Yield Elements

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Abstract: In our study, we focused on the link among various parameters of chlorophyll a fluorescence and yield elements in the barley doubled haploid (DH) lines. There were significant differences in all studied DH lines, both in yield components and parameters of chlorophyll a fluorescence. The most variable parameter was overall performance index of PSII (PI) while the least was the amount of energy trapped in PSII reaction centers (TRo/CS). Considering yield components, high variation was also observed in the subsequent order from highest to lowest variation: biomass, thousand-grain weight (TGW) and grain number per plant (GNP). Significant negative correlation was found among the following fluorescence parameters: PI and light energy absorption (ABS/CS), as well as between maximum photochemical efficiency (Fv/Fm) and TGW, and between biomass and electron acceptors pool size from PSII (Area). Conversely, significant positive correlation was found between: Area and PI, Area and energy used for electron transport (ETo/CS), Area and GNP, PI and ETo/CS, PI and GNP, ABS/CS and TRo/CS, as well as between ETo/CS and GNP. Yield components combined with fluorescence parameters of chlorophyll a expressed with canonical variate analysis did not clearly distinguish the barley DH lines into hulled and hull-less groups. The mean value for these groups significantly differs only for ETo/CS and TGW values. The other parameters are distributed almost uniformly in hulled and hull-less lines. However, certain hull-less DH lines possess higher yield parameters compared to parental forms, which suggests a possibility of occurrence of transgression effects. The results suggest the chance to find valuable hull-less forms that are desired by breeders and plant producers, since these forms possess favorable functional features.

Keywords: *Hordeum vulgare* L.; hulled and hull-less DH lines; biomass; chlorophyll a fluorescence; yield components; canonical variate analysis

Citation: Warzecha, T.; Skrzypek, E.; Bocianowski, J.; Sutkowska, A. Impact of Selected PSII Parameters on Barley DH Lines Biomass and Yield Elements. *Agronomy* **2021**, *11*, 1705. https://doi.org/10.3390/agronomy11091705

Academic Editors: Yasutomo Hoshika and Sara Di Lonardo

Received: 27 July 2021
Accepted: 24 August 2021
Published: 26 August 2021

1. Introduction

After rice, wheat, and maize, barley (*Hordeum vulgare* L.) occupies fourth place in cereal production, holding a significant role in world crop production [1]. It is recognized to be adjustable to broad types of environments [2], and numerous studies have discovered a significant impact of environment and genetic–environment interaction on phenotypic appearance of agronomically important characteristics [3–7]. Plant breeding currently has many techniques such as haploidization to generate new cultivars in shorter time. The classical method takes 6–10 years to obtain a homozygosity level high enough to maintain the desired features of autogamus plants. The time can be shortened by applying doubled haploid (DH) methods, which produce homozygotes for one year and make plant selection much easier [8]. Additionally, DH can be utilized as material in genetic map creation. Utilization of the technique is focused on the creation of a DH mapping population with markers associated with essential and economically important traits. As homozygous DH

lines can be repeatedly phenotyped, they are good material for studying polygenic traits inherited quantitatively, which may involve a trial with replication in time and different localization for relevant phenotyping and marker generation [8].

Crop breeding programs, whether using classical or biotech methods, are oriented to improve plant production efficiency. The purpose is to increase the above ground plant biomass; therefore, one of the breeder's main goals is in increasing the biomass and harvest index. Effectiveness of photosynthesis is a main physiological aspect of pure carbon gain. Abilities of plants to enhance biomass production can be read from their photosynthetic efficiency. The content of pigments involved photosynthetically (chlorophylls and carotenoids) are straightforwardly implicated in the photosynthetic apparatus action and can stimulate variation in a magnitude of chlorophyll fluorescence (CF) parameters [9]. The CF parameters were involved in assessing plant ability to struggle with environmental stresses, such as biotic stress caused by fungal infection, and these parameters correlated with direct assessment of plant resistance [10]. CF is recognized as a handy, harmless, sensitive, and fast method that enables evaluation of photosynthetic performance in plants [11–13]. This technique is applied for quick screenings of plants and enables recognition of a slight variation in the activity of the plant photosynthetic apparatus [10,14]. The CF measurements can answer the way in which the light energy immersed by chlorophyll particles is converted in a plant and can be consider in three forms of action. Photochemistry is one way that enables photosynthesis, since excess energy can dissipate as heat or be realized as re-emission of light chlorophyll fluorescence.

The actions described above occur in competition, indicating that an increase in effectiveness of one in consequence reduces the yield of the other two. The measurements of CF also provide information concerning efficiency of photochemistry and dissipation of heat [15]. The parameters of chlorophyll *a* fluorescence allow the approximation of the photochemical output of PS II photosystem, the effectiveness of stimulation energy exploitation in the photosynthetic progression, the amount of open reaction centers, and the total energy gained by the photosynthetic apparatus dispersed as heat [10,11,16]. Furthermore, CF provides information about alteration in the effectiveness of photochemistry and heat dispersion [13]. Certain CF parameters can assist to assess the CO_2 absorption speed and leaf photosynthetic accomplishment [16]. The screening programs focused on identifying enhanced plant performance can utilize the relationship between CF parameters and leaf photosynthetic performance. Physiological performance of leaves can be detected by a sensitive survey such as chlorophyll *a* fluorescence [11]. The impact of various environmental stresses such as water deficit, heat, salinity or cold on plants [17], as well as biotic stress caused by fungal infection [10,18], can be estimated using CF application.

Barley is systematically located in the Gramineae family (tribe Hordeae) and has hulled or hull-less grains. Hulled grains have glumes integrated with seeds with a connecting matter that is synthesized by pericarp-epidermis 48 h after flowering [19]. Hull-less forms are used for human food and fodder for animals, principally in pig and fowl nutrition. They have higher protein content compared to hulled barley grains and contain a similar amount of raw fiber as wheat [20–22]. Hull-less barley cultivars have been developed in Poland and other countries but thus far have lower yielding parameters compared to hulled grains [23,24]. It was also reported that hull-less forms of barley are more susceptible to biotic stresses, including infection of Fusarium genera [6,10,18,25,26]. We previously reported significant correlation between yield elements and chlorophyll *a* fluorescence parameters in the set of oat DH lines [27]. It will be interesting from the theoretical and pragmatic point of view to understand how the connections function in another cereal species; therefore, we have chosen the economically important crop barley. Accordingly, the aim of this research was to determine the potential correlations between selected yield components of barley DH lines and chosen CF indices of chlorophyll *a*, as well as to compare the differences in effectiveness of photosynthesis and selected yield components between hulled and hull-less barley DH lines.

2. Materials and Methods

2.1. Plant Material

The studies were performed on two parental genotypes (DH lines: 1N86—hull-less and RK63/1—hulled) and F1 progenies of the mentioned parental lines—30 DH lines. In order to obtain DH lines, the *Hordeum bulbosum* method was applied. The typical protocol was utilized for pollinating *H. vulgare* with *H. bulbosum*, along with the embryo rescue culture application [28,29]. Seeds of 32 barley DH lines were sown in the third week of April, with one seed per pot (3 L) filled with soil:sand combination (3:1 v/v) in five repetitions (160 plants for experimental unit). In order to maintain 70% field water capacity (FWC), plants were watered with tap water. While conducting the studies, plants were manured with a liquid Hoagland solution every week [30]. Plants were grown in an open-sided greenhouse until full maturity in August. The experiments were conducted in the Experimental Station in Prusy, University of Agriculture in Kraków, Poland (South Poland near Kraków, 50°06′52″ N, 20°04′23″ E). The research was conducted in a completely randomized design, with five replications. Chosen yield components and chlorophyll *a* fluorescence parameters were determined for all barley DH lines.

