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Studies of Oat-Maize Hybrids Tolerance to Soil Drought Stress

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Abstract: The ontogenesis and yield formation in crop plants are modified by environmental conditions. Due to climatic change detected over two decades, the harmful influence of abiotic factors is increasing. One of the most threatening issues reducing plant productivity is drought stress. The strength of plant response to water shortages could differ depending on the strength of the drought stress, type of crop, genetic background, presence of additional stresses, and stage of plant development. There are examples of sexual hybridization between crop plants like oat (*Avena sativa* L.) and maize (*Zea mays* L.) with which stable fertile hybrids were generated. Additional maize chromosomes in oat plants (oat × maize addition, OMA) often infer morphological and physiological (e.g., PS II photosystem activity and chlorophyll production) changes modulated by the interaction of certain maize chromosomes added to the oat genome. The aim of the research was to evaluate the chosen physiological, biochemical, and agronomic parameters of OMA plants subjected to soil drought. Analysis of variance indicated that the main effects of genotype as well as treatment × genotype interaction were significant for all the traits studied (photosynthetic pigment content, selected PSII indices, mass of stem, number of grains/plant, mass of grains/plant). Most of the examined lines severely reduced PSII photosystem parameters, pigment content, and yield-related traits under drought stress. The results indicated that two lines (9 and 78b) retained high yielding potential under drought stress compared to commercial cv. Bingo.

Keywords: agronomic traits; drought stress; maize; oat; OMA; photosynthetic pigments; PSII photosystem

Citation: Warzecha, T.; Bathelt, R.; Skrzypek, E.; Warchoł, M.; Bocianowski, J.; Sutkowska, A. Studies of Oat-Maize Hybrids Tolerance to Soil Drought Stress. *Agriculture* **2023**, *13*, 243. https://doi.org/10.3390/agriculture13020243

Academic Editor: Mercè Llugany

Received: 29 December 2022
Revised: 15 January 2023
Accepted: 17 January 2023
Published: 19 January 2023

1. Introduction

Oat is very important cereal crop due to its high nutrient value, biological properties, and application in the cosmetics industry [1]. The global harvest of oat grain in 2020 amounted to 25.18 million tons, which, with a sown area of 9.77 million ha, gave a yield ca. 25.77 dt/ha. Oats are grown over the largest area in Russia, Canada, and Australia. Crops in these three countries accounted for 45% of the total world cultivation in 2020. Higher yields of oat grain in Canada than in Russia put it in first place in terms of harvest volume (4.57 million tons and 4.13 million tons, respectively) [2].

The yield of oat as well as other cereal can be reduced by drought-caused losses by reducing the number of fertile ears, kernels per ear, and disturbed grain filling (lower thousand-grain weight) [1]. However, oat, in contrast to other cereal crops, requires more water during vegetation and expresses higher susceptibility to drought stress [3]. Cereal plants are divided into winter and spring, and this division implies that spring months are critical in terms of water demand. Winter cereals, after the start of spring vegetation, may undergo a short period of supplementation or immediately start the phase of shooting at the stalk. Drought conditions in this period are mostly rare due to the water reserves

in the soil accumulated in winter. Spring cereals, after sowing in early spring, need more time to enter the critical phase of shooting at the stem, which implies a greater risk of drought-related losses due to lack of rainfall after the termination of water reserves in the soil [4]. Grain pouring is also a critical development phase because the lack of water in this phase is particularly responsible for worse filling of the grain, i.e., a decrease in the thousand-grain weight. This applies to both winter and spring cereals [5]. Plant breeding in areas with regular late-spring droughts provides early crop varieties that start critical phases of water demand as early as possible to get through them before prolonged drought conditions develop [6]. Studies of winter barley on the effect of drought occurring during grain filling showed a decrease in yield by more than 80% both in greenhouse and field conditions [7]. Climate change may cause the problem of drought to spread to even larger areas, and its effects may be more severe [8].

Abiotic stress like drought is recognized as one of the most crucial factors reducing the effectiveness of crop productivity, e.g., wheat, rice, and maize [9]. Chlorophyll content in leaves might be reduced by severe drought as well as the incorporation of malfunctioning, predominantly in PSI and PSII, of the photosynthetic apparatus [10]. However, the interaction between these photosystems under drought stress remains unclear, as does the impact of drought on the reaction of photosynthetic electron transport [11]. The photosynthesis of vegetation is without a doubt depended on chlorophyll as a crucial component. Thus, chlorophyll could be considered a key indicator of plant function and productivity [12]. Plants have established extraordinary abilities to control growth and development in hostile environmental conditions through changes in various mechanisms that act at different levels of the physiological, biochemical, and genetic processes. The intensity of plant reaction to drought stress could vary depending on the strength of the stress, type of crop, genetic factors, existence of additional stresses, and stage of plant development [13].

In crop species we can find many examples of sexual hybridization wherein stable fertile hybrids were generated, e.g., oat (*Avena sativa* L.) and maize (*Zea mays* L.), which are very interesting since they are distinctly linked plant species that could be sexually mated [14]. The detection of the existence of retained maize chromosomes in oat plants after pollinating with maize and generating an oat line with retained maize chromosomes [15] named OMA (oat × maize addition) has made the examination of the maize genome simpler [14].

Many applications of OMA lines in the research can be listed as follows: studies on maize-gene expression in the oat genetic background, including examining gene-regulation aspects [16] and probable gain of new features [17], as well as resistance to diseases [18]. The OMA lines are hybrids between plants possessing two type of photosynthesis, i.e., C3 and C4, and therefore they can help researchers examine C4 photosynthesis [17]. C4 photosynthesis is considered more efficient, and plants performing that type of CO_2 assimilation are more resistant to photooxidation. Therefore, OMA lines could be also used to follow the genetics of the C4 photosynthesis of maize and detect chromosomes/chromosome areas that are significant in this process [17,19,20]. Other applications of OMA lines can be found in the field of molecular genetics to recall studies of maize knob and the centromere structure [15], chromosome behavior during meiosis [21], the application of in situ hybridization (FISH) in the physical mapping of single-copy sequences on maize chromosomes [22], and the process of flow cytometry separating single maize chromosomes [23]. Furthermore, the existence of maize chromatin frequently implies the morphology of shoots, leaves, and panicles and physiology (e.g., abnormal panicle growth and chlorophyll synthesis) anomalies, but their character is modulated according to the specific maize-chromosome addition as well as the oat genetic background [19]. Consequently, their influence on the photosynthetic apparatus behavior is also assumed, along with the improved tolerance of the OMA lines to various stresses, including resistance to environmental factors, e.g., *Puccinia coronata* f. sp. *avenae* or *Puccinia graminis* f. sp. *avenae* [24–26]. Numerous environmental factors can negatively affect the photosynthetic process and as a result incorporate changes into the photosynthetic apparatus via functional and structural abnormalities [27], leading to restricted growth and production of plant biomass [28,29].

