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Article

Delineation of Physiological, Agronomic and Genetic Responses of Different Wheat Genotypes under Drought Condition

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Abstract: Abioticstress such as drought is a potential threat posing a severe challenge to wheat production across the globe. The current study comparatively delineated the performance of elite Pakistani bread wheat genotypes at physiological (chlorophyll, canopy temperature, cell membrane percentage stability and leaf relative water content), agronomic (plant height, tillers plant^{-1}, flag leaf area, spike length, spikelets spike^{-1}, grains spike^{-1}, grain yield spike^{-1}, thousand grain weight and plant biomass) and genetic (*TaDREB1A*, *TaGROS-A*, *TaLEA3*, *TaHSFA1a*, *TaWRKY44* and *TaEXPA2*) levels. Atri-replicate experiment was conducted in a two factorial arrangement using RCBD, and data were analyzed statistically using the computer-based programsStatistix8.1 and R-studio. In general, all wheat genotypes illustrated significant ($p \leq 0.05$) alterations in physiological and agronomic traits under drought stress as compared to the control; however, this alteration was significantly ($p \leq 0.05$) different among all genotypes owing to their varying genetic potential. Furthermore, these genotypes were evaluated for the extent of the association of physiological and agronomic traits using PCA, correlation and heatmap analysis, which proved statistically significant variation in the paired association of traits among all genotypes during drought stress as compared to the control. In addition, based on statistical evaluations, the genotypes Pakistan-13, Shahkar-13, AAS-11, Chakwal-86, Chakwal-50 and AUR-09 were found to be tolerant, while genotypes Anmol-97, Chakwal-97, Bhakkar-02 and BWP-97 were comparatively susceptible. Furthermore, these screened genotypes showed differential expression of drought-related genes, with relatively high expression in tolerant genotypes compared to susceptible genotypes. The current study concluded that physiological, agronomic and molecular characteristics are significantly interconnected, and these associations determine the end productivity of wheat genotypes during abiotic stress. Therefore, their integrated study can enhance the pace of wheat breeding for drought tolerance in the near future.

Keywords: drought; PCA; heatmap; correlation; gene expression

1. Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop used as the main staple food in many countries across the globe, but its yield is compromised because of many biotic and abiotic factors, among which drought is the most devastating constraint [1].Drought stress is a potential threat to crop production in arid and semi-arid regions of the world owing to its tendency to inhibit the development and growth of the plant [2]. In addition, the frequency and severity of drought around the world have increased because of the varying precipitation and rising temperature caused by climate change [1]. Various studies have demonstrated that climate change not only negatively affects the current worldwide food production but will also have a more severe impact on future crop production [2–4]. A large area of the world is severely affected by stresses imposed by multivariate environments, with drought being the most important one [5]. It necessitates the introduction of crop cultivars with a high degree of drought tolerance in cropping systems [1]. Abiotic stresses, particularly drought, disrupt the plant water content, leading to decreased membrane fluidity [5]. Furthermore, continuous electrolyte leakage leads to cell death. Drought stress changes the concentrations of various ions, such as K and Mg, which are essential to sustain various physiological attributes, including the chlorophyll content and photosynthesis rate [6]. Plants are equipped with natural processes that counter the detrimental effects of stress at a molecular level. From this perspective, the varying expression of stress-associated genes is worthy of consideration, which marks the varying tendencies of genotypes to resist stress [7]. Drought stress perturbs physiological processes within plants, such as a reduction in chlorophyll and enhanced canopy temperature (CT), which leads to a reduction in photosynthesis and other metabolic activities within plants [8]. In addition, drought stress triggers the production of ROS, which also results in the deterioration of membrane integrity, causing a reduction in the membrane stability index (MSI) and leaf relative water content (LRWC) [9].Efficient physiological processes trigger metabolic activities, leading to high agronomic yield in terms of grain number, grain weight and plant biomass [8]. Plants, like every biological system, have a built-in tendency to counter the effects of abiotic stresses, including drought; however, they show resilience up to a certain extent [9]. Wheat is an ideal system to unravel the mysterious dynamics of drought stress, which triggers the expression of various genes regulating signaling pathways in it [10]. Fromthis perspective, the regulation of characterized genes is an important indicator to mark a genotype as being tolerant or susceptible. For instance, the overexpression of *ARGOS* genes enhances drought tolerance in maize and wheat through the activation of an ethylene-dependent pathway [11]. Moreover, *LEA* proteins, belonging to the class of late-induced stress-responsive genes, are activated in wheat during water deficit condition sand initiate various mechanisms that induce a state of stress tolerance [12]. Dehydration-responsive element binding (DREB) proteins also regulate some functional genes imparting stress tolerance [13]. Similarly, heat shock factors (Hsf) play a pivotal role in inducing thermal and drought stress tolerance in wheat due to their role as osmoprotectants [14]. The overexpression of genes from the *WRKY* family imparts tolerance to abiotic stresses owing to the activation of the antioxidant system and has been studied in model plant systems such asrice and Arabidopsis; however, there has been limited research in wheat owing to its large and complex genome [15]. The *TaEXPA2* gene from the expansin family loosens plant cell walls and regulates the drought tolerance phenotype when it is overexpressed, whereas its down regulation imparts drought susceptibility in wheat [16]. Drought is a major limiting constraint, particularly in arid zones of the world, and wheat is highly susceptible to it [17]. Correspondingly, in Pakistan, 20 percent of wheat production area lies in rainfed regions where seasonal drought spellsare a major problem. Although various wheat cultivars have been developed for drought tolerance in multivariate environments, their performance has been variable. In the current study, elite Pakistani drought-tolerant wheat genotypes were collected and reinvestigated for their performance at the molecular, physiological and agronomic levels. In order to screen out tolerant wheat genotypes in an environment with diverse conditions, it is important to highlight selection indicators at

physiological, agronomic and molecular levels. From this perspective, the current research hypothesized that agronomic, physiological and molecular traits would respond differently to control and drought stress conditions, and their relative performance will provide a pathway for future drought tolerance breeding programs.

2. Materials and Methods

Elite Pakistani drought-tolerant wheat genotypes (Table S1) collected from various research institutions of Pakistan were evaluated in a pot experiment in the research area of Department of Plant Breeding and Genetics, PMAS Arid Agriculture University, Rawalpindi, Pakistan. Atri-replicate experiment was conducted with an RCBD design using a two factorial arrangement, with genotype as one factor and drought treatment as the second factor.

2.1. Crop Husbandry and Treatment Imposition

In the pot experiment, six seeds were planted in each plastic pot of size 2 L, and optimum practices such as hoeing and weeding were continued throughout the crop growing period. Physiological and morphological traits of all wheat genotypes were examined by arranging the pots in two different environments: control and moisture stress. At the seedling stage, thinning was performed, and three plants per pot were maintained. Under the ordinary situation, the control set of plants was retained in an open environment, while drought-stress-treated pots were kept under arain shelter, and at the pre-anthesis stage (95 $\pm$ 10 DAS), plants were exposed to a dry spell cycle. Pots of the control sets were watered normally on an optimum basis whenever required to maintain a normal well-watered level. By withholding the water supply for about 10 to 15 days, drought stress was induced until drought symptoms started to appear in the form of temporary leaf rolling or wilting. When plants reached the pre-anthesis stage, the control set of pots was watered normally, while for the stress pot arrangement, irrigation was intermitted. After 10 to 15 days, plants in stress treatment pots were watered normally. To minimize the effect of positional errors, randomly placed pots in the glass house were repositioned on alternate days until the plant attained physiological maturity.

2.2. Quantification of Physiological Traits

Canopy temperature (CT) was taken on sunny days with the help of infrared thermometer (Testo-845, Titisee-Neustadt, Germany). Chlorophyll content was measured by using the SPAD-502 (Spectrum Technologies, Bridgend, UK).At three different points, data were recorded on each leaf, and as the final reading for each leaf, their average was calculated. RWC was calculated following the method used by Bannister [18] with the help of formula RWC = [(FW $-$ DW)/(TW $-$ DW) $\times$ 100, where FW = fresh weight; DW = dry weight; TW = total weight]. Moreover, cell membrane stability percentage (CMPS) of three randomly selected leaf samples was calculated using the formula CMPS = [(1 $-$ (T1/T2))/(1 $-$ (C1/C2))} $\times$ 100, where T1 = stress sample conductance before autoclaving; T2 = stress sample conductance after autoclaving; C1 = control sample conductance before autoclaving; C2 = control sample conductance after autoclaving].

2.3. Quantification of Agronomic Parameters

2.3.1. Growth Traits

Among growth traits, flag leaf area (FLA) was calculated using a scale by following Farooq et al.'s [19] method. Likewise, plant height (PH) of five randomly selected plants was calculated from shoot base to apex at the time of maturity and averaged. Furthermore, tillers plant^{-1} (TPP) were estimated from three to five randomly selected plants of all genotypes, and mean values were obtained.