2.2. Chlorophyll a Fluorescence

Measurements of chlorophyll fluorescence were performed with the fluorometer (Handy Plant Efficiency Analyzer, Handy PEA; Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). The measurements were performed during one day from 10.00 a.m. to 12.00 a.m. at ambient temperature and natural light intensity. To adapt leaves to the dark, they were covered with light blocking clips for 15 min. Saturating pulse duration was 3000 μmol $m^{-2} \cdot s^{-1}$, and stable gain was 0.8×. The following indices were measured per excited leaf cross-section (CS): Fv/Fm (maximum photochemical efficiency of PS II), PI (overall performance index of PSII photochemistry), ABS/CS (light energy absorption), TRo/CS (excitation energy trapped in PSII reaction centers), ETo/CS (energy used for electron transport), and Area (pool size of electron acceptors from PSII). The measurements were performed on the fully developed flag leaves after the plants' inflorescence entirely emerged, with five replications of each barely DH lines.

2.3. Selected Yield Components

In the full maturity stage, select yield components of barley DH line were determined, such as: thousand-grain weight (TGW), the number of grains per plant (GNP), and total biomass of plant.

2.4. Statistical Analysis

The normality of the distribution of the nine observed traits (Fv/Fm, Area, PI, ABS/CS, TRo/CS, ETo/CS, TGW, GNP and Biomass) was tested with Shapiro–Wilk's normality test [31] to check whether the analysis of variance (ANOVA) met the assumption that the ANOVA model residuals followed a normal distribution. The homogeneity of variance was tested using Bartlett's test. Multivariate normality and homogeneity of variance–covariance matrices were tested by Box's M test. A one-way multivariate analysis of variance (MANOVA) was produced. Following this, one-way analyses of variance (ANOVA) were performed in order to verify the null-hypotheses of a lack of genotype effect in terms of the values of the nine observed traits, independently for each trait. The arithmetic means and standard deviations were calculated. Moreover, Fisher's least significant differences (LSDs) were estimated at a significance level of $\alpha = 0.05$. The relationships between the observed traits were estimated using Pearson's correlation coefficients. Heterosis effects for lines for each trait were estimated and tested by comparing a particular line with the trait mean of both parents. The results were also analyzed using multivariate methods. The canonical variate analysis (CVA) was applied to present a multi-trait assessment of the similarity of the investigated genotypes in a lower number of dimensions with the least possible loss of information. This enabled the graphic illustration of the variation in

the traits of all genotypes under analysis. The Mahalanobis distance was suggested as a measure of similarity of multi-trait genotypes, whose significance was verified by employing critical value D_{cr} known as the least significant distance. Pearson's simple correlation coefficients were estimated between values of the first two canonical variates and values of the original individual traits to determine the relative share of each original trait in the multivariate variation of the genotypes [32]. The GenStat v. 18 statistical software package was used for all the analyses.

3. Results

3.1. Statistical Analysis

All the observed traits had normal distribution. The results of the MANOVA performed indicated that all the genotypes (Wilk's $\lambda = 0.00102$; $F = 2.21$; $p < 0.0001$) were significantly different with regard to all of the nine quantitative traits. ANOVA indicated that the main effects of genotype were significant for all examined traits of both yield components and CF parameters (Table 1).

3.2. Chlorophyll a Fluorescence

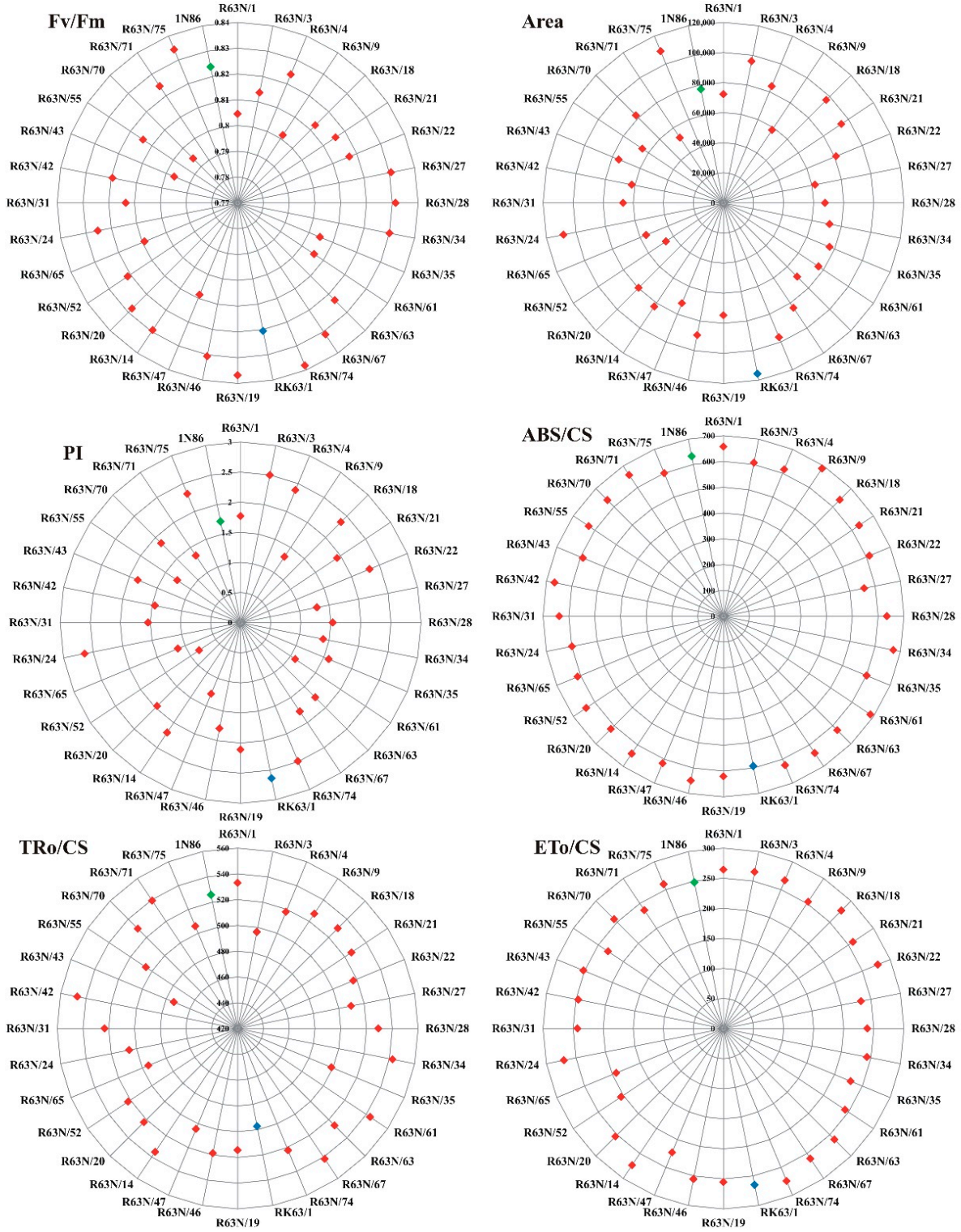
Compared to parental forms, various deviations in photosynthetic apparatus performance of barley DH lines were revealed. Variation in photosynthetic parameters arranged according to CV (coefficient of variation) values from lowest to highest were as follows: TRo/CS, Fv/Fm, ABS/CS, ETo/CS, Area, and PI. The range of values for maximum photochemical efficiency (Fv/Fm) and pool size of electron acceptors from PSII (Area) were from 0.794 to 0.838 and 46,000 to 115,733. Changes in PI corresponded almost completely to ABS/CS and ETo/CS (Table 1, Figure 1). Considering the overall performance index of PSII photochemistry based on equal absorption, energy used for electron transport, light energy absorption, and excitation energy trapped in PSII reaction centers, they did not separate examined barley DH lines into groups because an almost equal number of DH hulled, and hull-less lines had higher and lower values (Figure 1). However, the mean value for hulled DH lines was significantly higher than for hull-less lines for Area (respectively 81,383 and 74,920) and slightly higher for PI, ABS/CS, TRo/CS, and ETo/CS (respectively 1.884 and 1.705, 630.139 and 627.972, 522.458 and 516.748, and 257.033 and 243.831). The highest and lowest values for PI, ABS/CS, TRo/CS, and ETo/CS were respectively as follows: 0.828 to 2.643, 556.55 to 688.44, 473.60 to 546.76, and 192.87 to 277.67 (Table 1).