The study aimed to investigate selected physiological, biochemical, and agronomic parameters of OMA plants originating from the crossing of oat with maize under conditions of water availability and simulated soil drought. Base on the above results, an additional aim was to select the lines best adapted to growth and development in conditions of water shortage.

2. Materials and Methods

2.1. Material

The material utilized in this experiment consisted of the F2 and F3 generations of OMA (oat × maize addition) lines obtained by distant crossing of oat (*Avena sativa* L.) with maize (*Zea mays* L. var. *saccharata*) cv. "Waza" as pollinator. The inherence of maize chromatin introgressed into examined lines was verified by the application of PCR with specific *Grande-1* primers in order to amplify a maize retrotransposon [30]. The following 14 OMA lines were selected for the study: 1b, 9, 12, 18, 23, 26, 35, 42, 43, 55, 78b, 83, 114, and 119, as well as oat cultivar Bingo as the control genotype.

OMA lines selected on the basis of molecular analyses were then tested in the spring of 2020 in a greenhouse experiment. Two generations (F2 and F3) of 14 OMA lines (No. 1b, 9, 12, 18, 23, 26, 35, 42, 43, 55, 78b, 83, 114, 119) and the Bingo oat cultivar as a control genotype were tested. The plants were subjected to soil drought (20% relative water content), whereas the control (70% relative water content) was the same set of plants regularly watered throughout the experiment. The experiment was carried out in the greenhouse of the Department of Plant Breeding, Physiology and Seed Science at 3 Podłużna Street in Krakow. For each OMA line four replications were prepared for the experimental factor, i.e., simulating drought conditions, and for the control. The experiment consisted of 240 pots (15 genotypes—14 OMA lines and cv. Bingo × 4 replicates × 2 generations × 2 treatments). The seeds of the studied genotypes were sown in February 2020. The pots (20 cm × 14 cm) contained sand mixed with peat in equal parts with a total weight of 2500 g. Drought conditions were introduced for two weeks by stopping watering until the relative water content reached 20%. During the simulation of drought conditions, the control pots were watered normally, whereas the pots subjected to drought stress were supplemented with water only to the moisture level of 20%. During the greenhouse experiment, the following tests/measurements were carried out (the same for the combination subjected to drought stress and the control plants):

1. Measurement of chlorophyll fluorescence parameters of plants at the beginning of heading (tip of inflorescence emerging from the sheath, first spikelet just visible) after reaching 20% of substrate moisture and collection of leaves for biochemical analysis.
2. Measurement of chlorophyll fluorescence parameters of plants at the end of flowering (all spikelets have completed flowering) at the end of the drought stress (2 weeks after reaching 20% of substrate moisture) and collection of leaves for biochemical analysis.
3. Harvesting mature shoots, weighing the biomass of the aboveground parts and the total mass of kernels from all shoots.

2.2. Methods

2.2.1. Chlorophyll Fluorescence Analysis

Chlorophyll *a* fluorescence analysis was performed on one leaf of each plant with four replicates with the application of a fluorometer (Handy PEA; Hansatech Instruments, King's Lynn, Norfolk, UK). Measurements were carried out using a 3000 $\mu mol/m^2/s$ saturation pulse, 1 s pulse duration, and constant gain (1.0×). Chlorophyll fluorescence was measured on the first day of the draught stress when the substrate reached 20% moisture and after 2 weeks before returning to identical treatment for control and stressed plants. Measurements were made on flag leaves after a 20 min period of darkness. The following parameters were studied: Fv/Fm (maximum photochemical efficiency of PSII), area, PI (performance index of PSII), ETo/CS (amount of energy used for electron transport), and RC/CSo (number of active reaction centers in fully oxidized PSII) [31].

2.2.2. Analysis of the Content of Chlorophyll *a* and *b* and Carotenoids

The content of photosynthetic pigments was determined by the Lichtenthaler and Wellburn [32] method. Leaves were homogenized in 80% ethanol and then centrifuged at 1000× *g* for 5 min at 4 °C (Centrifuge 5702 R, Eppendorf, Hamburg, Germany). The supernatant was kept at 4 °C in darkness and absorbance was measured at the following wavelengths (λ): 470 nm, 648.6 nm, and 664.2 nm (Synergy 2 spectrophotometer, BioTek, Santa Clara, CA, USA). The content of chlorophyll *a* and *b* and the sum of carotenoids were calculated according to the following formula: C chl. *a* = (13.36 × A664.2) − (5.19 × A648.6) C chl. *b* = (27.43 × A648.6) − (8.12 × A664.2) C chl. *a* + *b* = (5.24 × A664.2) + (22.24 × A648.6). The abbreviations are as follows: Aλ—absorbance value for the appropriate wavelength [λ], C chl.*a*—concentration of chlorophyll *a*, C chl.*b*—concentration of chlorophyll *b*, C *a* + *b*—concentration of total chlorophylls. The results are presented in micrograms of a certain chlorophyll in 1 mL of the extract, after which its content in 1 g of dry matter was calculated.

2.2.3. Analysis of Aboveground Biomass and Selected Yield Elements

Plants from the greenhouse experiment were collected when the kernels of individual shoots reached full maturity. The aboveground part of the biomass produced by plants was weighed, as well as the share of grains in the total biomass yield. The weight of shoots and the weight of kernels along with their quantity were recorded. Measurements were carried out for each shoot separately, and then all branching of individual plants was summed up. Measurements were conducted for the control and the plants under soil drought.