2.3.2. Yield Traits

Five spikes were selected from randomly tagged plants of all genotypes, and their length (SL) was measured by numeric scale and averaged. From the same number of

spikes, spikelets spike^{-1} (SPS) were counted and then averaged. Then, number of grains spike^{-1} (GPS) was counted for each genotype and averaged afterward. The thousand grain weight (TGW) of each wheat genotype was measured by electronic weighing balance (Bioevopeak, Jinan, China). Grain yield spike^{-1} (GYP) was recorded from all fertile spikes of five randomly tagged plants of all genotypes and then converted into final yield to obtainyield in grams plant^{-1}. For plant biomass (PBM), at the time of harvesting, three randomly selected plants were weighed separately with the help of an electric balance (Bioevopeak, China) before threshing toobtain their biological yield in grams, and their average was calculated.

2.4. Statistical Analysis

The collected data were analyzed statistically using computer-based programs Statistix8.1 (McGraw-Hill 2008) and R-studio RStudio version 1.3.959 (RStudio Team 2020) following the procedure used by Alghabari et al. [20].

2.5. Gene Expression Analysis

For expression studies of drought-related genes (*TaDREB1A*, *TaGROS-A*, *TaLEA3*, *TaHSFA1a*, *TaWRKY44* and *TaEXPA2*), RNA was extracted from selected tolerant and susceptible wheat genotypes (based upon physiological and agronomic evaluation) with the help of Qiagen RNeasy kit (Qiagen, German Town, Vail, CO, USA) according to a set protocol used by Li et al. [21]. Subsequently, cDNA was prepared by following the methodology of Ahmed et al. [11]. For this purpose, a total of 2 µg of RNA was used in accordance with the manufacturer's instructions. Afterward, qRT-PCR analysis was conducted. Additionally, the expression of genes was normalized with the help of the TaActin1-expressing gene. The primers used in the expression study are mentioned in Table S2.

3. Results

3.1. Physiological Parameters

As shown in Table 1, physiological parameters such as Chl, CMPS and LRWC showed significant reductions ($p \leq 0.05$), while CT showed a significant rise in all genotypes under drought stress as compared to the control (Table 1). Among genotypes, Pakistan-13, Shahkar-13, AAS-11, Chakwal-86, Chakwal-50 and AUR-09 showed the maximum while genotypes Anmol-97, Chakwal-97, Bhakkar-02 and BWP-97 had the minimum values of Chl, CMPS and LRWC. However, the aforementioned genotypes exhibited opposite trends for CT, as shown in Table 1. Chlorophyll content under control conditions had mean values ranging from 35.5 to 51%, while under drought stress treatment, mean values ranged from 27 to 41% (Table 1). Among genotypes, AAS-11 showed the highest Chl content (41%), while Lasani-08 had the lowest (27%) (Table 1). Furthermore, under control conditions, CT had mean values ranging from 22 to 25 °C, while under drought stress, mean values ranged from 24 to 28 °C (Table 1). In addition, under stress treatment, Pakistan-13 showed the maximum (28 °C) while Chakwal-86 had the minimum (24 °C) mean value of CT (Table 1). Under control conditions, CMPS had mean values varying from 67 to 82%, whereas under drought stress, the mean values varied from 44 to 67% (Table 1). Among genotypes, Pakistan-13 showed the highest (67%) while Lasani-08 showed the lowest value (44%) of CMPS (Table 1). Correspondingly, LRWC demonstrated maximum mean values ranging from 37 to 88% under control conditions, as compared to drought stress, in which they ranged from 22 to 45% (Table 1). The genotype Khyber-87 had the highest (45%) while Anamol-91 had the lowest (22%) mean values of LRWC under stress (Table 1).

Table 1. Effect of control and water-stressed conditions on physiological parameters of different wheat genotypes.

Genotypes	Chl %			CMPS (%)			CT			LRWC %		
	Control	Drought	Difference	Control	Drought	Difference	Control	Drought	Difference	Control	Drought	Difference
Pakistan-13	51.08	40.1	10.98	81.81	66.54	15.27	22.88	27.54	−4.66	51.18	28.54	22.64
Shahkar-13	37.28	28.1	9.18	69.97	46.87	23.1	24.4	26.8	−2.4	62.22	36.16	26.06
Anmaol-91	50.55	39.13	11.42	69.24	56.35	12.89	23.41	25.91	−2.5	73.4	22.92	50.48
AARI-11	49.51	36.43	13.08	75.51	57.46	18.05	23.48	24.94	−1.46	56.39	30.43	25.96
Punjab-11	49.61	33.9	15.71	74.34	60.94	13.4	23.72	25.19	−1.47	39.96	24.25	15.71
AAS-11	50.48	41.16	9.32	82.81	66.9	15.91	22.98	24.34	−1.36	70.63	31.4	39.23
Millet-11	36.01	28.56	7.45	81.62	63.31	18.31	23.14	24.81	−1.67	93.61	42.46	51.15
Chakwal-50	48.81	38.2	10.61	75.21	60.71	14.5	23.51	24.82	−1.31	86.26	43.48	42.78
Pirsabak-08	36.75	28	8.75	68.4	45.11	23.29	24.65	27.29	−2.64	54.12	28.06	26.06
Lasani-08	35.15	26.9	8.25	67.34	44.22	23.12	25.11	27.63	−2.52	73.48	35.06	38.42
AUR-09	50.01	37.16	12.85	75.22	61.94	13.28	23.24	24.38	−1.14	65.1	34.86	30.24
Pirsabal-05	49.08	30.16	18.92	74.02	57.95	16.07	23.58	25.31	−1.73	68.58	32.71	35.87
Bhakkar-02	50.55	35.03	15.52	72.13	60.55	11.58	23.31	24.38	−1.07	85.29	45.63	39.66
Chakwal-97	45.55	36.26	9.29	71.03	58.07	12.96	23.54	24.99	−1.45	75.97	35.86	40.11
Sariab-92	43.58	29.73	13.85	71.62	56.11	15.51	22.91	24.64	−1.73	61.53	32.48	29.05
Inqalab-91	49.85	38.76	11.09	68.64	55.64	13	23.64	24.61	−0.97	71.7	36.16	35.54
Pirsabak-91	46.05	36.43	9.62	76.23	60.03	16.2	23.64	24.83	−1.19	82.31	43.52	38.79
AUR-10	48.55	31.93	16.62	76.19	63.04	13.15	23.41	24.81	−1.4	83.58	46.23	37.35
Bahawalpur-2000	50.1	40.93	9.17	72.19	52.17	20.02	23.31	24.58	−1.27	81.02	42.69	38.33
Bwp-97	45.61	38.06	7.55	72.89	56.49	16.4	23.21	24.54	−1.33	51.75	25.15	26.6
Chakwal-86	48.85	36.43	12.42	69.58	55.76	13.82	22.98	24.18	−1.2	59.67	27.37	32.3
Baranai-83	37.08	27.86	9.22	73.71	58.86	14.85	23.14	24.34	−1.2	58.33	27.14	31.19
Sarhad-82	41.38	33.7	7.68	75.18	58.29	16.89	24.31	26.42	−2.11	73.89	36.36	37.53
Pak-81	50.28	40.56	9.72	70.6	54.41	16.19	23.41	24.54	−1.13	84.35	39.18	45.17
SA-75	49.38	40.83	8.55	71.63	59.84	11.79	23.64	24.58	−0.94	51.41	24.06	27.35
Lyp-73	49.38	40.83	8.55	71.63	59.84	11.79	23.64	24.58	−0.94	51.41	24.06	27.35
Bahawalpur-79	47.95	37.56	10.39	72.86	60.58	12.28	23.54	25.32	−1.78	65.49	32.44	33.05
Fsd-83	49.21	35.83	13.38	69.47	57.06	12.41	23.41	25.38	−1.97	50.51	23.29	27.22
Punjab-85	44.71	35.6	9.11	72.64	60.86	11.78	23.54	24.71	−1.17	37.51	21.94	15.57
Khyber-87	50.05	39.91	10.14	67.17	55.86	11.31	24.15	26.26	−2.11	56.57	25.59	30.98

CT, canopy temperature; CMPS, cell membrane percentage stability; LRWC, leaf relative water content. Indicated values are mean observations averaged after drought treatment application in normal treatments during tri-replicate experiment at $p \leq 0.05$.

3.2. Agronomic Traits

3.2.1. Growth Traits

Growth traits, for instance, PH, FLA and TPP, underwent significant ($p \leq 0.05$) decreases in all genotypes under drought stress as compared to the control (Table 1). Among genotypes, Anmol-97, Chakwal-97, Bhakkar-02 and BWP-97 showed the highest while Pakistan-13, Shahkar-13, AAS-11, Chakwal-86, Chakwal-50 and AUR-09 had the lowest reduction in growth traits, as indicated in Table 2. Growth traits, for instance, PH, FLA and TPP, showed significant ($p \leq 0.05$) variation among all genotypes under both control and stress conditions (Table 2). PH underwent a statistically distinct reduction under drought stress, with mean values ranging from 59 to 77 cm as compared to control conditions, in which mean values ranged from 62 to 89 cm (Table 2). Correspondingly, TPP had mean values from 3 to 8 under control conditions and mean values from 1.3 to 5.6 during drought stress (Table 2). Under drought stress treatment, Pakistan-13 showed the maximum mean value of TPP (5.7), while Chakwal-86 had the minimum (1.33) (Table 2). Likewise, under control conditions, FLA demonstrated higher means (31 to 37 cm^2)as compared to drought stress conditions (10 to 27 cm^2) (Table 2). Among genotypes, Anamol-91 showed the highest (27 cm^2) whereas Chakwal 50 showed the lowest mean value (10 cm^2) of FLA during drought stress (Table 2).