3.3. Yield Components

The studied barley DH lines had a significant impact on the important agronomic features for yield components. The maximum differences were reported for biomass (12.14 to 27.45) with variation coefficient of 34.01%. The lowest values for biomass production were in lines R63N/75 and 1N86, while the maximum values were in lines R63N/27 and R63N/52 (Table 1). Thousand-grain weight variation was not as high as the biomass values (21.8%) (Table 1). Yield forming parameters such as TGW (thousand-grain weight) ranged from 32.07 g (hull-less line R63N/24) to 57.51 g (hulled line R63N/61). Whereas GNP (grain number per plant) varied from 389.8 (hull-less line R63N/65) to 537.2 (hulled line R63N/22) (Table 1).

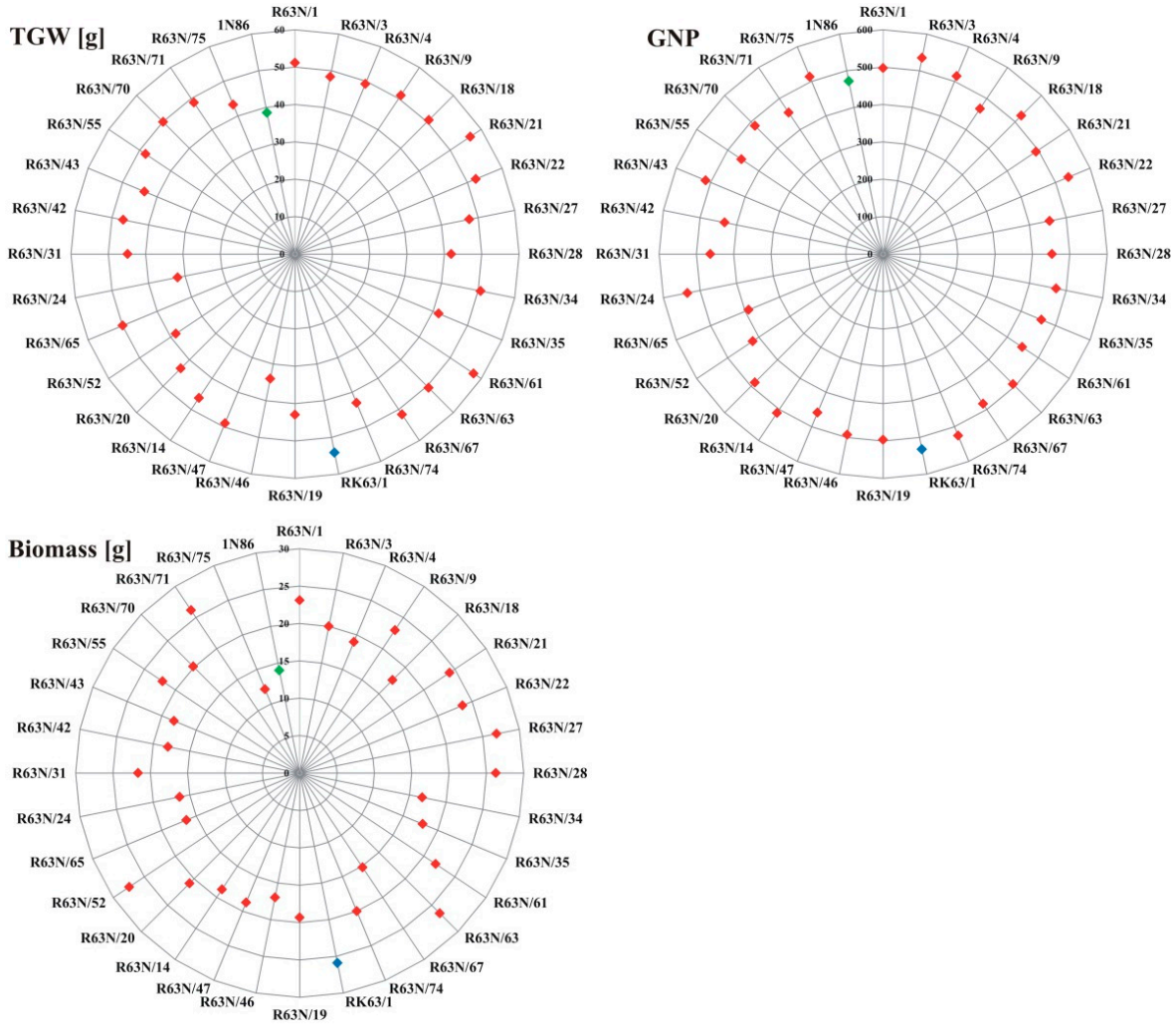
Table 1. The chlorophyll fluorescence indices, yield elements (mean values), standard deviations (s.d.), coefficient of variance CV (%), and *F*-statistics from one-way analysis of variance for studied variables features of barley DH lines.