2.2.4. Statistical Analysis

The normality of distribution of the 21 traits was tested using Shapiro–Wilk's normality test [33] to verify whether the analysis of variance (ANOVA) met the assumption that the ANOVA model residuals followed a normal distribution. Bartlett's test was used to testing of the homogeneity of variance. Box's M test tested multivariate normality and homogeneity of variance–covariance matrices. All the traits had a normal distribution. Three-way multivariate analysis of variance (MANOVA) was performed. Three-way analyses of variance (ANOVA) were carried out to determine the main effects of treatment, generation, and genotype as well as their interactions on the variability of the particular traits. Generation was not a differentiating factor for most of the observed traits, so this factor was omitted from further analyses, yielding a higher number of replicates. The mean values and standard deviations of traits were calculated for treatments, genotypes, and combinations of treatment × genotype. Additionally, Fisher's least significant differences (LSDs) were calculated for individual traits at the 0.05 level, and on this basis homogeneous groups were generated. The relationships between the 21 observed traits were estimated using Pearson's linear correlation coefficients based on the means of the genotypes. The relationships of the 21 observed traits are presented in a heatmap. The results were also analyzed using multivariate methods. Canonical variance analysis (CVA) was utilized to show a multi-trait assessment of the similarity of the tested genotypes in a lower number of dimensions with the least possible loss of information. The Mahalanobis distance was suggested as a measure of "polytrait" genotype similarity [34], the significance of which was verified by means of critical value Dα called "the least significant distance" [35]. Mahalanobis distances were calculated for all genotypes for (1) control and drought and (2) only the drought experiment. The GenStat v. 22 statistical software package (VSN International) was used for the analyses. Altogether, 21 traits were studied and annotated as t1–t21, respectively.

3. Results

The results of MANOVA indicated that treatment (Wilk's λ = 0.0411; $F = 177.9$), generation (Wilk's λ = 0.7361; $F = 2.73$), genotype (Wilk's λ = 0.0004; $F = 6.33$), treatment × genotype interaction (Wilk's λ = 0.0057; $F = 3.62$), generation × genotype interaction (Wilk's λ = 0.1001; $F = 1.41$), and treatment × generation × genotype interaction (Wilk's λ = 0.1212; $F = 1.29$)

were statistically significant when examined in all 21 quantitative traits jointly. Only treatment × generation interaction was not significant (Wilk's λ = 0.8375; $F = 1.48$; $p = 0.092$). Analysis of variance revealed that the main effects of genotype as well as treatment × genotype interaction were significant for all the traits studied (Table 1). The main effect of treatment was significant for all traits except RC/CSo (number of active reaction centers in the state of the fully oxidized PSII reaction center) on the first day of drought (Table 1). The mean values and standard deviations for the examined traits showed high variability among the tested genotypes and treatments, for which significant differences were observed for all the studied quantitative traits (Tables S1–S21). When considering the studied traits on the first day of drought it was noticed that most of them were reduced under the stress conditions, and only chlorophyll *b* content was lower in the control plants and, consequently, the sum of chlorophyll *a* and *b* (Tables S2 and S3). The first day of the drought condition did not affect the number of active reaction centers in the state of the fully oxidized PSII reaction center (RC/CSo) (Table S9), but the rest of the analyzed PSII system parameters were reduced in the drought condition (Tables S5–S8). The impact of drought stress was more severe after two weeks of the treatment and most of the studied traits were reduced, with the highest reduction observed in carotenoid content, to 16% of the control combination (Table S13), whereas most of the rest of the traits had a reduction amounting to 29 to 82% of that of the control plants (Tables S10–S12 and S15–S21). The drought resulted in a significant reduction in yield elements, and the general biomass of stems ranged from a 57 to 68% decrease (Tables S19–S21).

The density plots of the selected observed traits by treatment are presented in Figures 1–3. The distribution of the observation for chlorophyll *a* content (Figure 1A) and for chlorophyll *b* content (Figure 1B) clearly indicated that drought reduced the number of observations with increasing values of the traits. The wider part of the grey chart (control plants) ranged from 0.3 to 0.6 μg/g of d.w. Since the wider part of the pink chart (drought combination) ranged between 0.1 and 0.15, only a few individuals reached the level of the control plants. Similar tendencies were observed in chlorophyll *b* content and the sum of both types of chlorophyll (Figures 1B and 2A). The drought also had a significant impact on the total stem mass of the plants. As is shown in the density plot, for the control plants most of the observation ranged between 10 g and 22 g, and in plants under drought stress most individuals ranged between 5 g and 13 g (Figure 2B). The highest values of the observation for the number of grains/plant located in the control-plant area (grey part) reached 200–250 grains, and the frequency of higher values were in favor of this group of plants (Figure 3A). When considering the mass of grains/plant a similar tendency was observed. Some of the control plants reached the level of 11 g/plant with quite a wide plot of observation ranging between 5 and 7 g/plant, and in the drought combination (pink chart) the wide part terminated at the level of 5 g/plant (Figure 3B).

Table 1. *F*-statistics from three-way analysis of variance for 21 observed traits (t1–t21, respectively).

Source of Variation		Treatment (T)	Generation (Gener)	Genotype (G)	T × Gener	T × G	Gener × G	T × Gener × G
The number of degrees of freedom		1	1	14	1	14	14	14
First day of drought (20% of soil relative humidity)	Chlorophyll *a* content (µg/g of d.w.)	45.06 ***	7.54 **	12.5 ***	0.09	5.5 ***	3.34 ***	3.89 ***
	Chlorophyll *b* content (µg/g of d.w.)	176.64 ***	3.7	6.22 ***	2.12	5.32 ***	1.44	4.62 ***
	Chlorophyll *a* and chlorophyll *b* content (µg/g of d.w.)	5.76 *	6.98 **	10.15 ***	0.28	5.89 ***	2.12 *	4.64 ***
	Carotenoid content (µg/g of d.w.)	578.97 ***	0.6	12.8 ***	2.49	7.29 ***	3.73 ***	2.76 ***
	Fv/Fm (maximum photochemical efficiency of PS II)	10.41 ***	7.02 **	13.48 ***	0.76	3.19 ***	1.56	1.09
	Area (pool size of electron acceptors from PSII)	91.19 ***	0.57	6.04 ***	1.65	2.22 **	1.42	0.34
	PI (overall performance index of PSII photochemistry)	7.65 **	3.44	9.13 ***	0.63	2.91 ***	0.9	1.32
	ETo/CS (energy used for electron transport)	5.18 *	0.73	6.48 ***	1.98	2.7 ***	1.26	0.62
	RC/CSo (number of active reaction centers in the state of the fully oxidized PSII reaction center)	2.68	2.42	6.12 ***	0.68	2.93 ***	1.23	0.51
After two weeks of drought (maintaining 20% of soil relative humidity)	Chlorophyll *a* content (µg/g of d.w.)	1749.63 ***	17.53 ***	8.42 ***	0.85	9.46 ***	1.46	0.59
	Chlorophyll *b* content (µg/g of d.w.)	1345.86 ***	4.45 *	3.02 ***	1.66	4.71 ***	0.87	0.96
	Chlorophyll *a* and chlorophyll *b* content (µg/g of d.w.)	1735.11 ***	13.28 ***	6.23 ***	1.16	7.78 ***	1.06	0.63
	Carotenoids content (µg/g of d.w.)	1985.13 ***	2.6	7.28 ***	1.87	10.64 ***	1.46	1.11
	Fv/Fm (maximum photochemical efficiency of PS II)	4.16 *	8.32 **	11.66 ***	0.52	2.84 ***	2.59 **	0.99
	Area (pool size of electron acceptors from PSII)	150.4 ***	0.03	10.95 ***	3.54	2.85 ***	1.02	1.46
	PI (overall performance index of PSII photochemistry)	69.23 ***	4.08 *	11.77 ***	0.01	2.61 **	1.29	1.38
	ETo/CS (energy used for electron transport)	65.91 ***	0.99	7.71 ***	0.73	5.55 ***	1.63	1.08
	RC/CSo (number of active reaction centers in the state of the fully oxidized PSII reaction center)	118.05 ***	0.79	9.87 ***	0.5	6.01 ***	1.89 *	1.96 *
Mass of stems/plant (g)		318.02 ***	1.24	10.41 ***	1.34	3.58 ***	1.66	0.82
Number of grains		38.79 ***	0.67	35.12 ***	0.29	4.48 ***	4.56 ***	0.4
Mass of grains/plant (g)		50.01 ***	1.45	33.73 ***	0.02	6.42 ***	4.09 ***	0.67

