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Physio-Morphological and Biochemical Trait-Based Evaluation of Ethiopian and Chinese Wheat Germplasm for Drought Tolerance at the Seedling Stage

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Abstract: For Ethiopia's wheat production, drought is a major natural disaster. Exploration of drought-resistant varieties from a bulk of wheat germplasm conserved in the gene bank is of paramount importance for breeding climate change-resilient modern cultivars. The present study was aimed at identifying the best performing drought-resistant genotypes under non-stress and polyethylene glycol simulated (PEG) stress conditions in a growth chamber. Forty diverse Ethiopian bread and durum wheat cultivars along with three Chinese bread wheat cultivars possessing strong drought resistance and susceptibility were evaluated. After acclimation with the natural environment, the seedlings were imposed to severe drought stress (20% PEG₆₀₀₀), and 15 seedling traits including photosynthetic and free proline were investigated. Our findings indicated that drought stress caused a profound decline in plant water consumption (83.0%), shoot fresh weight (64.9%), stomatal conductance (61.6%), root dry weight (55.2%), and other investigated traits except root to shoot length ratio and proline content which showed a significant increase under drought stress. A significant and positive correlation was found between photosynthetic pigments in both growth conditions. Proline exhibited a negative correlation with most of the investigated traits except root to shoot length ratio and all photosynthetic pigments which showed a positive and non-significant association. Our result also showed a wide range of genetic variation (CV) ranging from 3.23% to 47.3%; the highest in shoot dry weight (SDW) (47.3%) followed by proline content (44.63%) and root dry weight (36.03%). Based on multivariate principal component biplot analysis and average sum of ranks (ASR), G12, G16 and G25 were identified as the best drought tolerant and G6, G42, G4, G11, and G9 as bottom five sensitive. The potential of these genotypes offers further investigation at a molecular and cellular level to identify the novel gene associated with the stress response.

Keywords: wheat germplasm; drought resistance; evaluation; seedling stage; principal component analysis; average sum of rank; stress tolerance index

1. Introduction

Wheat (*Triticum L.*) is one of the most important domesticated cereal crops in the fertile crescent between 8000 and 10,000 years [1]. Globally, wheat ranks second after rice in terms of production; provides 55% carbohydrates, 20% proteins, and calories for the daily requirements of 4.5 billion people [2–4]. China is the largest wheat producer and consumer in the world [5]; however, drought stress is a major factor threatening wheat productivity in the major growing areas such as North China plains. Furthermore, 60% of wheat production in this region is threatened by a severe water deficit. According to

Xinhua's recent report [6], China holds more than 50,000 accessions of wheat germplasm in the gene bank and 24,000 of them were evaluated for drought tolerance. According to He [7] review of the literature, China has shown a long history of wheat breeding since 1949, and major progress has been made in the past in developing modern cultivars resilient to environmental stress [8].

In Ethiopia, wheat was the fifth most important cereal crop after tef (*Eragrostis tef* (Zucc.) Trotter), maize, barley, and sorghum in terms of area acreage and production until the 1990s [9]. However, according to a recent USDA report [10], the rapid urbanization, growing population, and shifting of food habits rapidly exceeded wheat consumption (~6.7 million tonnes) over its production (>5 million tonnes) in Ethiopia. The demand for wheat grain has increased exponentially due to the growing interest in commercial and staple foods like local bread, porridge (*genfo*), local beer (*tela*), roasted grain (*Kolo*), boiled grain (*nifro*), pasta, and different confectionary products [11,12]. Regardless of these, wheat becomes the second most staple food crop next to tef [13] and two wheat species namely durum wheat (*T. turgidum subsp. durum*, $2n = 4x = 28$, AABB) and bread wheat (*Triticum aestivum*, $2n = 6x = 42$, AABBDD) [9,14] are widely grown in diversified agro-ecologies differing in soil content, rainfall distribution, and weather conditions [9]. Due to this, Ethiopia was recognized as a center of origin and/or diversity in many field crops [15] particularly in tetraploid wheat [16–21]. Although Ethiopia is one of the top three wheat producers in Africa and the largest in sub-Saharan Africa (SSA) however, the country remains a net importer [5,11]. More than 90% of Ethiopia's wheat is rain-fed and grown by small-scale farmers in marginal lands which is highly susceptible to recurrent drought. A review of literature by Adhikari et al. [22] also depicted that 72% of the wheat grain yield in SSA is projected to decline by recurrent drought associated with climate change.