Table 2. Effect of control and water-stressed conditions on growth traits of different wheat genotypes.

Genotypes	FLA (cm^2)			TPP			PH (cm)		
	Control	Drought	Difference	Control	Drought	Difference	Control	Drought	Difference
Pakistan-13	36.98	26.82	10.16	7.86	5.76	2.10	83.33	77.66	5.67
Shahkar-13	30.88	20.77	10.11	6.45	4.33	2.12	65.11	61.66	3.45
Anmaol-91	36.98	27.04	9.94	7.78	5.45	2.33	72.31	67.67	4.64
AARI-11	36.49	27.02	9.47	7.61	5.66	1.95	75.12	68.33	6.79
Punjab-11	36.66	14.43	22.23	4.72	2.66	2.06	64.35	60.33	4.02
AAS-11	36.73	20.83	15.90	6.61	4.66	1.95	82.38	76.66	5.72
Millet-11	29.58	23.31	6.27	6.63	4.66	1.97	77.33	73.56	3.77
Chakwal-50	36.16	10.32	25.84	5.67	3.45	2.22	62.66	59.45	3.21
Pirsabak-08	30.70	22.83	7.87	5.20	3.33	1.87	68.30	61.33	6.97
Lasani-08	30.13	32.35	−2.22	6.67	4.66	2.01	72.45	68.56	3.89
AUR-09	36.63	24.84	11.79	5.59	3.66	1.93	81.39	77.34	4.05
Pirsabal-05	36.33	27.67	8.66	6.60	4.66	1.94	72.31	69.33	2.98
Bhakkar-02	36.93	26.57	10.36	6.32	4.33	1.99	89.66	81.27	8.39
Chakwal-97	34.54	25.05	9.49	7.29	5.33	1.96	71.29	65.33	5.96
Sariab-92	33.24	16.03	17.21	4.64	2.66	1.98	67.21	62.33	4.88
Inqalab-91	36.74	11.79	24.95	4.48	2.45	2.03	77.48	70.45	7.03
Pirsabak-91	34.84	21.77	13.07	7.62	5.66	1.96	73.36	68.66	4.70
AUR-10	35.98	18.57	17.41	6.30	4.33	1.97	78.51	73.33	5.18
Bahawalpur-2000	36.74	16.93	19.81	4.51	2.56	1.95	79.22	74.33	4.89
Bwp-97	34.41	21.50	12.91	5.45	3.78	1.67	78.33	75.67	2.66
Chakwal-86	35.91	24.43	11.48	3.45	1.33	2.12	78.45	72.33	6.12
Baranai-83	30.11	26.50	3.61	4.58	2.66	1.92	78.78	72.56	6.22
Sarhad-82	32.84	21.73	11.11	5.60	3.66	1.94	78.25	69.33	8.92
Pak-81	36.84	22.90	13.94	6.31	4.33	1.98	80.69	73.33	7.36
SA-75	36.51	19.50	17.01	6.38	4.33	2.05	80.66	76.56	4.10
Lyp-73	35.74	11.58	24.16	4.65	2.66	1.99	80.33	75.34	4.99
Bahawalpur-79	36.31	13.50	22.81	4.69	2.66	2.03	79.56	73.33	6.23
Fsd-83	34.13	25.13	9.00	6.35	4.33	2.02	80.66	75.39	5.27
Punjab-85	37.10	21.73	15.37	5.67	3.66	2.01	75.33	70.23	5.10
Khyber-87	33.87	20.53	13.34	4.63	2.66	1.97	72.66	65.45	7.21

FLA, flag leaf area (cm^2); TPP, tillers $plant^{-1}$; PH, plant height (cm). Indicated values are mean observations made as plants attained physical maturity after application of drought stress as compared to control treatment during tri-replicate experiment at $p \leq 0.05$.

3.2.2. Yield Traits

All yield traits, including SL, SPS, GPS, GYP, TGW and PBM, showed statistically significant ($p \leq 0.05$) differences among all genotypes under both stress and normal conditions (Table 3). Under control conditions, SL ranged from 9 to 11.5 cm, while under drought stress, SL ranged from 8 to 10 cm. Under drought stress, the cultivar SA-75 showed the highest (10 cm) whereas Pirsabak-05 and Inqalab-91 showed the lowest (8.5 cm) mean values for SL (Table 3). Moreover, GPS under control condition shad mean values ranging from 32 to 51, while under drought stress treatment, mean values varied from 25 to 50 (Table 3). Among genotypes, AARI-11 had the maximum (50) while Punjab-11 had the minimum (27) GPS under drought stress (Table 3). Additionally, SPS had mean values in the range of 13 to 18 in the control treatment and mean values in the range of 11 to 16 during drought stress application (Table 3). Among genotypes, Fsd-83 and Pak-81 had the maximum (15.6) while Chakwal-97 had the minimum (11) SPS with the application of drought stress (Table 3). In addition, under control conditions, the mean values of GYP varied from 4 to 10.5 g, whereas under drought treatment, mean values varied from 1.3 to 8g (Table 3). Under drought stress treatment, the cultivar Pakistan-13 showed the highest (8g) while the cultivar Inqalab-91 showed the lowest (1.26) GYP (Table 3).On the other hand, TGW demonstrated mean values ranging from 29 to 38 g under normal conditions, and mean values ranged from 25 to 34 g under stress conditions (Table 3). Among genotypes, Shakkar-13 had the highest (33.9 g) while Bhakkar-02 showed the lowest (25g) TGW with the application of drought stress (Table 3). Furthermore, PBM had mean values ranging from 43 to 58 g under control application conditions, and mean values ranged from 37 to 52 g during drought stress treatment (Table 3). Under drought stress treatment, the

genotype Khyber-87 showed the maximum (52 g) whereas the genotype Punjab-11 showed the minimum (37g) mean value of PBM (Table 3).

3.3. Correlation, PCA and Heatmap Analysis

The paired analysis of physiological, growth and yield parameters revealed significant correlations among traits; however, all traits that showed a largeextent of association under stress conditions wereslightly different (Figure 1). The physiological attribute Chl showed significant positive associations with CT, CMPS, PH and SL under drought stress as compared to the control (Figure 1). Furthermore, CT manifested positive correlations with CMPS and SL under both drought and normal treatments (Figure 1). Among growth traits, FLA had significant paired associations with CMPS, PH and SL that were statistically more distinct under stress as compared to the control (Figure 1).On the other hand, both CMPS and LRWC revealed statistically significant positive paired associations with TPP and GYP under both control and stress conditions that were comparatively equal in extent (Figure 1). Additionally, yield traits such as SL, GPS, GYP and TGW also had significant positive paired correlations among them; however, their association was stronger under control conditions as compared to stress (Figure 1). Moreover, the PCA scatter plot revealed more dispersion of traits from the origin under conditions of drought stress as compared to the control (Figure 2). In general, all traits, with the exception of CT, remained in close proximity under control conditions and manifested maximum parallelism in their associated expression. Furthermore, PCA illustrated that the extent of the association of traits varied under drought as compared to the control, which means that both drought and control conditions have different influences on the pairing of traits. The manifestation of all traits under study was different in all wheat genotypes; therefore, from the perspective of traits, all differentially expressedgenotypes were segregated and categorized into each quadrant of the biplot (Figure 3). Furthermore, in the context of the differential extent of trait association, a heatmap dendrogram categorizing all genotypes into six sub-clusters is indicated in Figure 4.

Table 3. Effect of control and water-stressed conditions on yield traits (spike length, spikelets spike^{-1}, grains per spike, grain yield spike^{-1}, thousand grain weight and plant biomass) of different wheat genotypes.