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

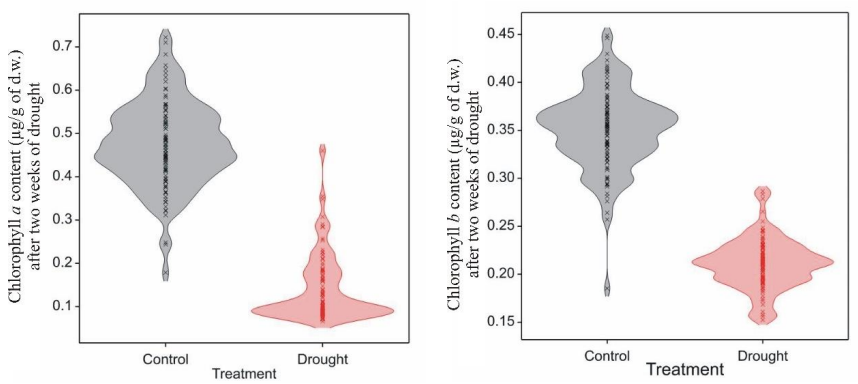

Figure 1. (**A**) The density plot of chlorophyll *a* content (μg/g of d.w.) after two weeks of drought for treatments. (**B**) The density plot of chlorophyll *b* content (μg/g of d.w.) after two weeks of drought for treatments.

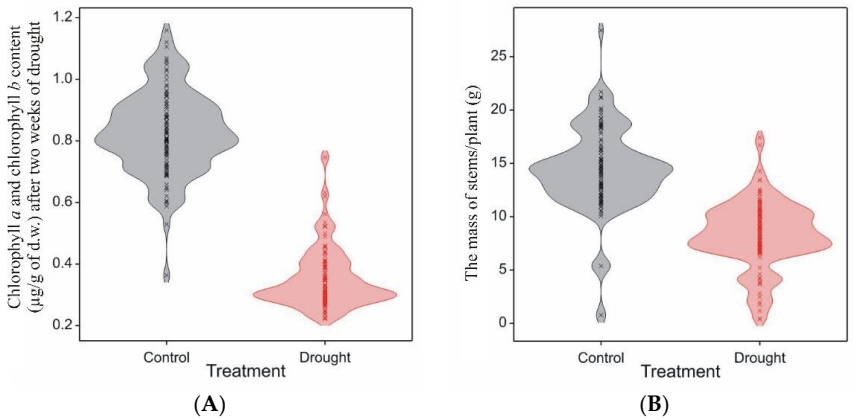

Figure 2. (**A**) The density plot of chlorophyll *a* and chlorophyll *b* content (μg/g of d.w.) after two weeks of drought for treatments. (**B**) The density plot of the mass of stems/plant (g) for treatments.

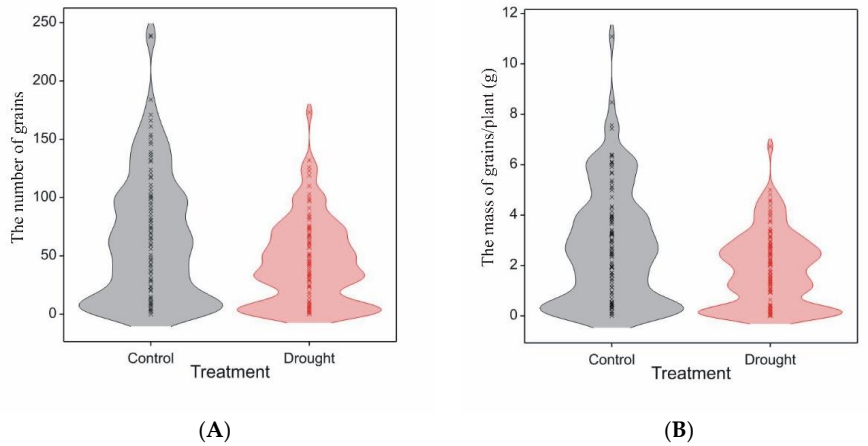

Figure 3. (**A**) The density plot of the number of grains for treatments. (**B**) The density plot of the mass of grains/plant (g) for treatments.

Significant positive relationships were detected between 31 pairs of traits in drought treatment (Table 2, Figure 4). However, 22 pairs of observed traits were correlated negatively (Table 2, Figure 4). The maximum photochemical efficiency of the PSII photosystem (Fv/Fm) in drought conditions on the first day of drought maintained similar activity even after two weeks of drought since there was a significant positive correlation, so the genotypes with higher values

of Fv/Fm could be considered less susceptible to drought stress. The maximum photochemical efficiency on the first day of drought also positively correlated with the overall performance index of the PS II photosystem (PI), and the same relation was observed after two weeks of drought (Table 2). The chlorophyll *a* content on the first day of drought negatively correlated with the number of grains/plant and mass of grains/plant; an identical tendency was observed when considering chlorophyll *a* content after two weeks of drought.