Despite its socio-economic importance, global wheat production at large is threatened by biotic and abiotic stresses. Previous studies indicated that abiotic stresses such as drought, heat, cold, salinity, and mineral toxicity are the most leading constraints for crop production than biotic stress [23–27]. Among abiotic stresses, drought is the most common incidence [28] and causes a prominent effect on the plant morphological, physiological, biochemical, and molecular processes [29,30] that can occur at any plant growth and development. Drought stress is caused by the reduction of soil moisture content and groundwater level after prolonged water stress/dry spells which eventually leading to the cessation of plant growth [31]. Depending on the growth stage, intensity and duration of the stress, and nature of plant stress response mechanisms; drought stress causes a decrease in RWC, chlorophyll degradation, stomatal closure, increased osmolytes, and growth inhibition in wheat [32–35] which ultimately leading to yield loss and productivity. Previous reports demonstrated that water deficit (drought stress) in wheat [36,37] has unfavorable effects on the efficiency of photosystem II (PSII), as indicated by changes in chlorophyll fluorescence (CF) [38] parameters such as initial fluorescence (F_0), maximum quantum yield of PSII (F_v/F_m), maximum primary yield of PSII photochemistry (F_v/F_0), as well as plant growth and dry matter. Therefore, understanding crop stress physiology and its related tools such as chlorophyll fluorescence (CF) has become known as a useful indicator for investigating stress in plants in vivo [39].

Gonfa and Tesfaye [40] reported that the effect of water stress on grain yield and other agronomic components was highly pronounced at the tillering stage (72% reduction) than flowering (37%) and grain filling (17.1%) as compared to the well-watered treatment. Therefore, it is critical to select high-performing drought-resistant wheat genotypes under dry environmental conditions [41] at the early growth stage. However, multiple biotic and abiotic factors may coexist in the field conditions and presumably misleading our results and conclusions. To better define drought stress and screening, the in-vitro screening method is widely used under controlled conditions. Polyethylene glycol (PEG) is the most commonly used chemical to simulate the natural drought stress in the in-vitro screening methods. PEG is a high molecular weight, inert in nature, non-ionic, and induce uniform drought stress without entering the plant cells [41–44].

Several drought stress experiments in wheat were focused on the post-anthesis and/or reproduction stage [32,45] due to its imminent importance to grain yield [46]. An accumulation of evidence suggested that drought stress screening at the early growth stage hydroponically in the growth chamber [43,47] and soil conditions in the greenhouse [48–51] enables us to better define the drought stress and manage a large set of genotypes under controlled growing conditions. Though using hydroponic nutrient solution does not represent the actual field condition, it is still viable and used in drought stress screening at the vegetative stage [42,52–54]. Previous studies showed that drought stress imposition at the early growth stage significantly affects the grain yield by deteriorating the photosynthetic reserves [55] and delaying phenology through smaller roots [56]. Drought tolerance is a stage-specific trait [57] and therefore breeding for drought tolerance using morpho-physiological, and biochemical parameters at any growth stage [30,46,58–64] have paramount importance.

Despite their low grain yield, landraces have been thought of as high genetic variation, wide adaptability nature even under harsh environmental conditions, resistance, and/or tolerance to abiotic and biotic stresses [65], and therefore, evaluation of wheat landraces stored in gene banks is highly beneficial for wheat improvement [66]. Chinese wheat cultivars have high yield and good drought resistance traits that could be used for improving Ethiopia wheat cultivars by cross-breeding. In the current study, we hypothesized that a comparative physio-morphological and biochemical study on a bulk of wheat landraces conserved in the gene bank compared with well-known Chinese drought resistant and susceptible wheat cultivars may scale up drought stress screening and ultimately improving grain yield in the climate change scenario. To the best of our knowledge, the wheat genotypes used in this study were previously conserved in the Ethiopian biodiversity institute (EBI) gene bank in the form of population and after a thorough preliminary characterization in the field, best-performing single lines were selected from the entire population and following the ear to row seed multiplication, their agronomic performance was studied. However, their performance against drought stress is largely unknown. Therefore, the objective of the present study was (i) to identify best performing drought-tolerant genotypes using growth and physio-biochemical traits (ii) to find out the extent of genetic variation in drought-resistant among diverse Ethiopian wheat genotypes and standard Chinese drought tolerance and susceptible cultivars and (iii) to determine the drought-resistant traits associated with morphological and physio-biochemical traits under stress and non-stress conditions.