Genotypes	SL (cm)			SPS			GPS		
	Control	Drought	Difference	Control	Drought	Difference	Control	Drought	Difference
Pakistan-13	10.45	7.88	2.57	38.06	33.85	4.21	56.91	50.16	6.75
Shahkar-13	8.08	5.43	2.65	38.2	33.95	4.25	54.88	48.51	6.37
Anmaol-91	9.01	6.36	2.65	31.96	27.67	4.29	52.74	46.06	6.68
AARI-11	9.48	6.83	2.65	35.5	31.35	4.15	51.99	45.23	6.76
Punjab-11	7.15	4.56	2.59	36.56	32.3	4.26	42.77	36.54	6.23
AAS-11	9.45	6.84	2.61	37.71	33.35	4.36	49.98	43.74	6.24
Millet-11	8.78	6.15	2.63	37.1	32.85	4.25	47.84	41	6.84
Chakwal-50	8.31	5.66	2.65	37.63	33.45	4.18	52.34	46.27	6.07
Pirsabak-08	8.41	5.76	2.65	34.82	30.45	4.37	50.91	44.56	6.35
Lasani-08	9.28	6.63	2.65	34.56	30.35	4.21	57.98	51.57	6.41
AUR-09	8.85	6.24	2.61	36.96	32.55	4.41	58.42	52.08	6.34
Pirsabal-05	8.48	5.83	2.65	36.26	32.74	3.52	50.98	44.52	6.46
Bhakkar-02	5.15	2.53	2.62	28.96	24.77	4.19	57.9	52.44	5.46
Chakwal-97	6.75	3.86	2.89	28.57	24.96	3.61	49.25	43.43	5.82
Sariab-92	6.67	3.76	2.91	35.36	31.76	3.6	57.46	51.26	6.2
Inqalab-91	4.15	1.26	2.89	34.38	30.76	3.62	49.13	43.21	5.92
Pirsabak-91	7.56	4.66	2.9	35.89	31.46	4.43	48.2	42.35	5.85
AUR-10	8.13	5.26	2.87	33.56	29.91	3.65	48.66	42.3	6.36
Bahawalpur-2000	7.66	4.83	2.83	34.35	30.66	3.69	55.5	49.76	5.74
Bwp-97	7.31	4.53	2.78	31.16	27.56	3.6	49.45	43.22	6.23
Chakwal-86	7.03	4.28	2.75	37.25	33.66	3.59	57.95	51.78	6.17
Baranai-83	5.65	2.76	2.89	31.74	28.16	3.58	49.64	43.29	6.35

Table 3. *Cont.*

Genotypes	SL (cm)			SPS			GPS		
	Control	Drought	Difference	Control	Drought	Difference	Control	Drought	Difference
Sarhad-82	8.52	5.73	2.79	35.44	31.86	3.58	47.45	41.27	6.18
Pak-81	8.78	6.03	2.75	33.64	30.06	3.58	54.93	48.76	6.17
SA-75	7.03	4.25	2.78	34.56	30.96	3.6	53.34	47.22	6.12
Lyp-73	6.63	3.81	2.82	35.95	32.36	3.59	58.9	52.71	6.19
Bahawalpur-79	6.5	3.16	3.34	35.14	31.56	3.58	56.78	50.79	5.99
Fsd-83	8.03	5.25	2.78	33.23	29.66	3.57	53.54	47.16	6.38
Punjab-85	6.76	3.86	2.9	31.78	28.16	3.62	49.4	43.21	6.19
Khyber-87	8.93	6.23	2.7	35.95	32.36	3.59	58.24	52.28	5.96
Genotypes	**GYP (g)**			**TGW (g)**			**PBM (g)**		
	Control	**Drought**	**Difference**	**Control**	**Drought**	**Difference**	**Control**	**Drought**	**Difference**
Pakistan-13	10.45	7.88	2.57	38.06	33.85	4.21	56.91	50.16	6.75
Shahkar-13	8.08	5.43	2.65	38.2	33.95	4.25	54.88	48.51	6.37
Anmaol-91	9.01	6.36	2.65	31.96	27.67	4.29	52.74	46.06	6.68
AARI-11	9.48	6.83	2.65	35.5	31.35	4.15	51.99	45.23	6.76
Punjab-11	7.15	4.56	2.59	36.56	32.3	4.26	42.77	36.54	6.23
AAS-11	9.45	6.84	2.61	37.71	33.35	4.36	49.98	43.74	6.24
Millet-11	8.78	6.15	2.63	37.1	32.85	4.25	47.84	41	6.84
Chakwal-50	8.31	5.66	2.65	37.63	33.45	4.18	52.34	46.27	6.07
Pirsabak-08	8.41	5.76	2.65	34.82	30.45	4.37	50.91	44.56	6.35
Lasani-08	9.28	6.63	2.65	34.56	30.35	4.21	57.98	51.57	6.41
AUR-09	8.85	6.24	2.61	36.96	32.55	4.41	58.42	52.08	6.34
Pirsabal-05	8.48	5.83	2.65	36.26	32.74	3.52	50.98	44.52	6.46
Bhakkar-02	5.15	2.53	2.62	28.96	24.77	4.19	57.9	52.44	5.46
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Sarhad-82	8.52	5.73	2.79	35.44	31.86	3.58	47.45	41.27	6.18
Pak-81	8.78	6.03	2.75	33.64	30.06	3.58	54.93	48.76	6.17
SA-75	7.03	4.25	2.78	34.56	30.96	3.6	53.34	47.22	6.12
Lyp-73	6.63	3.81	2.82	35.95	32.36	3.59	58.9	52.71	6.19
Bahawalpur-79	6.5	3.16	3.34	35.14	31.56	3.58	56.78	50.79	5.99
Fsd-83	8.03	5.25	2.78	33.23	29.66	3.57	53.54	47.16	6.38
Punjab-85	6.76	3.86	2.9	31.78	28.16	3.62	49.4	43.21	6.19
Khyber-87	8.93	6.23	2.7	35.95	32.36	3.59	58.24	52.28	5.96

SL, spike length (cm); SPS, spikelets spike^{-1}; GPS, grains spike^{-1}. Indicated values are mean observations made as plants attained maturity after application of drought stress as compared to control treatment during tri-replicate experiment at $p \leq 0.05$. GYP, grain yield spike^{-1} (g); TGW, thousand grain weight (g); PBM, plant biomass (g). Indicated values are mean observations made as plants attained maturity after application of drought stress as compared to control treatment during tri-replicate experiment at $p \leq 0.05$.

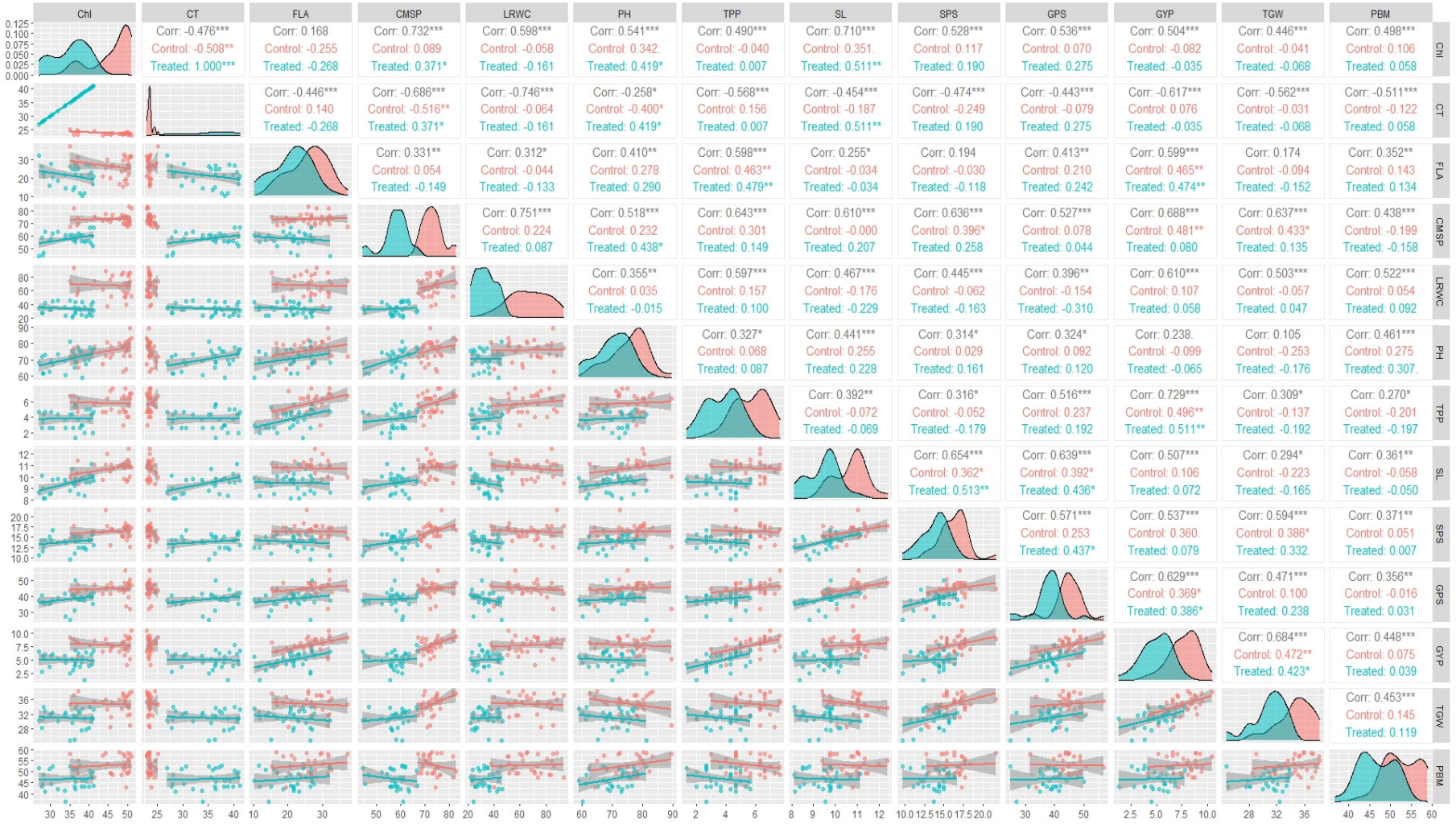

Figure 1. Effect of control and water stress conditions on the significance of association of physiological, growth and yield parameters of different wheat genotypes. Chl, chlorophyll; CT, canopy temperature; CMPS, cell membrane percentage stability; LRWC, leaf relative water content; FLA, flag leaf area; PH, plant height; TPP, tillers plant^{-1}; SL, spike length; SPS, spikelets spike^{-1}; GPS, grains spike^{-1}; GYP, grain yield spike^{-1}; TGW, thousand grain weight; PBM, plant biomass. *** = Significant at $p \leq 0.001$; ** = Significant at $p \leq 0.01$; * = Significant at $p \leq 0.05$.