Type of Line	Genotype	No.	Fv/Fm		Area		PI		ABS/CS		TRo/CS		ETo/CS		TGW		GNP		Biomass	
			Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Hulled	R63N/1	1	0.805	0.030	72,200	27,819	1.769	1.100	657.4	17.0	533.0	11.7	264.2	19.1	51.16	2.03	497.5	40.56	23.10	1.28
	R63N/3	2	0.814	0.038	96,000	7624	2.499	0.369	606.4	9.3	496.4	16.1	265.4	5.8	48.36	1.99	534.9	12.05	20.00	2.46
	R63N/4	3	0.824	0.021	83,933	22,268	2.382	0.336	616	12.1	517.9	6.0	266.7	7.7	49.24	2.61	515.1	20.62	18.98	2.46
	R63N/9	4	0.802	0.072	58,267	30,877	1.315	1.088	688.4	60.6	527.1	32.0	253.5	28.7	51.03	4.03	467.5	47.98	22.98	2.22
	R63N/18	5	0.813	0.030	96,733	4365	2.360	0.423	638.3	9.3	529.9	2.1	277.3	10.2	50.68	3.28	523.4	20.1	17.59	0.76
	R63N/21	6	0.816	0.035	94,333	11,075	1.930	0.286	633.3	13.3	526.2	4.6	259.1	7.1	56.47	2.70	492.5	15.64	24.15	2.55
	R63N/22	7	0.817	0.031	81,067	3646	2.322	0.637	612.6	19.7	517.1	18.5	277.7	7.2	52.41	0.96	537.2	10.4	23.61	6.47
	R63N/27	8	0.831	0.022	62,067	30343	1.295	0.728	556.6	44.7	509.7	41.0	233.1	24.6	47.6	3.71	454.5	40.26	26.89	5.07
	R63N/28	9	0.831	0.013	67,533	14589	1.532	0.088	634	25.0	529.2	25.0	239.3	14.1	41.84	7.90	452.1	11.44	26.27	2.85
	R63N/34	10	0.830	0.033	72,000	28875	1.402	1.123	672.2	81.6	542.6	36.9	243.3	25.2	50.67	1.77	472.3	42.23	16.73	4.15
	R63N/35	11	0.805	0.131	76,400	12817	1.587	0.409	601.3	17.2	498.8	13.5	229	16.3	41.63	2.60	459	24.29	17.82	5.49
	R63N/61	12	0.806	0.027	76,067	13001	1.092	0.112	685.7	13.3	543.7	11.8	243.2	16.6	57.52	10.97	447.8	39.56	21.90	3.41
	R63N/63	13	0.823	0.024	69,333	39461	1.757	1.416	624.7	22.8	526.4	19.9	261.1	33.8	50.57	4.54	492.2	57.82	26.54	4.73
	R63N/67	14	0.831	0.018	83,867	13299	1.775	0.882	637.7	26.9	541.7	31.4	260.2	25.2	51.62	2.74	481.8	58.82	15.17	5.75
	R63N/74	15	0.838	0.014	96,600	10173	2.491	0.338	625.2	20.3	522.3	13.6	274.4	3.6	43.1	3.60	525.5	7.94	19.99	4.88
	RK63/1 (parental line)	23	0.821	0.027	115,733	8075	2.636	0.616	592.3	22.2	497.3	22.2	264.9	24.5	54.13	6.92	532.2	33.52	25.89	3.53
Hull-Less	R63N/19	16	0.837	0.012	74,667	1419	2.110	0.573	620.6	6.8	514.4	4.1	255.5	12.6	42.97	2.32	496.8	27.36	19.32	1.81
	R63N/46	17	0.831	0.021	89,533	24730	1.793	0.780	649.3	13.0	518.6	16.9	255.2	18.5	34	8.79	492.8	46.15	16.96	4.13
	R63N/47	18	0.809	0.027	72,133	18036	1.280	0.416	617	14.1	504.4	13.3	223.1	27.5	49	3.28	458.8	16.75	18.74	3.32
	R63N/14	19	0.829	0.016	82,800	15096	2.198	0.526	640.7	31.0	535.2	16.4	273.3	10.4	46.3	8.54	511.1	25.83	18.72	0.89
	R63N/20	20	0.828	0.031	79,800	13101	1.961	0.332	617.7	13.1	522.8	11.0	254	13.0	43.26	2.64	485.7	21.27	20.86	2.69
	R63N/52	21	0.821	0.056	46,000	16975	0.828	0.663	640.3	81.3	522.2	34.2	204.6	16.6	38.36	5.68	419.8	46.12	27.45	6.46
	R63N/65	22	0.809	0.041	55,667	15531	1.128	0.568	612.3	25.0	494.9	14.0	192.9	10.1	49.94	6.97	389.9	26.65	16.42	5.90
	R63N/24	24	0.825	0.019	108,467	1332	2.643	0.309	599	12.5	505.8	3.7	270.4	2.9	32.07	8.99	534.5	6.87	16.38	2.00
	R63N/31	25	0.813	0.068	66,733	16928	1.538	0.397	637	19.9	523.2	16.8	242.6	30.0	44.84	4.56	462.7	43.17	21.64	6.25
	R63N/42	26	0.820	0.035	62,200	35807	1.451	0.402	667.7	21.9	546.8	18.0	246	17.6	46.92	8.55	432.9	15.96	17.98	2.13
	R63N/43	27	0.797	0.056	75,333	36436	1.849	1.298	590	17.5	473.6	35.6	251.9	26.0	43.65	4.46	514.6	45.36	18.21	1.49
	R63N/55	28	0.814	0.035	64,867	4291	1.265	0.140	628.7	26.3	505.6	12.0	230.7	10.3	48.14	3.07	456.2	10.22	22.08	4.00
	R63N/70	29	0.794	0.078	82,267	9659	1.866	0.460	636.3	7.4	529.5	10.1	257	20.9	49.96	1.79	485.1	33.66	20.15	2.59
	R63N/71	30	0.824	0.024	52,133	9758	1.337	0.530	658.3	14.2	539.3	4.4	236.7	24.8	48.73	3.33	454.9	16.21	26.18	1.34
	R63N/75	31	0.834	0.018	109,067	8401	2.315	0.496	600.3	8.6	505.9	8.5	259.7	14.6	43.25	2.40	513.4	29.43	12.14	3.89
	1N86 (parental line)	32	0.824	0.024	77,067	9843	1.713	0.373	632.3	31.3	525.8	15.9	247.8	4.8	38.56	1.04	471.6	20.12	14.01	2.20
$LSD_{0.05}$			0.0264		31,295.7		1.0719		49		32.34		30.31		8.281		52.43		6.2	
F-statistics (*p*-value)			1.86 (0.003)		2.25 (0.003)		1.68 (0.04)		2.68 (<0.001)		2.17 (0.005)		3.4 (<0.001)		4.04 (<0.001)		3.77 (<0.001)		3.35 (<0.001)	
CV (%)			7.24		36.92		47.49		7.71		5.63		13.95		21.80		13.18		34.01	



(**A**)

Figure 1. *Cont.*



(**B**)

Figure 1. Average values of the studied chlorophyll fluorescence indices (**A**), yield elements and biomass (**B**) for the set of 32 barley DH lines (blue—hulled parental form, green—hull-less parental form, red—offspring DH lines).