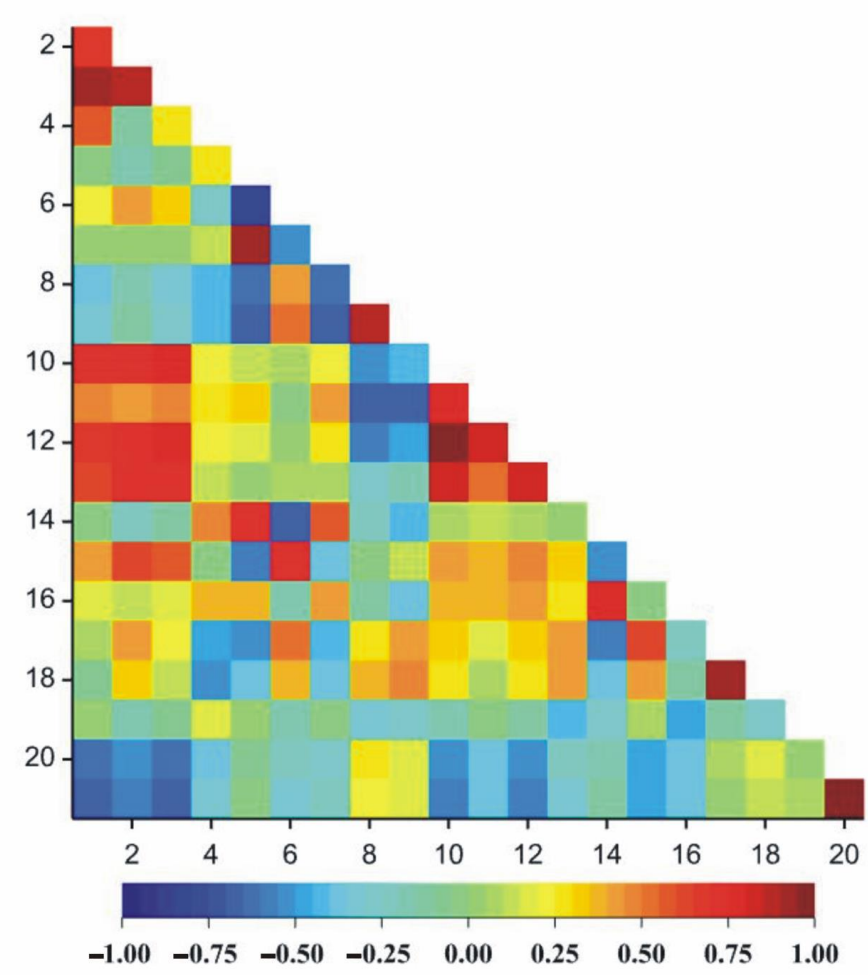

Figure 4. A heatmap showing correlation coefficients between all pairs of observed traits in drought treatment. [1—chlorophyll *a* content (µg/g of d.w.) on the first day of drought; 2—chlorophyll *b* content (µg/g of d.w.) on the first day of drought; 3—chlorophyll *a* and chlorophyll *b* content (µg/g of d.w.) on the first day of drought; 4—carotenoid content (µg/g of d.w.) on the first day of drought; 5 —Fv/Fm (maximum photochemical efficiency of PS II) on the first day of drought; 6—area (pool size of electron acceptors from PSII) on the first day of drought; 7—PI (overall performance index of PSII photochemistry) on the first day of drought; 8—ETo/CS (energy used for electron transport) on the first day of drought; 9—RC/CSo (the number of active reaction centers in the state of the fully oxidized PSII reaction center) on the first day of drought; 10—chlorophyll *a* content (µg/g of d.w.) after two weeks of drought; 11—chlorophyll *b* content (µg/g of d.w.) after two weeks of drought; 12—chlorophyll *a* and chlorophyll *b* content (µg/g of d.w.) after two weeks of drought; 13—carotenoid content (µg/g of d.w.) after two weeks of drought; 14—Fv/Fm (maximum photochemical efficiency of PS II) after two weeks of drought; 15—area (pool size of electron acceptors from PSII) after two weeks of drought; 16—PI (overall performance index of PSII photochemistry) after two weeks of drought; 17—ETo/CS (energy used for electron transport) after two weeks of drought; 18—RC/CSo (the number of active reaction centers in the state of the fully oxidized PSII reaction center) after two weeks of drought; 19—the mass of stems/plant (g); 20—the number of grains; 21—the mass of grains/plant (g)].

Table 2. Correlation coefficient between observed traits in drought treatment.

Trait	t [1]	t [2]	t [3]	t [4]	t [5]	t [6]	t [7]	t [8]	t [9]	t [10]	t [11]	t [12]	t [13]	t [14]	t [15]	t [16]	t [17]	t [18]	t [19]	t [20]
t [2]	0.69																			
t [3]	0.94	0.90																		
t [4]	0.57	−0.11	0.30																	
t [5]	−0.02	−0.17	−0.09	0.26																
t [6]	0.21	0.44	0.34	−0.28	−0.83															
t [7]	0.05	0.02	0.04	0.12	0.91	−0.54														
t [8]	−0.37	−0.19	−0.31	−0.41	−0.60	0.43	−0.62													
t [9]	−0.31	−0.12	−0.25	−0.41	−0.66	0.50	−0.67	0.85												
t [10]	0.72	0.74	0.79	0.22	0.14	0.06	0.22	−0.51	−0.41											
t [11]	0.47	0.42	0.49	0.27	0.31	−0.01	0.42	−0.67	−0.66	0.76										
t [12]	0.69	0.71	0.76	0.24	0.18	0.05	0.27	−0.56	−0.48	0.99	0.84									
t [13]	0.63	0.73	0.73	0.10	0.04	0.08	0.08	−0.26	−0.20	0.85	0.53	0.82								
t [14]	−0.02	−0.25	−0.13	0.47	0.74	−0.66	0.58	−0.22	−0.43	0.06	0.14	0.08	0.01							
t [15]	0.43	0.62	0.56	0.01	−0.59	0.75	−0.38	−0.05	0.13	0.45	0.38	0.45	0.33	−0.51						
t [16]	0.18	0.15	0.18	0.35	0.40	−0.19	0.40	−0.14	−0.38	0.40	0.39	0.41	0.29	0.80	−0.04					
t [17]	0.07	0.45	0.25	−0.45	−0.53	0.54	−0.42	0.29	0.41	0.33	0.15	0.31	0.45	−0.57	0.63	−0.22				
t [18]	−0.09	0.34	0.10	−0.52	−0.39	0.37	−0.36	0.36	0.49	0.30	0.09	0.26	0.44	−0.40	0.42	−0.12	0.94			
t [19]	0.01	−0.18	−0.08	0.16	0.04	−0.19	−0.02	−0.34	−0.27	−0.18	−0.01	−0.15	−0.41	−0.30	0.10	−0.48	−0.12	−0.29		
t [20]	−0.63	−0.52	−0.63	−0.36	−0.10	−0.25	−0.29	0.26	0.16	−0.52	−0.35	−0.51	−0.25	−0.16	−0.45	−0.37	0.06	0.17	0.05	
t [21]	−0.66	−0.59	−0.69	−0.32	−0.05	−0.31	−0.25	0.23	0.16	−0.56	−0.39	−0.55	−0.31	−0.13	−0.50	−0.39	0.01	0.13	0.08	0.99