2. Material and Methods

2.1. Plant Material

The present study was conducted at the laboratory of the Center for agricultural resources research (CARR), institute of genetics and developmental biology (IGDB), Chinese Academy of Sciences (CAS), Shijiazhuang city, Hebei province, China. Forty-three spring wheat genotypes including (24 bread wheat and 16 durum wheat) provided from the Ethiopian Biodiversity Institute (www.ebi.gov.et, accessed on 20 April 2021) national gene bank, Addis Ababa, and three Chinese spring wheat cultivars (2 drylands and 1 irrigated land) were supplied by Prof Chen Zhiguo, Northwest Institute of Plateau Biology (NWIPB), Chinese Academy of Sciences, Xining city Qinghai province, China, were used (Supplementary Table S1) for drought tolerance evaluation under controlled conditions in the growth chamber at the seedling stage.

2.1.1. Experimental Design and Growth Conditions

The experiment was designed in a randomized complete block design (RCBD) with three biological replicates using Hoagland nutrient solution (control) and 20% (*w/v*) PEG 6000 (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) (drought stress). To do these, we designed a simple and cost-effective hydroponic culture medium using a triangular flask (50 mL) covered with perforated polystyrene.

Seeds of all wheat genotypes were first rinsed and then surface sterilized with 70% alcohol for 2–3 min, then washed three to four times with distilled water. Seeds were then sown and germinated in a sterilized Petri dishes lined up with Whatman filter paper soaked with distilled water for 48 h in the darkroom. After 48 h of sowing, the seedlings were subsequently transferred to the growth chamber adjusted to 60–70% relative humidity and 28/23 °C day/night temperatures. Light intensity of 400 $\mu\text{mol}^{-2}\text{s}^{-1}$ was supplied using fluorescent lamps (PHILIPS Lifemax TL-D 36W/865 for 16 h light/8 h dark photoperiod using an automatic timer. The seedlings were maintained under normal growth conditions for 5 days until bearing of the shoot and reasonable roots.

At one leaf stage, the 7 days age seedlings (10 plants per bottle per treatment) were transplanted to perforated holes made from polystyrene and fixed over triangular flask tanks (50 mL in size) containing Hoagland nutrient solution [67] for 7 days acclimation. To prevent the liquid in the bottle from evaporating, the plants were fixed with the bottle using plastic wrap or water plaster and aeration was taken place in the hole that plants were fixed.

2.1.2. Drought Stress Treatment

Upon acclimation of the seedlings in the new environment (7 days later), drought stress treatments were subjected to osmotic stress by 20% (*w/v*) PEG-6000 solution either in half-strength Hoagland solution alone (control) or half-strength Hoagland solution with PEG 6000 (drought stress) according to the method developed by Ayalew et al. [43,59] in wheat. All the seedlings were grown under hydroponic conditions in a controlled growth chamber as described on Section 2.1.1. The drought stress was ended after two weeks of subsequent stress applications. To decrease the differences in microclimate [68] and positional effect [69], the bottle positions were randomly switched every three days. Moreover, to avoid nutrient depletion, the Hoagland solution was renewed every five days during the plant growth period as per the manufacturer's instruction (Yamei Horticulture Co., Ltd. Shijiazhuang, Hebei province, China).

2.2. Physio-Morphological Traits

2.2.1. Total Water Consumption

The total water consumption per plant (PWC, g plant^{-1}) during the stress period was determined by the difference between initial and final flask weight per ten plants during the solution changes. Nonetheless, flasks were measured (using a sensitive balance) twice, i.e., during the initial osmotic stress induction (W_1 , W_2 , W_3 and W_4) and after second solution changes (W_5 , W_6 , W_7 and W_8). Where, W_1 : the weight of flask + solution, W_2 : the weight of flask + solution + plants, W_3 : the weight of flask + solution + plants (after five days), W_4 : the weight of the remaining solution (after five days), and W_5 , W_6 , W_7 and W_8 are repeated measurements (W_{1+1} , W_{2+1} , W_{3+1} and W_{4+1}) respectively after second solution changes. Conversely, to estimate the actual water used by the plants, the initial flask weight (W_0) was first subtracted from all measurements. Since the density of water is 1 g/mL, the total volume (mL) of the water used by the plant is determined by dividing the weight and/or mass of water by the density of water. Therefore, the data provided for PWC (g plants^{-1}) is equivalent to mL plants^{-1} .