3.4. Correlation between Chlorophyll a Fluorescence Parameters and Yield Components

The studies also revealed important relationships between CF parameters and yield-forming elements since significant correlation was reported (Table 2, Figure 2). Maximum photochemical efficiency of PS II photosystem (Fv/Fm) correlated negatively with thousand-grain weight (TGW). Pool size of electron acceptors from PSII (Area) correlated positively with: PI (0.865), ETo/CS (0.702), and GNP (0.804) and negatively with biomass (−0.375). PI correlated negatively with light energy absorption (ABS/CS) and positively with: energy used for electron transport (ETo/CS) and GNP. Additionally, positive significant correlation coefficients were observed between ABS/CS and TRo/CS (0.766) as well as ETo/CS and GNP (0.903) (Table 3, Figure 2).

3.5. Prediction of the Heterosis Effect

The values of heterosis effects for the observed traits are shown in Table 3. The number of significant heterosis effects ranged from two (for Fv/Fm: R63N/52 and R63N/61) to ten (for Area). The R63N/52 DH line characterized the largest number of statistically significant heterosis effects: seven—for Fv/Fm, Area, PI, ETo/CS, TGW, GNP, and Biomass (Table 3). Line R63N/61 had significant heterosis effects for six observed traits: Fv/Fm, PI, ABS/CS, TRo/CS, TGW, and GNP (Table 3).

3.6. Canonical Variate Analysis

The values for the first two canonical variates were also significant and accounted jointly for 44.14% of the whole variation (Figure 3). Significant positive linear relationships with the first canonical variate were found for Area, PI, ETo/CS, and GNP (Table 4). Significant linear relationships with the second canonical variate were found for Area and PI (negative) as well as TGW and biomass (positive) (Table 4). The greatest variation in genotypes of the nine traits (measured Mahalanobis distances) was jointly found for R63N/1 and R63N/65, with the Mahalanobis distance between them amounting to 8.203 (Table 5). The greatest similarity (1.374) was found for R63N/20 and R63N/31 (Table 5).

Table 2. Correlation coefficients between variables for chlorophyll fluorescence and yield elements of barley DH lines.

Trait	Fv/Fm	Area	PI	ABS/CS	TRo/CS	ETo/CS	TGW	GNP
Area	0.205							
PI	0.268	0.865 ***						
ABS/CS	−0.144	−0.34	−0.392 *					
TRo/CS	0.235	−0.232	−0.229	0.766 ***				
ETo/CS	0.196	0.702 ***	0.835 ***	−0.006	0.173			
TGW	−0.357 *	−0.048	−0.096	0.239	0.251	0.095		
GNP	0.166	0.804 ***	0.919 ***	−0.301	−0.2	0.903 ***	−0.02	
Biomass	−0.078	−0.375 *	−0.262	0.019	0.113	−0.132	0.303	−0.15

* $p < 0.05$; *** $p < 0.001$.

Table 3. Heterosis coefficients for individual quantitative traits barley DH lines.

Line	Fv/Fm	Area	PI	ABS/CS	TRo/CS	ETo/CS	TGW	GNP	Biomass
R63N/1	0.001	−24,200	−0.41	45 *	21	8	4.8	−4	3.1
R63N/3	−0.008	−400	0.33	−6	−15	9	2	33	0
R63N/4	0.005	−12,467	0.21	4	6	10	2.9	13	−1
R63N/9	−0.038	−38,133 **	−0.86	76 ***	16	−3	4.7	−34	3
R63N/18	−0.006	333	0.19	26	18	21	4.3	21	−2.4
R63N/21	−0.005	−2067	−0.24	21	15	3	10.1 **	−9	4.2
R63N/22	−0.019	−15,333	0.15	0	6	21	6.1	35	3.7
R63N/27	−0.014	−34,333 *	−0.88	−56 *	−2	−23	1.2	−47 *	6.9 *
R63N/28	−0.001	−28,867 *	−0.64	22	18	−17	−4.5	−50 *	6.3 *
R63N/34	−0.02	−24,400	−0.77	60 **	31 *	−13	4.3	−30	−3.2
R63N/35	−0.006	−20,000	−0.59	−11	−13	−27 *	−4.7	−43	−2.1
R63N/61	−0.043 *	−20,333	−1.08*	73 ***	32 *	−13	11.2 **	−54 *	1.9
R63N/63	−0.03	−27,067 *	−0.42	12	15	5	4.2	−10	6.6 *
R63N/67	−0.01	−12,533	−0.4	25	30 *	4	5.3	−20	−4.8
R63N/74	0.008	200	0.32	13	11	18	−3.2	24	0
R63N/19	0.001	−21,733	−0.06	8	3	−1	−3.4	−5	−0.6
R63N/46	−0.02	−6867	−0.38	37	7	−1	−12.3 ***	−9	−3
R63N/47	−0.018	−24,267	−0.89	5	−7	−33 *	2.6	−43	−1.2
R63N/14	0	−13,600	0.02	28	24	17	0	9	−1.2
R63N/20	0.01	−16,600	−0.21	5	11	−2	−3.1	−16	0.9
R63N/52	−0.051 **	−50,400 ***	−1.35 **	28	11	−52 ***	−8 *	−82 ***	7.5 **
R63N/65	−0.027	−40,733 **	−1.05 *	0	−17	−63 ***	3.6	−112 ***	−3.5
R63N/24	0.009	12,067	0.47	−13	−6	14	−14.3 ***	33	−3.6
R63N/31	−0.015	−29,667 *	−0.64	25	12	−14	−1.5	−39	1.7
R63N/42	−0.017	−34,200 *	−0.72	55 *	35 *	−10	0.6	−69 **	−2
R63N/43	−0.034	−21,067	−0.33	−22	−38 **	−4	−2.7	13	−1.7
R63N/55	−0.031	−31,533 *	−0.91	16	−6	−26	1.8	−46 *	2.1
R63N/70	−0.004	−14,133	−0.31	24	18	1	3.6	−17	0.2
R63N/71	−0.016	−44,267 **	−0.84	46 *	28	−20	2.4	−47 *	6.2 *
R63N/75	0.007	12,667	0.14	−12	−6	3	−3.1	12	−7.8 **