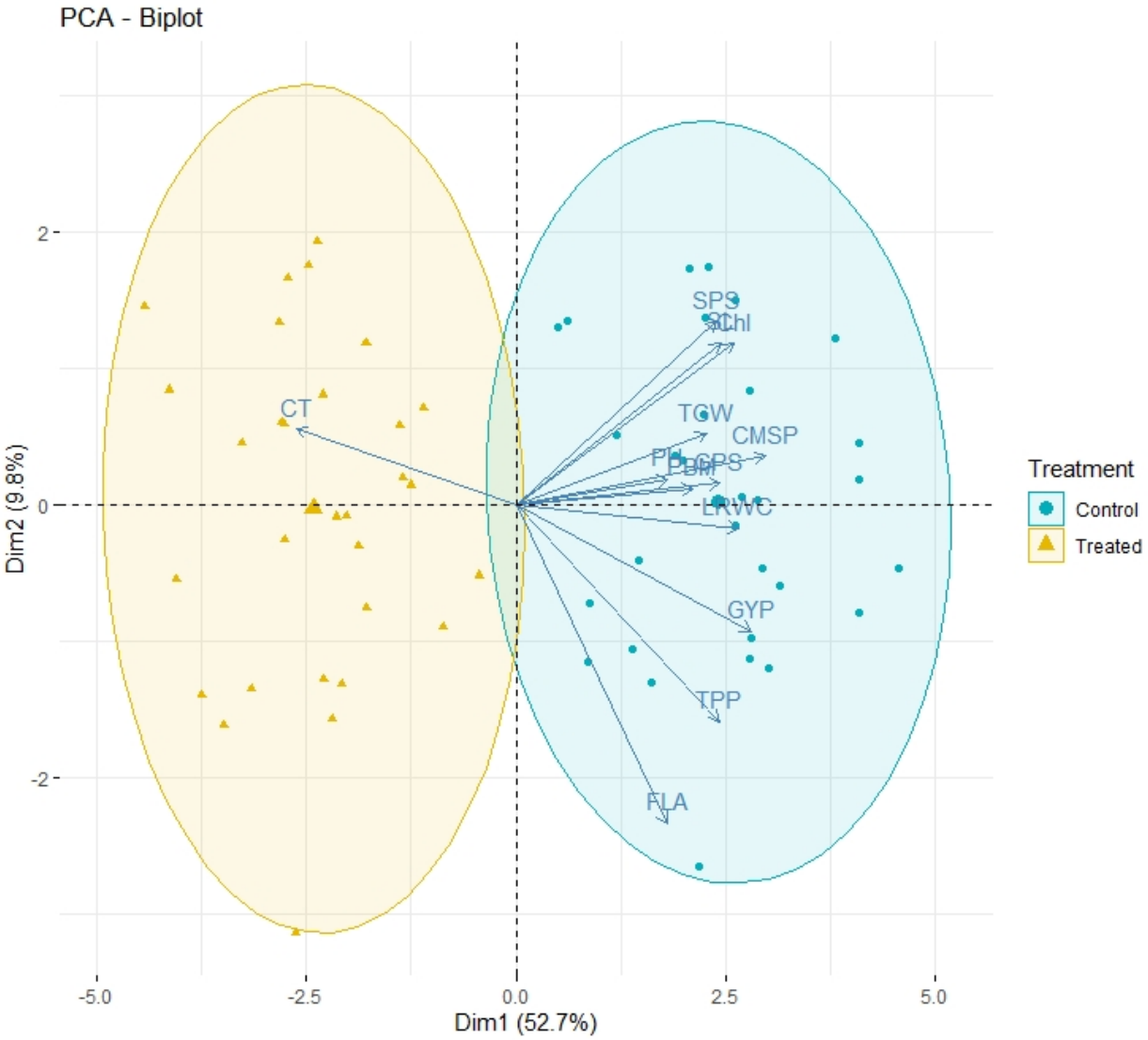

Figure 2. PCA scatter diagram illustrating the extent of similarity and dissimilarity in the expression of physiological, growth and yield traits of wheat genotypes under control and stress treatment conditions. Chl, chlorophyll; CT, canopy temperature; CMPS, cell membrane percentage stability; LRWC, leaf relative water content; FLA, flag leaf area; PH, plant height; TPP, tillersplant^{-1}; SL, spike length; SPS, spikelets spike^{-1}; GPS, grains spike^{-1}; GYP, grain yield spike^{-1}; TGW, thousand grain weight; PBM, plant biomass.

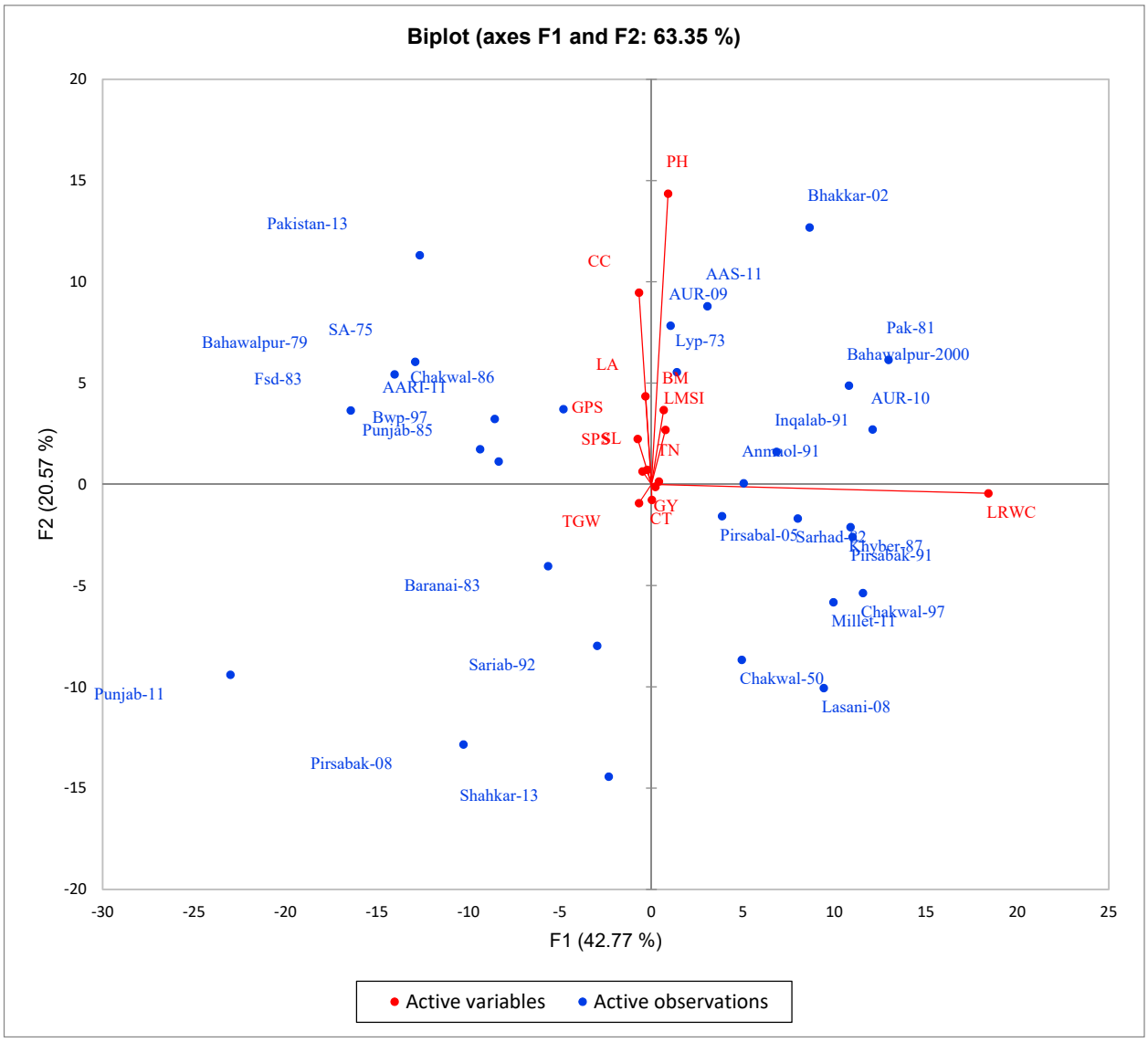

Figure 3. Biplot scattering genotypes into different quadrants based on the extent of trait association. Chl, chlorophyll; CT, canopy temperature; CMPS, cell membrane percentage stability; LRWC, leaf relative water content; FLA, flag leaf area; PH, plant height; TPP, tillers plant^{-1}; SL, spike length; SPS, spikelet spike^{-1}; GPS, grains spike^{-1}; GYP, grain yield spike^{-1}; TGW, thousand grain weight; PBM, plant biomass.