2.2.2. Leaf Relative Water Content (RWC)

To measure RWC, fresh and fully extended flag leaves were taken from 3 randomly chosen plants after 72 h of osmotic stress imposition. The flag leaves were cut into approximately 5 cm long fragments and fresh mass was taken immediately. Leaves were then soaked in distilled water in a vial for 4 h. After 4 h saturation, turgid mass was recorded by careful blot dry with soft newspaper and towel. After that, the samples were placed in a paper bag and kept in an oven at 65 °C for 48 h to record dry weight [70]. The relative water content was recorded using Barrs et al. [71] formula.

$$\text{RWC}(\%) = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100 \quad (1)$$

2.2.3. Stomatal Conductance

Stomatal conductance (gs) was measured after 72 h drought stress imposition on the adaxial surface of a fully developed penultimate leaf from three randomly chosen plants using a portable leaf porometer (model SC-1, Decagon devices, Inc. Pullman) and expressed as $\text{mmol m}^{-2}\text{s}^{-1}$. Before taking the measurement, the leaf porometer device was first calibrated following the standard protocol of Decagon devices [72].

2.2.4. Growth Traits

At the end of the drought stress, three seedlings from each treatment were harvested to score root length (RL) and shoot length (SL) (manually by ruler cm), RL:SL, root and shoot fresh weight (Rt and St FW) (by a sensitive balance in mg plant^{-1}). In contrast, dry weight was scored after oven drying of the measured fresh weight seedlings at 65°C for 48 h.

2.3. Biochemical Assay

2.3.1. Photosynthetic Pigments

Chlorophyll pigments were determined after extraction of 100 mg fresh leaves using 80% pre-refrigerated acetone solution overnight at 4°C according to the method used by [73]. After 72 h of initial PEG stress induction, the fresh leaves were collected, frozen in liquid nitrogen, and subsequently stored at -80°C refrigerator until further processing. Carotenoids (Car), chlorophyll a (Chla) and b (Chlb) were measured by taking the optical densities (OD) of the extracted solution at 470, 663.2, 646.8 nm respectively using UV-spectrophotometry (SHIMADZU, UV-2450, Dongguan Hong Cheng Optical Products Co. Ltd., Dongguan, China). The total chlorophyll content ($\text{mg g}^{-1}\text{FW}$) was calculated according to Lichtenthaler [73].

$$\text{Chlorophyll a (Chla)} = \frac{(12.25A_{663.2} - 2.79A_{646.8}) \times V}{1000 \times W} \quad (2)$$

$$\text{Chlorophyll b (Chlb)} = \frac{(21.5050A_{646.8} - 5.10A_{663.2}) \times V}{1000 \times W} \quad (3)$$

$$\text{Total chlorophyll (ChIT)} = \frac{(7.15A_{663.2} + 18.71A_{646.8}) \times V}{1000 \times W} \quad (4)$$

$$\text{Carotenoids (Car)} = \frac{(1000A_{470} - 1.82Ca - 85.02Cb) \times V}{1000 \times W \times 198} \quad (5)$$

where, V = volume of the extracted chlorophyll; W = weight of the sample used, A = absorption at 520 nm.

2.3.2. Proline Content

Proline content (PC) was determined following the acid-ninhydrin method of Bates et al. [74] with little modification. After 72 h of initial PEG stress imposition, the fresh leaves were collected, frozen in liquid nitrogen, and subsequently stored at -80°C refrigerator until further processing. 0.1 g of fresh leaves were grounded by mortar and pestle using 10 mL of 3% sulfosalicylic acid (*w/v*) and the extract was centrifuged at 3000 rpm for 10 min. After centrifugation, 2 mL of the supernatant was carefully transferred to a new falcon tube and 2 mL ninhydrin acid reagent and 2 mL of glacial acetic acid were added to prepare the reaction mixture. After that, the reaction mixture was heated in a water bath at 100°C for 1 h and subsequently cooled for sometimes at room temperature. Then 4 mL toluene was added to each reaction mixture and shake well for some time. As a result, two layers were developed and the upper phase chromophore containing pink-reddish color was carefully transferred to a new test tube and was used to read the optical density (OD) at 520 nm. The unknown proline content was estimated from the standard linear curve

($y = mx + b$). The total amount of PC in a given sample is expressed as a fresh weight (FW) basis using the following formula.

$$PC = \frac{X \times \text{volume of the extract}}{\text{The volume of aliquot} \times \text{weight of sample}} \quad (6)$$

where, y = absorption at 520 nm; X = unknown concentration determined from the standard curve; m = slope and b = y intercept.