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

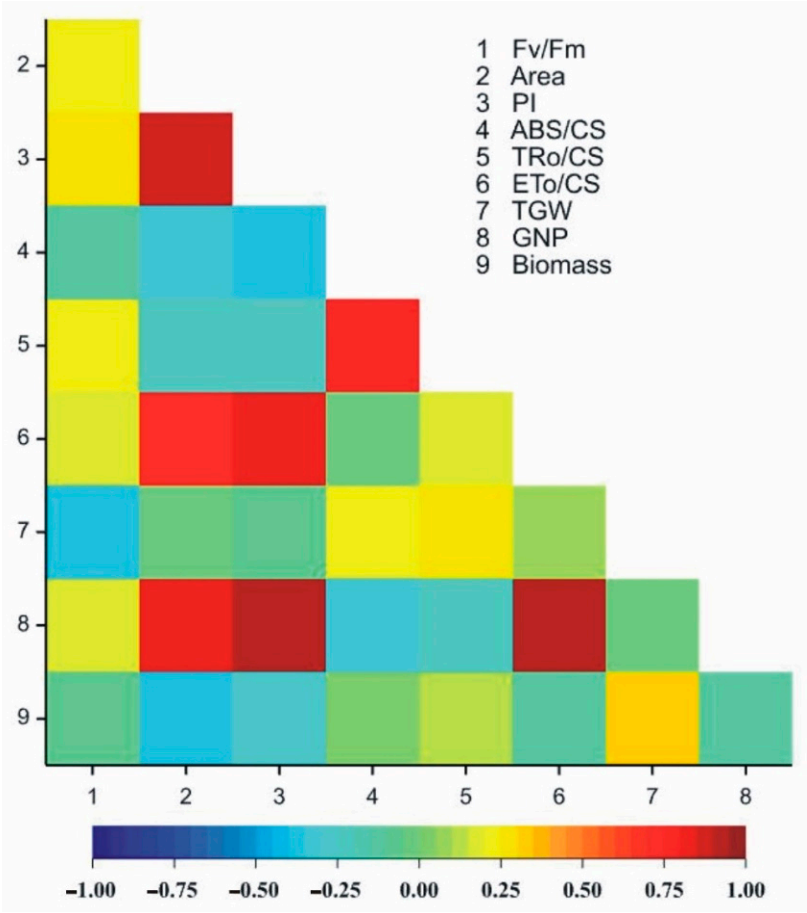


Figure 2. Heatmaps for Pearson's correlation coefficients between observed traits (CF parameters, yield elements and biomass) for 32 barley DH lines.

Table 4. Correlation coefficients between the first two canonical variables and original traits of barley DH lines.

Trait	First Canonical Variate, V_1	Second Canonical Variate, V_2
Fv/Fm	−0.042	−0.298
Area	0.541 **	−0.458 **
PI	0.616 ***	−0.450 **
ABS/CS	−0.012	0.12
TRo/CS	0.063	0.206
ETo/CS	0.846 ***	−0.303
TGW	0.375 *	0.800 ***
GNP	0.839 ***	−0.411 *
Biomass	0.151	0.683 ***
Percentage variation	25.21	18.93

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

CVA separated DH lines into four groups indicating that chlorophyll *a* fluorescence indices with yield forming elements are relevant for yield potential (Figure 3). The first group contained only one DH hull-less line 22 (R63N/65). The second group contained hull-less lines: 17, 24, 31, and parental form 32 (Figure 3). In group III were hulled lines: 8, 9, and 11 and hull-less lines: 16, 18, 20, 21, 25, 26, 28, and 30. The low value of V_1 and V_2 indicates better genotypes according to their photosynthetic performance and yield potential. This suggests the possibility of the effect of transgression not only in hulled lines but in hull-less ones.

Table 5. Mahalanobis distances between analyzed 32 barley DH lines.

Line		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
R63N/1	**1**																
R63N/3	**2**	4.119															
R63N/4	**3**	3.281	1.891														
R63N/9	**4**	2.79	4.402	3.548													
R63N/18	**5**	3.641	2.286	1.595	3.692												
R63N/21	**6**	3.132	3.235	2.832	3.868	2.825											
R63N/22	**7**	4.05	2.399	2.425	4.388	2.593	3.385										
R63N/27	**8**	5.537	5.093	4.572	6.388	5.404	4.306	4.753									
R63N/28	**9**	3.6	4.936	3.991	4.043	4.867	4.07	5.431	4.449								
R63N/34	**10**	3.613	3.397	2.731	4.103	2.6	3.414	4.106	5.649	4.606							
R63N/35	**11**	4.557	3.548	3.063	4.775	3.923	3.971	4.972	4.261	3.16	3.58						
R63N/61	**12**	4.05	4.2	3.529	3.419	3.017	2.404	4.299	5.527	4.724	3.074	4.449					
R63N/63	**13**	3.745	3.128	2.452	3.646	2.914	2.836	2.022	3.7	4.027	4.052	4.218	3.476				
R63N/67	**14**	4.419	3.636	2.492	4.459	1.954	3.261	3.772	4.981	4.976	2.528	3.73	2.824	3.489			
R63N/74	**15**	2.922	2.452	1.801	3.563	2.217	3.218	3.333	5.044	3.357	3.085	3.026	4.07	3.144	3.322		
R63N/19	**16**	3.275	2.698	1.625	3.417	2.774	3.666	3.599	4.726	2.983	3.063	2.303	4.187	3.105	3.362	1.532	
R63N/46	**17**	4.489	4.008	3.757	4.443	3.848	5.022	5.307	6.335	3.956	3.804	3.176	5.074	4.9	4.406	2.491	2.734
R63N/47	**18**	4.229	3.038	2.989	4.926	3.602	3.179	4.329	4.182	4.148	2.608	2.183	3.812	4.032	3.445	3.446	3.08
R63N/14	**19**	2.786	2.872	1.472	3.054	1.668	3.228	3.025	5.121	3.726	2.654	3.472	3.536	2.784	2.518	1.39	1.719
R63N/20	**20**	2.79	3.5	2.425	3.731	3.311	3.219	4.198	4.146	2.009	3.288	2.226	4.129	3.37	3.542	1.788	1.594
R63N/52	**21**	6.192	5.224	4.977	5.957	5.826	5.665	5.947	4.946	3.913	5.071	3.76	5.672	4.77	5.732	4.963	4.177
R63N/65	**22**	8.203	6.648	6.126	7.164	6.728	6.724	7.697	6.775	6.48	6.453	4.828	6.026	6.705	5.932	6.973	5.974
RK63/1	**23**	4.694	2.75	3.575	5.428	3.778	2.434	3.58	4.608	5.122	4.681	4.408	4.297	3.668	4.671	3.846	4.373
R63N/24	**24**	5.219	3.652	3.751	5.684	4.046	5.318	5.114	5.986	4.598	4.486	3.296	5.982	5.066	4.719	2.598	3.001
R63N/31	**25**	3.118	3.38	2.229	3.018	3.099	3.16	3.989	4.242	2.106	3.038	2.141	3.401	2.836	3.224	2.167	1.402
R63N/42	**26**	5.264	5.658	4.21	3.882	4.427	5.033	5.922	6.232	4.177	4.681	4.28	3.975	4.63	3.824	4.49	3.864
R63N/43	**27**	4.707	2.041	2.861	4.683	3.368	4.422	3.072	5.155	5.371	4.307	3.714	5.107	3.648	4.324	3.29	3.096
R63N/55	**28**	3.512	2.742	2.275	3.369	3.082	2.684	3.605	3.919	3.092	2.994	2.089	3.06	2.68	3.279	2.754	2.134
R63N/70	**29**	2.687	2.84	1.569	3.094	1.944	1.997	3.287	4.309	3.165	2.362	2.748	2.54	2.574	2.178	1.912	1.992
R63N/71	**30**	2.837	4.111	3.09	3.303	4.009	3.404	4.189	4.458	2.307	3.331	3.56	3.803	3.054	4.233	3.184	2.621
R63N/75	**31**	5.107	3.041	3.082	5.585	2.926	4.165	4.647	5.545	5.253	3.249	2.868	4.607	4.822	2.974	3.031	3.324
1N86	**32**	4.444	4.091	3.085	4.468	3.462	4.675	5.139	5.501	3.691	3.149	2.295	4.565	4.558	3.174	2.664	2.191
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
		17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	
R63N/47	**18**	4.182															
R63N/14	**19**	3.107	3.685														
R63N/20	**20**	3.01	3.004	2.2													
R63N/52	**21**	4.838	4.27	5.254	4.389												
R63N/65	**22**	7.031	5.524	6.841	6.404	5.375											
RK63/1	**23**	5.568	3.604	4.453	4.261	5.822	7.109										
R63N/24	**24**	1.993	4.308	3.557	3.334	5.116	7.331	5.185									
R63N/31	**25**	3.064	2.917	2.128	1.374	3.733	5.534	4.353	3.779								
R63N/42	**26**	4.729	5.334	3.819	4.073	5.436	4.973	6.608	5.742	3.186							
R63N/43	**27**	4.12	3.597	3.538	3.997	5.523	6.909	4.138	3.844	3.791	6.004						
R63N/55	**28**	3.705	2.022	2.834	2.332	3.756	5.192	3.517	4.214	1.541	4.071	3.088					
R63N/70	**29**	3.548	2.68	1.585	1.801	4.829	6.008	3.596	4.041	1.583	3.622	3.709	1.856				
R63N/71	**30**	4.359	3.482	3.041	2.399	3.727	6.429	4.687	5.049	1.938	4.273	4.796	2.49	2.558			
R63N/75	**31**	3.338	2.949	3.389	3.55	5.73	6.255	4.321	2.89	3.779	5.357	3.575	3.593	3.186	5.158		
1N86	**32**	2.147	3.512	2.628	2.424	4.647	5.816	5.648	2.822	2.382	3.544	4.228	3.219	2.814	3.906	2.81	
		17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	