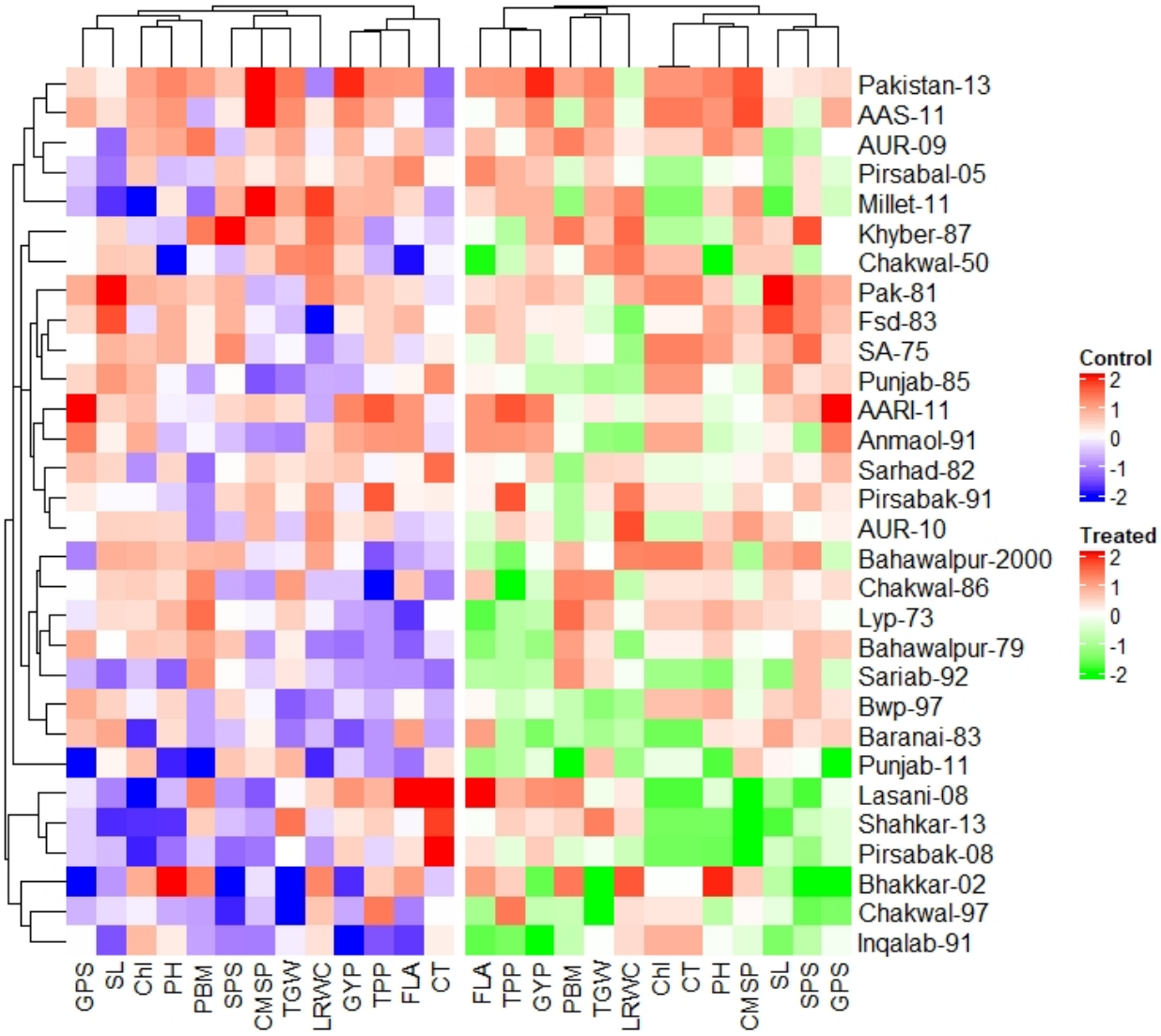

Figure 4. Heatmap dendrogram dividing wheat genotypes into different clusters based on the extent of association of physiological and agronomic traits under drought and control conditions. Chl, chlorophyll; CT, canopy temperature; CMPS, cell membrane percentage stability; LRWC, leaf relative water content; FLA, flag leaf area; PH, plant height; TPP, tillers plant^{-1}; SL, spike length; SPS, spikelet spike^{-1}; GPS, grains spike^{-1}; GYP, grain yield spike^{-1}; TGW, thousand grain weight; PBM, plant biomass.

3.4. Gene Expression Analysis

The relative gene expression of drought-related genes, such as *TaDREB1A*, *TaGROS-A*, *TaLEA3*, *TaHSFA1a*, *TaWRKY44* and *TaEXPA2*, showed considerable differences in selected wheat genotypes under drought stress as compared to the control (Figure 5). All genes were up regulated during drought stress in selected genotypes; however, their expression was relatively high in the genotypes Pakistan-13, Shahkar-13, AAS-11, Chakwal-86, Chakwal-50 and AUR-09 while comparatively low ingenotypes Anmol-97, Chakwal-97, Bhakkar-02 and BWP-97. Overall, all selected genotypes showed relatively high gene expression during drought stress as compared to the control.

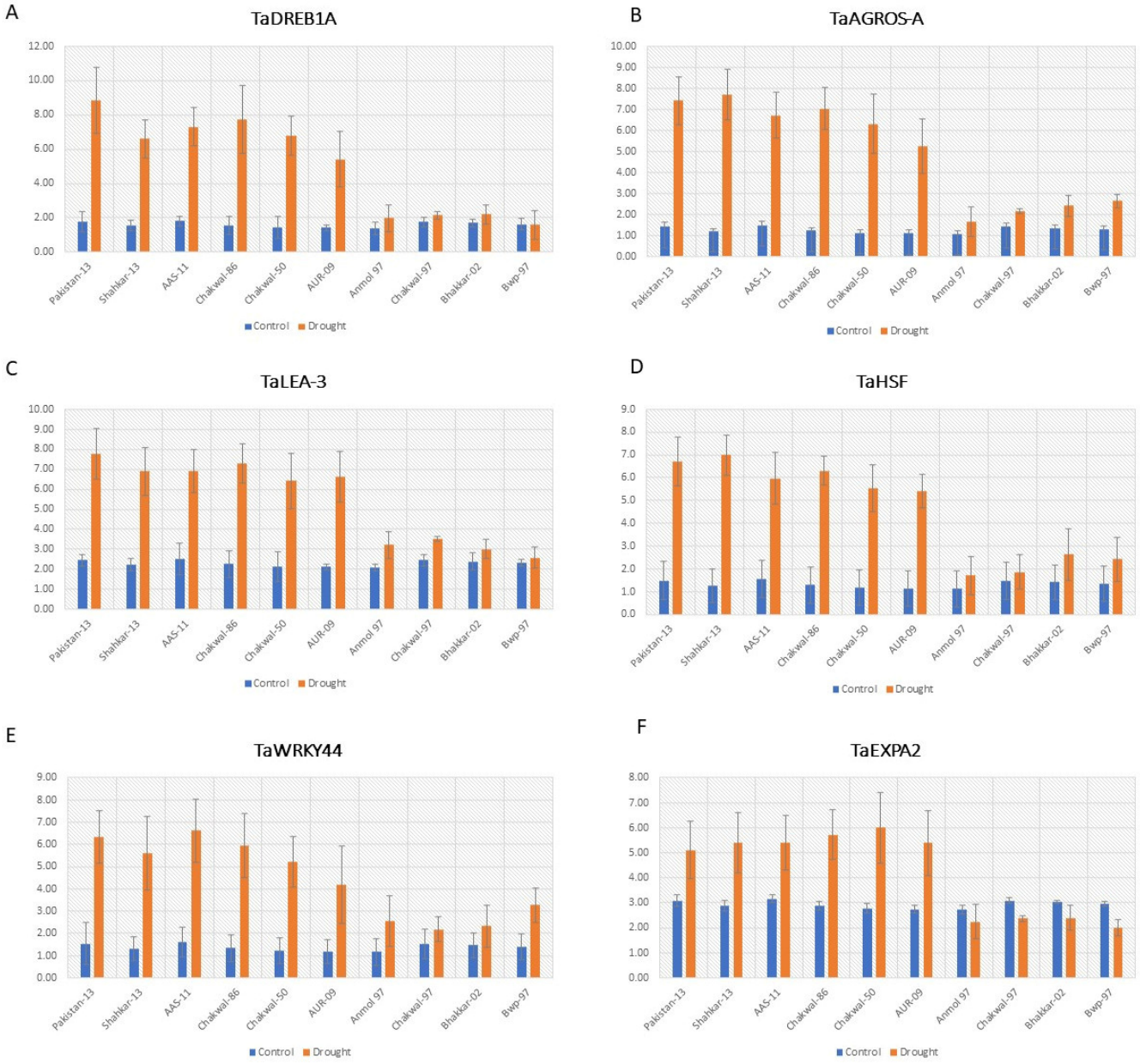

Figure 5. Relative expression analysis of drought-associated genes in selected wheat genotypes under control and drought stress conditions.