Green—positive significance, red—negative significance; t [1]—chlorophyll a content (µg/g of d.w.) on the first day of drought, t [2]—chlorophyll b content (µg/g of d.w.) on the first day of drought, t [3]—chlorophyll a and chlorophyll b content (µg/g of d.w.) on the first day of drought, t [4]—carotenoid content (µg/g of d.w.) on the first day of drought, t [5]—Fv/Fm (maximum photochemical efficiency of PS II) on the first day of drought, t [6]—area (pool size of electron acceptors from PSII) on the first day of drought, t [7]—PI (overall performance index of PSII photochemistry) on the first day of drought, t [8]—ETo/CS (energy used for electron transport) on the first day of drought, t [9]—RC/CSo (the number of active reaction centers in the state of the fully oxidized PSII reaction center) on the first day of drought, t [10]—chlorophyll a content (µg/g of d.w.) after two weeks of drought, t [11]—chlorophyll b content (µg/g of d.w.) after two weeks of drought, t [12]—chlorophyll a and chlorophyll b content (µg/g of d.w.) after two weeks of drought, t [13]—carotenoid content (µg/g of d.w.) after two weeks of drought, t [14]—Fv/Fm (maximum photochemical efficiency of PS II) after two weeks of drought, t [15]—area (pool size of electron acceptors from PSII) after two weeks of drought, t [16]—PI (overall performance index of PSII photochemistry) after two weeks of drought, t [17]—ETo/CS (energy used for electron transport) after two weeks of drought, t [18]—RC/CSo (number of active reaction centers in the state of the fully oxidized PSII reaction center) after two weeks of drought, t [19]—the mass of stems/plant (g), t [20]—the number of grains, t [21]—the mass of grains/plant (g). Each trait was of varying significance and had different shares in the joint multivariate variation in the examined lines. Analysis of the first two canonical variates for 15 lines regarding the 21 quantitative traits is shown in Figure 5 (for both treatments: control and drought) and Figure 6 (only for drought). In the graphs the coordinates of the point for a certain line are the values for the first and second canonical variate. For both treatments: control and drought, the first two canonical variates accounted for 66.47% of the total variability between the individual genotypes (Figure 5). The most significant positive linear relationship with the first canonical variate was found for t1, t4, t6, t10, t11, t12, t13, t15, t16, t17, t18, and t19, whereas the most significant negative linear relationship was found for t2. The second canonical variate was significantly negatively correlated with t18, t20, and t21. The highest variability in all 21 traits jointly calculated with Mahalanobis distances was found for line 119 in control and 9 in drought (the distance between them amounted to 13.696). The highest similarity was found between lines 42 and 43 (2.182) (data not shown). For only drought treatment, the first two canonical variates accounted for 66.16% of the total variability between the individual genotypes (Figure 6). The most significant negative linear relationship with the first canonical variate was found for t1, t2, t3, t10, t12, t13, t17, and t18. The second canonical variate was significantly positively correlated with t1, t3, t15, and t19, whereas it was significantly negatively correlated with t8, t20, and t21. The greatest variation in drought treatment in terms of all 21 traits jointly calculated with Mahalanobis distances was found for lines 42 and 83 (17.589). The greatest similarity was found between lines 23 and 26 (3.424) (data not shown).

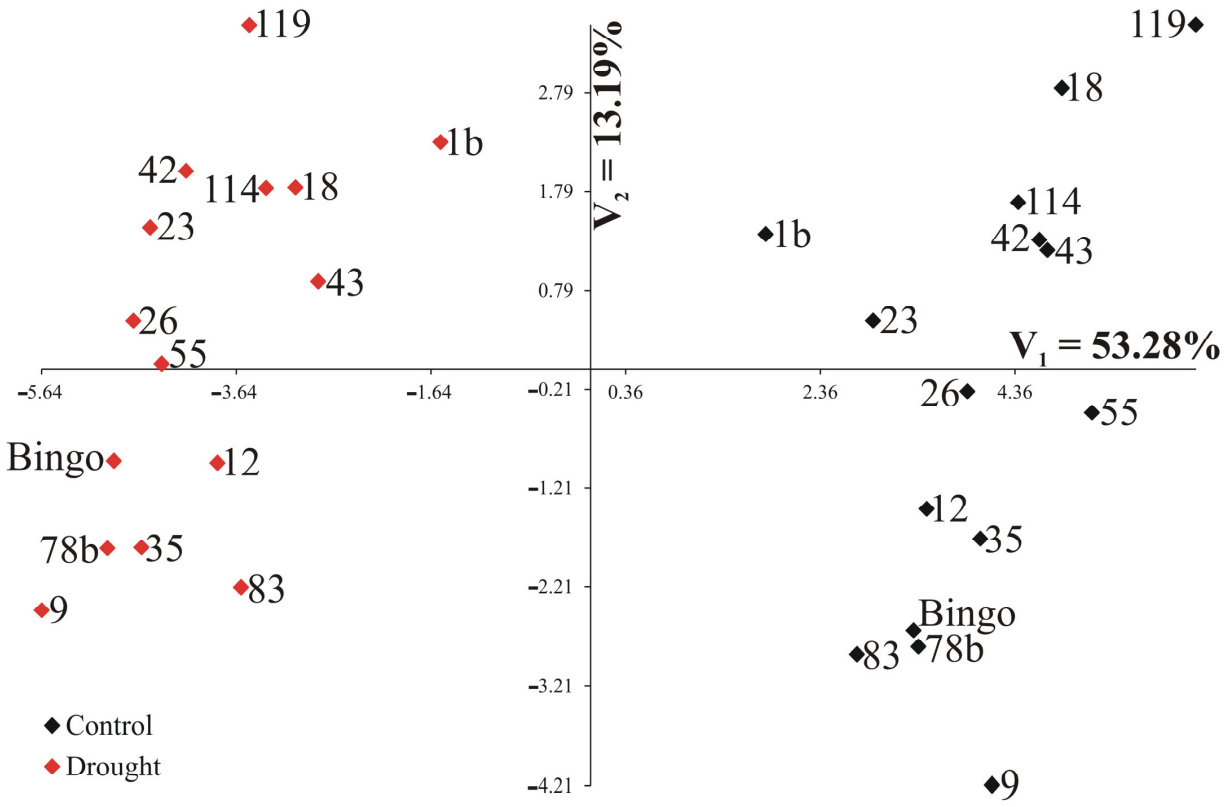

Figure 5. Distribution of 15 genotypes in the space of the first two canonical variables for both treatments: control and drought.