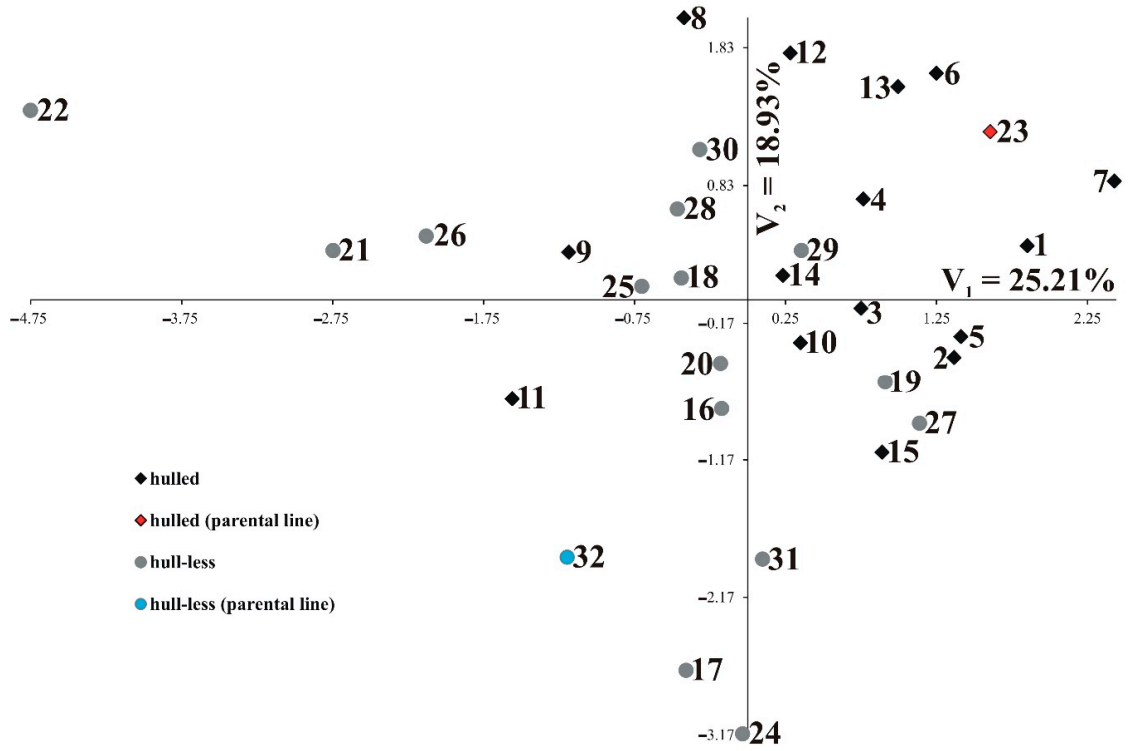


Figure 3. Distribution of 32 barley DH lines in space of two first canonical variables calculated on the basis of chlorophyll *a* fluorescence parameters (PI, Fv/Fm, Area, ABS/CS, TRo/CS, and ETo/CS) and yield components (biomass, grain number per plant (GNP), and thousand-grain weight (TGW)).

4. Discussion

Studies of the performance of photosynthetic apparatus expressed in CF indices may produce a helpful measure, and the genuine potency of it lies in its capability to provide characteristics of plants not easy to obtain in other manners. Measurements of chlorophyll fluorescence enables the understanding of how plants are able to cope with stress generated by various environmental factors and how the stressors influence the functioning of the PS II photosystem [15]. Thus, studies of plant yield can investigate activity of the photosynthetic apparatus elements, such as chlorophyll fluorescence kinetics [33]. This is why we have incorporated in our studies concerning yielding ability of barley DH lines above CF indices, and we have found significant difference in all studied FC parameters as well as selected yield elements. We have applied multivariate statistical analysis such as CVA in our studies. This type of analysis was applied in studies of Lopatynska et al. [34] to detect differences in DH lines and hybrids of winter rapeseed e.g., in thousand seed weight and the first two canonical variates together explained from 66.12% to 82.03% of the total variation depending on the year, since in our experiment, CVA explained 44.14% of the entire variation.

This type of investigation also gives the essential information regarding photochemical effectiveness of the photosystem II (PSII), and additionally provides the information on the quantity of excitation energy isolated in PSII reaction centers through photosynthesis. Moreover, the measurement enables the calculation of light energy absorption, the quantity of energy disappearing from PSII, the amount of active reaction centers oxidized and reduced, and the energy quantum consumed for electron transportation [11]. In our studies, we have found significant positive linear relationships with the first canonical variate for the following CF parameters: Area, PI, ETo/CS, and one of the yield elements as GNP. Therefore, mentioned CF parameters can help to predict possible yield of barley and may be suggested as markers in evaluating the certain genotypes in breeding programs.