4. Discussion

The current study comparatively elucidated the performance of elite Pakistani drought-tolerant wheat genotypes under drought stress at physiological, agronomic and molecular levels. Overall, all genotypes showed significant alteration sintraits under stress as compared to the control treatment. The current study proved that physiological and agronomic traits are significantly correlated in all wheat genotypes, and each wheat genotype responds differently in terms of the associations of traits, depending upon its genetic potential. In fact, drought stress deters various physiological processes in plants, which ultimately leads to a reduction in the growth as well as yield traits of plants. Extensive drought stress inhibits photosynthesis due to an alteration in chlorophyll content as well as damage to the chlorophyll and photosynthetic machinery. Similarly, Pour-Aboughadareh et al. [22] observed a decline in leaf Chl due to drought stress in durum wheat. Correspondingly, the current study recorded a decline in the Chl content of all wheat cultivars under drought stress (Table 1). In addition, drought stress has a tendency to in duceoxidative stress within

plant cells, leading to the generation of ROS that ultimately disrupt the integrity of the cell membrane [9]. This disruption leads to more electrolyte leakage, which results in adecline in CMPS and LRWC [20]. Likewise, the present study reported a significant decline in CMPS and LRWC among all wheat cultivars under drought stress as compared to the control (Table 1). Canopy temperature (CT) is an important physiological trait indicating the water status of plants [23] and is an established parameter for targeting stomatal conductance [24]. Since drought stress has a tendency to increase CT due to enhanced stomatal conductance, wheat cultivars with cooler canopies are more tolerant to drought stress, as reviewed by Khadka et al. [25]. Likewise, the current study found a differential increase in CT of all wheat cultivars, which is an indicator of their differential potential against drought stress. PH, TPP and FLA are important growth traits affected directly by drought stress. In fact, low moisture content reduces photosynthesis and nutrient translocation, particularly during stem elongation and the tillering stage, resulting in reduced PH, TPP and FLA [26]. The extent of the reduction in PH, TPP and FLA depends on both the drought stress duration and genotype [25]. Likewise, the current study revealeda significant reduction in PH, TPP and FLA among all genotypes; however, the extent of the reduction was different in all genotypes (Table 2). This can be attributed to the differential genetic tolerance of all genotypes against drought stress [9]. An efficient physiological process provides sufficient translocation to plants, which not only accelerates their growth but also increases their yield [27]. Drought stress inhibits different plant physiological processes, including photosynthesis, which leads to a dramatic reduction in yield parameters, including SL, SPS, GPS, GYP, TGW and PBM [14,22]. In the present study, our results indicate a statistically distinct reduction in all of the aforementioned yield traits among all tested genotypes under drought stress (Table 3). Additionally, a breeding program can be made more efficient in diverse environments by gaining deep insight into the associations between various physiological and agronomical traits serving as selection indices [28,29]. Although various physiological, morphological and agronomic traits have been studied to elucidate the dynamics of drought tolerance in wheat, few of them have been suggested to improve ina practical breeding program. Fromth is perspective, a few traits, such as Chl, CMPS, LRWC, CT, PH, TPP, FLA, SL and SPS, are used as selection indices owing to their direct relation with grain yield under drought stress [29–32]. In this context, the present study revealed significant positive correlations of GPS, GYP and TGW with Chl, CMPS, LRWC, PH, TPP, FLA, SL and SPS (Figure 1) under drought conditions. Furthermore, the extent of the association between traits among cultivars was different, depending upon the treatment and genotype (Figures 2–4). Moreover, correlation analysis confirmed the direct association of traits under study in determining the ultimate grain yield in wheat genotypes (Figure 1). The paired association of these traits appeared to be more significant under conditions of stress, which confirmed yield as an output of the association between physiological and agronomical traits (Figures 1 and 2). However, differential responses of wheat genotypes in terms of physiological and agronomic traits confirmed variations in their gene architectures (Figure 4), as reviewed by Shah et al. [9]. In this regard, the genotypes Pakistan-13, Shahkar-13, AAS-11, Chakwal-86, Chakwal-50 and AUR-09 exhibitednote worthy performance. On the other hand, like every living organism, plants have a natural tendency to cope with any sort of stress through the regulation of genetic determinants controlling the homeostatic machinery. The varying expression of drought-responsive genes in wheat genotypes under drought stress is an indicator of their varying tendencies of drought tolerance [9]. For instance, Liu et al. [13] reported the high expression of the *TaDREB1* gene in a wheat cultivar during drought-imposed osmotic stress. Moreover, the *TaHSFA1a* gene regulates the accumulation of heat shock protein, which prevents plants from the hazards of heat and other abiotic stresses [14]. Ahmed et al. [11] observed the high expression of the *TaRGOS-A* gene in drought-tolerant wheat genotypes, which they attributed to the dynamic role of *TaRGOS-A* in triggering the production of ABA, which elicits the drought resistance pathway in wheat. In addition, transgenic wheat with the overexpression of *HVA1*, which regulatesthe *LEA* protein, enhances drought tolerance in wheat under water

deficit conditions, as reported by Sivamani et al. [33] and Xiao et al. [13]. On the other hand, Wang et al. [34] confirmed the role of the wheat gene *TaWRKY44* in conferring drought tolerance by transferring it into tobacco due to its tendency to eliminate ROS through the activation of the antioxidant defense system. Correspondingly, Yang et al. [16] observed the high expression of the *TaEXPA2* gene in transgenic wheat genotypes, which triggered the drought tolerance potential of wheat genotypes in a water deficit environment due to increased lateral root development. Complementary to these findings, the present study revealed the high expression of drought-related genes *TaDREB1A*, *TaHSFA1a*, *TaRGOS-A*, *TaLEA3*, *TaWRKY44* and *TaEXPA2* in drought-tolerant genotypes Pakistan-13, Shahkar-13, AAS-11, Chakwal-86, Chakwal-50 and AUR-09 as compared to susceptible genotypes Anmol-97, Chakwal-97, Bhakkar-02 and BWP-97 underwater deficit conditions (Figure 5). Therefore, further integration of these genotypes with the demarcation of drought tolerance indices would prove a fruitful tool in the future for devising breeding programs in multivariate environments. The current study concluded that the simultaneous study of physiological and agronomic traits provides important criteria for the delineation of drought tolerance in wheat genotypes. Furtherstudy linking physiological, agronomic and molecular parameters serving as selection indices will accelerate the pace of wheat breeding for drought tolerance in the near future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051056/s1. Table S1. List of wheat genotypes used in the study. Table S2. List of primers used in relative gene expression analysis.

Author Contributions: G.S., S.I.M. and N.I.R. developed the idea and supervised the study; S.M.D.M.S. conducted experiments; M.R. and Z.H.S. conducted the statistical analysis; S.M.D.M.S. and Z.H.S. wrote the manuscript; Z.H.S., F.A., Y.A.Z. and H.A. proofread and K.S. and S.H.Y. critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Raza, A.; Razzaq, A.; Mehmood, S.S.; Zou, X.; Zhang, X.; Lv, Y.; Xu, J. Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. *Plants* **2019**, *8*, 34. [CrossRef] [PubMed]
2. Fahad, S.; Bajwa, A.; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S.; et al. Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Front. Plant Sci.* **2017**, *8*, 1147. [CrossRef] [PubMed]
3. Ali, S.; Liu, Y.; Ishaq, M.; Shah, T.; Abdullah; Ilyas, A.; Din, I.U. Climate Change and Its Impact on the Yield of Major Food Crops: Evidence from Pakistan. *Foods* **2017**, *6*, 39. [CrossRef] [PubMed]
4. Ray, D.K.; West, P.C.; Clark, M.; Gerber, J.S.; Prishchepov, A.V.; Chatterjee, S. Climate change has likely already affected global food production. *PLoS ONE* **2019**, *14*, e0217148. [CrossRef] [PubMed]
5. Seleiman, M.F.; Al-Suhaibani, N.; Ali, N.; Akmal, M.; Alotaibi, M.; Refay, Y.; Dindaroglu, T.; Abdul-Wajid, H.H.; Battaglia, M.L. Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. *Plants* **2021**, *10*, 259. [CrossRef] [PubMed]
6. Pandey, A.; Khobra, R.; Mamrutha, H.M.; Wadhwa, Z.; Krishnappa, G.; Singh, G.; Singh, G.P. Elucidating the Drought Responsiveness in Wheat Genotypes. *Sustainability* **2022**, *14*, 3957. [CrossRef]
7. Chaudhary, S.; Devi, P.; Bhardwaj, A.; Jha, U.C.; Sharma, K.D.; Prasad, P.V.V.; Siddique, K.H.M.; Bindumadhava, H.; Kumar, S.; Nayyar, H. Identification and Characterization of Contrasting Geno-types/Cultivars for Developing Heat Tolerance in Agricultural Crops: Current Status and Prospects. *Front. Plant Sci.* **2020**, *11*, 587264. [CrossRef]
8. Morales, F.; Ancín, M.; Fakhet, D.; González-Torralba, J.; Gámez, A.L.; Seminario, A.; Soba, D.; Ben Mariem, S.; Garriga, M.; Aranjuelo, I. Photosynthetic Metabolism under Stressful Growth Conditions as a Bases for Crop Breeding and Yield Improvement. *Plants* **2020**, *9*, 88. [CrossRef]