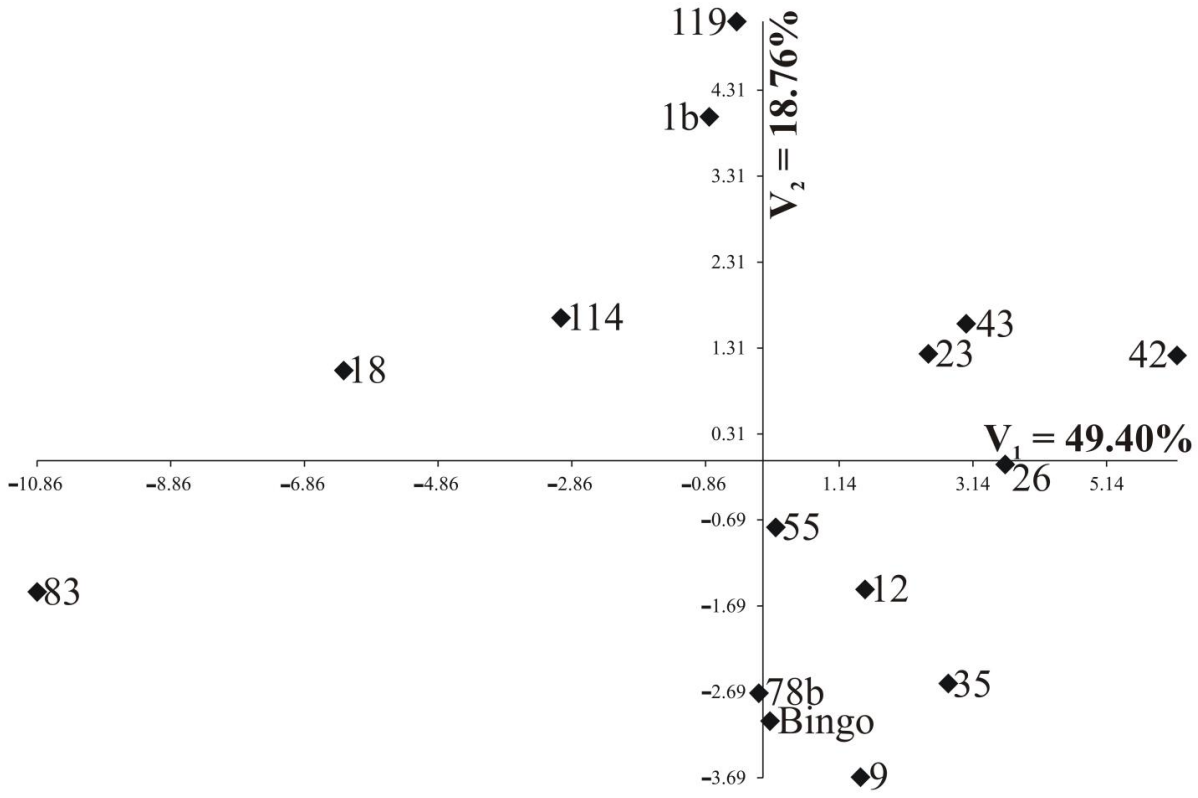

Figure 6. Distribution of 15 genotypes in the space of the first two canonical variables for drought.

4. Discussion

When some related species are crossed, the pollinator's chromosomes could be completely eliminated, resulting in haploid offspring production. This phenomenon is used in plant breeding to accelerate breeding work by obtaining a fully homozygous generation and is known as wide crossing [36]. When wide crossing of oat with maize is utilized, some maize chromosomes are not eliminated in embryogenesis; during mitotic divisions they behave like oat chromosomes and are permanently incorporated into the genome of newly created hybrids. OMA (oat × maize addition) lines can be useful genotypes in plant breeding. They are also helpful in mapping the maize genome [13]. Oat-maize hybrids demonstrate the habit of oats, but many of the genes located on the added maize chromosomes are expressed, and they could have an impact on the phenotype of the hybrids. Oat belongs to the group of plants possessing C3 photosynthesis, and this type of photosynthesis is associated with a significant occurrence of photorespiration, which under current climate conditions reduces the potential of plants by 40% [37]. The total chlorophyll content of a plant is correlated with the age of the plant and the plant condition. A reduction in all photosynthetic pigments is observed under stress conditions and in late stages of development. The occurrence of the intense green color of the aboveground organs during the entire vegetation period in agricultural plants may indicate a good supply of assimilates and the formation of higher yield [38]. In our research the drought resulted in a significant reduction in yield elements and the decrease in the general biomass of stems ranged from 57 to 68%. The study by Gholamin and Khayatnezhad [39] demonstrated that maize genotypes with a higher total chlorophyll content generated a higher yield in drought conditions than genotypes with naturally lower chlorophyll content. Changes in the content of photosynthetic pigments and damage of the photosynthetic apparatus as a result of drought stress have been observed in many plant species [40,41]. In the present study the impact of drought stress was very severe after two weeks of prolonged drought. The significant reduction in the sum of chlorophyll *a* and *b* observed amounted to 42% of the control plants, since the reduction in individual pigments was higher—in the case of chlorophyll *a* it was only 29.5% of control plants, but the carotenoid content was drastically low, reaching only 16% of control plants. A 15% decrease in the content of chlorophyll was also found in wheat growing under drought stress compared to the conditions of adequate water supply [42]. Water shortages in plants restrict proper progress of all life processes, leading to a number of reactions resulting in lower productivity. This results directly from lower efficiency of photosynthesis, which is often inhibited by damage to the PSII photosystem. Under normal conditions, most (about 80%) of the energy absorbed by chlorophylls is bound in NADPH and ATP, but some is transmitted as fluorescence (2–10%) and heat [43]. Under stress conditions, the efficiency of energy binding decreases—its excess is dissipated and can be observed in the form of fluorescence. The fluorescence emission disappears 8–10 s after the end of the emission of the stimulation. The reaction of plants to drought stress, i.e., changes in chlorophyll fluorescence, are observed as one of the first—much earlier than would visible, for example, per the regular habit of the plant [44]. For this reason, the method of measuring chlorophyll fluorescence is used in many studies on plant responses to both abiotic and biotic stresses. A great facilitation in the use of these measurements is a non-invasive method, and the small size of the apparatus allows measurements in field conditions [45,46]. The measurement methodology using handheld devices is based on keeping a fragment of the leaf blade in the dark for at least 20 min. After this period of time and after illumination, fluorescence very quickly from the basic value Fo reaches the maximum value Fm. With the initiation of photosynthesis after a period of darkness, the slow decline of fluorescence to its stationary state begins. The maximum photochemical efficiency of PSII (Fv/Fm) is reduced due to various stress factors damaging PSII, which reduces the efficiency of electron transport. The rate of electron transport through the reaction centers (ETo/CS) is also reduced due to stress. The overall PSII performance index (PI) also describes the plant's response to the ambient conditions. In the current results of our investigation the effect of drought stress was significant in all