The process of photosynthesis is dependent on chlorophyll, which holds a fundamental role in the assimilation and utilization of light energy, thus having an impact on photosynthetic efficiency [35]. We have also found significant linear relationships with the second canonical variate for Area and PI (negative) as well as TGW and biomass (positive). Thus, the results correspond with results presented by Smillie and Nott [36], Maxwell and Johnson [15], Pereira et al. [37], Fracheboud and Leipner [38], and O'Neill et al. [39], of which all deliver additional explanations of the molecular purpose of chlorophyll fluorescence and its relationships with assimilation pigments, which may be potentially valuable for yield improvement. Chlorophyll fluorescence indices have been utilized to identify minor variations in effectiveness of the photosynthetic apparatus among genotypes of crop plants [15,16]. Moreover, it was revealed that triticale yield was significantly correlated with selected chlorophyll fluorescence indices and leaf gas exchange [40]. The quantitative and qualitative deviations in photosynthesis can be investigated by CF measurements. CF technique was utilized to assess the photochemistry of the PS II photosystem in senescent leaves of cultivars with differing grain production potential. It was revealed that cultivar had an impact on the photosynthetic activity [41]. Rice cv. 'BRS Firmeza' expressed better effectiveness of the photosynthetic apparatus as compared to cv. 'BRS Pelota' in the grain filling stage [42]. An excellent marker for senescence in cv. 'BRS Pelota' will be photochemical effectiveness decrease, revealing that the rice cultivar possessing superior grain yield potential senesces earlier than with inferior grain yield. Another instrument to assess the general performance of the photosynthetic apparatus can be measurements of CF, and the measurements may provide a reason for checking the presence of various genotypes inside species [43]. In our studies, measurements of CF revealed differences in barley DH lines photosynthetic apparatus performance. Hulled DH lines possessed significantly higher mean values for Area than for hull-less lines (81,383 and 74,920, respectively). It was also the second most variable CF parameter (CV equal to 36.92%). This was the only parameter effectively dividing the barley DH lines into hulled and hull-less groups; for PI, ABS/CS, TRo/CS and ETo/CS the differences were only slightly higher for hulled lines. In contrast, PI was the most variable parameter among all (with no division of hulled or hull-less) DH lines (CV equal to 47.49%). PI parameter can functionally characterize PSII, as well as give information about current plant condition [44]. As Kalaji and Guo [45] reported, breeders frequently utilize the measurement of CF as a biological marker or indicator, or a selection feature. It provides a consistent reference regarding plant photosynthesis effectiveness in correlation with yield of crop plants. Chlorophyll fluorescence kinetics as components of the photosynthetic apparatus can be utilized in studies of plant productivity [33]. Additional explanation of the molecular role of chlorophyll fluorescence parameters and their associations with assimilation pigments may be worth considering for crop yield and biomass improvement [15,36–39].

Characteristics of CF are investigated for different aspects of plant photosynthetic effectiveness, establishing plant productivity, and finally the yield [14]. As showed by Planchon et al. [43], CF transient can be used as a genetic marker of yield in barley. The authors have found correlation between yield and leaf area, whereas the economical yield depended more on the length of vegetation period or the combination of the two factors mentioned above. The studies also revealed that CF indices correlated negatively with photosynthesis despite positive correlation with the leaf area. The authors described a lack of correlation between the yield and the maximum photosynthetic efficiency at the blossoming stage, but there was correlation between yield and the CF indices. Conversely, Slapakauskas and Ruzgas [46] shows correlation between CF parameters of PSII photosystem and agronomic traits and productivity of crop plants. Higher yield matches to increased plant biomass or economical yield. Therefore, biomass harvest index is essential for breeders, and efficiency of photosynthesis is a significant physiological element of net carbon gain. Given this, understanding of crop productivity can be gained by exploring the effectiveness of photosynthesis expressed by its components such as chlorophyll fluorescence kinetics as well as chlorophyll *a*, *b* and carotenoids content [10,14,33]. This

research delivers crucial data about photochemical effectiveness of plant photosystems and the amount of energy consumed by photosynthetic apparatus. CF parameters as well as chlorophyll *a*, *b* and carotenoids content have grown to be the most recognizable and functional characteristics in photosynthetic studies [15,47].

Our research proved that CF parameters (Fv/Fm, Area, PI, ETo/CS) positively correlated with GNP and biomass, while Fv/Fm and Area negatively correlated with biomass. In addition, TGW positively correlated with the following CF indices: ABS/CS and TRo/CS. Significant correlations among CF indices and yield elements make it possible to use them as biomarkers in barley breeding programs, especially when DH lines are incorporated into the protocol. Among CF parameters and yield elements of studied barley DH lines, a significant close relation was reported, proven by principal component analysis. However, as described by Slapakauskas and Ruzgas [46], it is crucial to understand the basis of physiology of recently produced cultivars and the correlation between physiology and economically important features of the plants. Jiang et al. [48] investigated rice cultivars varying in grain yield and revealed that those cultivars possessed significantly different potentials to absorb and utilize the light. Their results presented variation existing in CF indices in rice cultivars with different yield levels. Shao et al. [49] demonstrated a significant link between enhanced grain yield potentials in maize and the photosynthetic abilities of leaves. In our studies, enhanced values of the following CF parameters were linked with enhanced seed yield: Fv/Fm and Area. Conversely, superior plant biomass was produced when Fv/Fm and Area were lower. Furthermore, in our research, we have found a significant correlation between high values of the following CF indices: ABS/CS and TRo/CS and barley TGW. Additionally, a higher amount of energy used for electron transport resulted in barley plants setting a higher number of seeds. Chlorophyll fluorescence surveys can be utilized to determine a plant's vigor while alive, as well as the plant's reaction to various environmental conditions. Barley's tolerances to salinity were estimated using this method [50]. Another example of using CF parameters derives from studies by Herzog and Olszewski [51]. They used the Fv/Fm parameter to evaluate winter and spring oats for freezing tolerance and cold acclimatization, and they contrasted this to the TRo/CS. In addition, the method was utilized for quickly checking the chilling tolerance of oat varieties. If the parental form did not significantly differ from each other, there is a possibility to obtain from which possesses a heterosis effect. Such studies were conducted by Lopatynska et al. [34] in winter rape and helped to choose the valuable genotypes for breeding programs. In our studies, we have found significant heterosis effect for CF parameters (Fv/Fm, Area, PI and ETo/CS), as well as for yield elements (TGW, GNP) and biomass.

In conclusion, the findings of our studies demonstrate a connection among CF indices and elements of barely DH lines yield. Out of six studied chlorophyll *a* fluorescence parameters, three (PI, Area, ETo/CS,—order according to value of CV coefficient) significantly correlated with GNP (grain number per plant). Additionally, one CF parameter (Fv/Fm) significantly correlated with TGW (thousand grain weight), and finally there was a significant correlation between Area and total plant mass above ground (biomass). These results demonstrate that the survey of CF parameters can increasingly be used as a technique to assess the yield potential of crop plants. The analysis of principal components efficiently distinguished barley genotypes photosynthetically. Another important finding suggests a possibility of existence of transgression effects for selected yield elements and CF parameters in hull-less DH lines. The information can be valuable for breeders who try to generate better yielding hull-less forms possessing favorable nutritional characteristics.

Author Contributions: Conceptualization, T.W.; methodology, T.W., E.S. and J.B.; validation, T.W.; formal analysis, T.W., E.S. and J.B.; investigation, T.W. and E.S.; resources, T.W., E.S. and A.S.; writing—original draft preparation, T.W.; writing—review and editing, T.W., E.S., A.S. and J.B.; supervision, T.W.; project administration, T.W.; funding acquisition, T.W. All authors have read and agreed to the published version of the manuscript.

Funding: The research was financially supported by National Science Centre Poland (Project No. NN 310 725740).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to express our gratitude to Sarah C. Williams Head, Funk ACES Library, University Library, University of Illinois Urbana-Champaign, Urbana, United States for her precious help with language editing of the manuscript. We also thank the Institute of Plant Genetics of the Polish Academy of Science for the barley DH lines used for the experiment.

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

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