9. Shah, Z.H.; Rehman, H.M.; Akhtar, T.; Daur, I.; Nawaz, M.A.; Ahmad, M.Q.; Rana, I.A.; Atif, R.M.; Yang, S.H.; Chung, G. Redox and Ionic Homeostasis Regulations against Oxidative, Salinity and Drought Stress in Wheat (A Systems Biology Approach). *Front. Genet.* **2017**, *8*, 141. [CrossRef]
10. Kang, Y.; Udvardi, M. Global regulation of reactive oxygen species scavenging genes in alfalfa root and shoot under gradual drought stress and recovery. *Plant Signal. Behav.* **2012**, *7*, 539–543. [CrossRef]
11. Ahmed, K.; Shabbir, G.; Ahmed, M.; Noor, S.; Din, A.M.U.; Qamar, M.; Rehman, N. Expression profiling of TaARGOS homoeologous drought responsive genes in bread wheat. *Sci. Rep.* **2022**, *12*, 3595. [CrossRef] [PubMed]
12. Xiao, B.; Huang, Y.; Tang, N.; Xiong, L. Over-expression of a LEA gene in rice improves drought resistance under the field conditions. *Theor. Appl. Genet.* **2007**, *115*, 35–46. [CrossRef] [PubMed]
13. Liu, M.; Wang, Z.; Xiao, H.-M.; Yang, Y. Characterization of TaDREB1 in wheat genotypes with different seed germination under osmotic stress. *Hereditas* **2018**, *155*, 26. [CrossRef] [PubMed]
14. Xue, Q.; Rudda, J.C.; Liua, S.; Jessupa, K.E.; Devkotaa, R.N.; Mahano, J.R. Yield determination and water use efficiency of wheat under water-limited conditions in the U.S. Southern High Plains. *Crop Sci.* **2013**, *34*, 47. [CrossRef]
15. Niu, C.-F.; Wei, W.; Zhou, Q.-Y.; Tian, A.; Hao, Y.-J.; Zhang, W.-K.; Ma, B.; Lin, Q.; Zhang, Z.-B.; Zhang, J.-S.; et al. Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants. *Plant Cell Environ.* **2012**, *35*, 1156–1170. [CrossRef]
16. Yang, J.; Zhang, G.; An, J.; Li, Q.; Chen, Y.; Zhao, X.; Wu, J.; Wang, Y.; Hao, Q.; Wang, W.; et al. Expansin gene TaEXPA2 positively regulates drought tolerance in transgenic wheat (*Triticum aestivum* L.). *Plant Sci.* **2020**, *298*, 110596. [CrossRef] [PubMed]
17. Lan, Y.; Chawade, A.; Kuktaite, R.; Johansson, E. Climate Change Impact on Wheat Performance—Effects on Vigour, Plant Traits and Yield from Early and Late Drought Stress in Diverse Lines. *Int. J. Mol. Sci.* **2022**, *23*, 3333. [CrossRef]
18. Bannister, P. The Water Relations of Certain Heath Plants with Reference to their Ecological Amplitude: III. Experimental Studies: General Conclusions. *J. Ecol.* **1964**, *52*, 499–509. [CrossRef]
19. Farooq, J.; Khaliq, I.; Kashif, M.; Ali, Q.; Mahpara, S. Genetic analysis of relative cell injury per-centage and some yield contributing traits in wheat under normal and heat stress conditions. *Chil. J. Agric. Res.* **2011**, *71*, 511. [CrossRef]
20. Alghabari, F.; Shah, Z.H.; Elfeel, A.A.; Alyami, J.H. Biochemical and Physiological Responses of Thermostable Wheat Genotypes for Agronomic Yield under Heat Stress during Reproductive Stages. *Agronomy* **2021**, *20*, 80. [CrossRef]
21. Li, S.; Wang, R.; Jin, H.; Ding, Y.; Cai, C. Molecular Characterization and Expression Profile Analysis of Heat Shock Transcription Factors in Mungbean. *Front. Genet.* **2019**, *9*, 736. [CrossRef] [PubMed]
22. Pour-Aboughadareh, A.; Mohammadi, R.; Etminan, A.; Shooshtari, L.; Maleki-Tabrizi, N.; Poczai, P. Effects of Drought Stress on Some Agronomic and Morpho-Physiological Traits in Durum Wheat Genotypes. *Sustainability* **2020**, *12*, 5610. [CrossRef]
23. Mason, R.E.; Singh, R.P. Considerations when deploying canopy temperature to select high yielding wheat breeding lines under drought and heat stress. *J. Agron.* **2014**, *191*, 201. [CrossRef]
24. Deery, D.M.; Rebetzke, G.J.; Jimenez-Berni, J.A.; Bovill, W.D.; James, R.A.; Condon, A.G.; Furbank, R.T.; Chapman, S.C.; Fischer, R.A. Evaluation of the phenotypic repeatability of canopy temperature in wheat using continuous-terrestrial and airborne measurements. *Front. Plant Sci.* **2019**, *10*, 875. [CrossRef]
25. Khadka, K.; Earl, H.J.; Raizada, M.N.; Navabi, A.A. Physio-Morphological Trait-Based Approach for Breeding Drought Tolerant Wheat. *Front. Plant Sci.* **2020**, *11*, 715. [CrossRef]
26. Sarto, M.V.M.; Sarto, J.R.W.; Rampim, L.; Rosset, J.S.; Bassegio, D.; da Costa, P.F.; Inagaki, A.M. Wheat phenology and yield under drought: A review. *Aust. J. Crop Sci.* **2017**, *11*, 941–946. [CrossRef]
27. Ahmadi, J.; Pour-Aboughadareh, A.; FabrikiOurang, S.; Mehrabi, A.A.; Siddique, K.H.M. Wild relatives of wheat: Aegilops–Triticum accessions disclose differential antioxidative and physiological responses to water stress. *Acta Physiol. Plant.* **2018**, *40*, 90. [CrossRef]
28. Foulkes, M.J.; Sylvester-Bradley, R.; Weightman, R.; Snape, J.W. Identifying physiological traits associated with improved drought resistance in winter wheat. *Field Crop Res.* **2007**, *11*, 24. [CrossRef]
29. Lafitte, H.R.; Blum, A.; Atlin, G. Using secondary traits to help identify drought tolerant genotypes. In *Hardy Breeding Rice for Drought-Prone Environments*; Fischer, K., Lafitte, S.R., Fukai, G., Atlin, B., Eds.; IRRI: Los Baños, Philippines, 2003; Volume 38, p. 39.
30. Poursiahbidi, M.; Pour-Aboughadareh, A.; Tahmasebi, G.; Jasemi, S. Evaluation of genetic diversity and interrelationships of agro-morphological characters in durum wheat (*Triticum durum* desf.) lines using multivariate analysis. *Int. J. Agric. Res. Rev.* **2013**, *184*, 194.
31. Pour-Aboughadareh, A.; Ahmadi, J.; Mehrabi, A.A.; Etminan, A.; Moghaddam, M.; Siddique, K. Physiological responses to drought stress in wild relatives of wheat: Implications for wheat improvement. *Acta Physiol. Plant.* **2017**, *39*, 106. [CrossRef]
32. Pour-Aboughadareh, A.; Omidi, M.; Naghavi, M.R.; Etminan, A.; Mehrabi, A.A.; Poczai, P.; Bayat, H. Effect of Water Deficit Stress on Seedling Biomass and Physio-Chemical Characteristics in Different Species of Wheat Possessing the D Genome. *Agronomy* **2019**, *9*, 522. [CrossRef]
33. Sivamani, E.; Bahieldin, A.; Wraith, J.M.; Al-Niemi, T.; Dyer, W.E.; Ho, T.-H.D.; Qu, R. Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley HVA1 gene. *Plant Sci.* **2000**, *155*, 1–9. [CrossRef]
34. Wang, X.; Zeng, J.; Li, Y.; Rong, X.; Sun, J.; Sun, T.; Li, M.; Wang, L.; Feng, Y.; Chai, R.; et al. Expression of TaWRKY44, a wheat WRKY gene, in transgenic tobacco confers multiple abiotic stress tolerances. *Front. Plant Sci.* **2015**, *6*, 615. [CrossRef] [PubMed]