considered PSII indices and the reduction of the mean value ranged from 56% to 96% of the value of the control plants. The most affected parameters of the PSII photosystem were PI (56% of the value of the control plants) and the area–pool size of the electron acceptors from PSII (62% of the control plants). To a large extent, it is correlated with the availability of water and the occurrence of drought conditions [45,47]. Studies of wheat under drought-stress conditions showed 28% higher fluorescence compared to control conditions with proper water supply [42]. Changes in chlorophyll fluorescence parameters have also been observed during biotic stresses. In barley DH lines inoculated with *Fusarium culmorum* spores, a decrease in both Fv/Fm and PI indices was found [48], and other CF parameters, including RC/CSo (studied in the present research), were reduced after infection [49]. In our studies, the decrease in the value of Fv/Fm was 94% of that of the control plants. Since the value of PI amounted to 81% of that of control plants on the first day of drought, both changes in parameters under drought were statistically significant. Our investigation revealed significant positive relationships between 31 pairs of traits in the drought treatment, thought 22 pairs of observed traits were correlated negatively. The maximum photochemical efficiency of the PSII photosystem (Fv/Fm) in drought conditions on the first day of drought maintained similar activity even after two weeks of drought since there was a significant positive correlation, so the genotypes with higher values of Fv/Fm could be considered less susceptible to drought stress. The maximum photochemical efficiency on the first day of drought was also positively correlated with the overall performance index of the PS II photosystem (PI). The same relationship was observed after two weeks of drought. Therefore, PS II photosystem parameters could be considered a good tool to distinguish plant genotypes that are less susceptible to drought stress. Another finding is that most of the lines under stress conditions drastically reduced yield-related traits, but there were two lines (9 and 78b) with high yielding potential comparable to that of commercial cv. Bingo in the control condition (no drought stress). Those lines possessed higher yielding potential under drought stress as well, which was expressed in the much lower reduction in the number of grains/plant and mass of grains/plant.

5. Conclusions

Based on the present findings it can be concluded that most of the lines under stress conditions drastically reduced yield-related traits. Furthermore, two lines with high yielding potential were found to be comparable to commercial cv. Bingo in the control condition (no drought stress). Therefore, those two lines, 9 and 78b, expressed higher yielding potential under drought stress as well, which was expressed in the much lower reduction in the number of grains/plant and mass of grains/plant. Information in this regard would help breeders to make better selections of desirable parents to develop an efficient breeding program to obtain new and drought-resistant genotypes with high grain-yield potential for food and nutritional security.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13020243/s1, Table S1: Mean values, standard deviations and Fisher's least significant differences (LSDs) for chlorophyll *a* content (μg/g of d.w.) in the first day of drought. Table S2: Mean values, standard deviations and Fisher's least significant differences (LSDs) for chlorophyll *b* content (μg/g of d.w.) in the first day of drought. Table S3: Mean values, standard deviations and Fisher's least significant differences (LSDs) for chlorophyll *a* and chlorophyll *b* content (μg/g of d.w.) in the first day of drought. Table S4: Mean values, standard deviations and Fisher's least significant differences (LSDs) for carotenoids content (μg/g of d.w.) in the first day of drought. Table S5: Mean values, standard deviations and Fisher's least significant differences (LSDs) for Fv/Fm (maximum photochemical efficiency of PS II) in the first day of drought. Table S6: Mean values, standard deviations and Fisher's least significant differences (LSDs) for area (pool size of electron acceptors from PSII) in the first day of drought. Table S7: Mean values, standard deviations and Fisher's least significant differences (LSDs) for PI (overall performance index of PSII photochemistry) in the first day of drought. Table S8: Mean values, standard deviations and Fisher's least significant differences (LSDs) for ETo/CS (energy used for electron transport) in the first day of

drought. Table S9: Mean values, standard deviations and Fisher's least significant differences (LSDs) for RC/CSo (number of active reaction centres in the state of fully oxidized PSII reaction center) in the first day of drought. Table S10: Mean values, standard deviations and Fisher's least significant differences (LSDs) for chlorophyll *a* content (μg/g of d.w.) after two weeks of drought. Table S11: Mean values, standard deviations and Fisher's least significant differences (LSDs) for chlorophyll *b* content (μg/g of d.w.) after two weeks of drought. Table S12: Mean values, standard deviations and Fisher's least significant differences (LSDs) for chlorophyll *a* and chlorophyll *b* content (μg/g of d.w.) after two weeks of drought. Table S13: Mean values, standard deviations and Fisher's least significant differences (LSDs) for carotenoids content (μg/g of d.w.) after two weeks of drought. Table S14: Mean values, standard deviations and Fisher's least significant differences (LSDs) for Fv/Fm (maximum photochemical efficiency of PS II) after two weeks of drought. Table S15: Mean values, standard deviations and Fisher's least significant differences (LSDs) for area (pool size of electron acceptors from PSII) after two weeks of drought. Table S16: Mean values, standard deviations and Fisher's least significant differences (LSDs) for PI (overall performance index of PSII photochemistry) after two weeks of drought. Table S17: Mean values, standard deviations and Fisher's least significant differences (LSDs) for ETo/CS (energy used for electron transport) after two weeks of drought. Table S18: Mean values, standard deviations and Fisher's least significant differences (LSDs) for RC/CSo (number of active reaction centres in the state of fully oxidized PSII reaction center) after two weeks of drought. Table S19: Mean values, standard deviations and Fisher's least significant differences (LSDs) for the mass of stems/plant (g). Table S20: Mean values, standard deviations and Fisher's least significant differences (LSDs) for the number of grains. Table S21: Mean values, standard deviations and Fisher's least significant differences (LSDs) for the mass of grains/plant (g).

Author Contributions: Conceptualization and methodology, T.W. and E.S.; investigation, T.W., R.B., E.S. and M.W.; resources T.W.; writing—original draft preparation, T.W. and J.B.; writing—review and editing, T.W., A.S. and E.S.; visualization, J.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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