2.4. Data Analysis

The mean values of all data obtained from three replications were statistically analyzed using the analysis of variance (ANOVA) technique designed for RCBD design. The main and interaction effect between experimental factors were analyzed according to Fischer's $LSD_{5\%}$ level of significance using the Agricolae package in R studio [75]. On the other hand, agglomerative hierarchical clustering (AHC) and Pearson's correlation coefficients (r) were performed based on the mean value of studied seedling traits under stress and non-stress conditions using XLSTAT software (Addisonsoft XLSTAT, Paris, France). Principal component analysis (PCA) based on correlation was computed to identify the influential traits for selection. Moreover, to screen better-performing genotypes under stress conditions, stress tolerance index (STI) was computed by iPASTIC: an online toolkit [76], and genotypes having the smaller average sum of ranks (ASR) was selected as best tolerant. Apart from this, GraphPad Prism version 8.4.3 for Windows (San Diego, CA, USA, (www.graphpad.com/, accessed on 20 April 2021) was used for the graphical presentation of data. Relative change (RC) due to PEG induced stress was also determined for each trait using the following formula:

$$RC (\%) = \frac{X_c - X_s}{X_c} \times 100 \quad (7)$$

where X_c and X_s are the adjusted mean values of a trait in a given genotype under the control and stress conditions respectively.

3. Results

3.1. Water Relations

The effect of PEG simulated treatment (Trt), genotypes (G), and their interaction (Trt $\times$ G) were highly significant ($p < 0.001$) on plant water consumption (PWC), leaf relative water content (RWC) and stomatal conductance (gs) traits as shown on ANOVA analysis (Table 1).

Physiological processes such as plant water consumption (PWC), relative water content (RWC), stomatal conductance (gs), transpiration rate and canopy temperature are the key determinant of plant water relations. To exhibit the performance of wheat genotypes against severe osmotic stress and normal conditions, we measured PWC, RWC and gs (Supplementary Table S2, Figure 1A–C). Drought stress showed a detrimental effect on the wheat seedlings though a very narrow range (0.43 to 4.73 g plant^{−1}) with an average of 1.03 g plant^{−1} of water usage (PWC) was noticed. Whereas, under control conditions, a wide range of variation was noted and ranged from 2.13 to 11.6 g plant^{−1} with a mean value of 6.03 g plant^{−1}. Similarly, RWC showed a wide range of variation ranging from 76.1 to 97.1% with an average mean of 91.1% in control and 27.1 to 95.2% with an average mean of 71.4% under drought stress conditions. On the other hand, the stomatal conductance (gs) indicated a high variability ranged from 32.8–214.6 mmol m^{−2}s^{−1} in control and 16.1–112 mmol m^{−2}s^{−1} in stress (Supplementary Table S2, Figure 1C).

Table 1. Analysis of variance (ANOVA) and summary statistics for measured morphological and physio-biochemical traits of 43 bread and durum wheat genotypes evaluated under control and PEG stress conditions in the growth chamber.

Traits	Trt	G	Trt X G	MSerr	CV (%)	LSD _{5%}	Sources of Variation						
							Control			Stress			RC (%)
							Min	Max	Mean $\pm$ SD	Min	Max	Mean $\pm$ SD	
DF	1	42	42	172									
PWC	1613.85 ***	419.5 ***	278.6 ***	0.16	11.3	17.47	2.13	11.6	6.03 $\pm$ 2.24	0.43	4.73	1.03 $\pm$ 0.71	83.0
RWC	24947.2 ***	357.5 ***	53.6 ***	53.6	9.0	17.47	76.1	97.1	91.1 $\pm$ 4.8	27.1	95.2	71.4 $\pm$ 15.3	21.6
Gs	291312 ***	5238 ***	163 ***	163	16.9	30.49	32.8	214.6	109.1 $\pm$ 44.8	16.1	112.0	41.9 $\pm$ 22.1	61.6
RL	2988.6 ***	81.4 ***	4.63 ***	4.63	11.2	5.14	9.94	35.11	22.6 $\pm$ 5.53	7.70	22.4	15.7 $\pm$ 3.33	30.4
SL	7104.8 ***	106.7 ***	5.0 ***	5.0	11.4	5.35	7.33	33.22	24.9 $\pm$ 4.8	6.30	27.6	14.4 $\pm$ 5.41	42.2
RL:SL	9.6 ***	1.37 ***	0.09 ***	0.09	25.7	0.73	0.45	3.29	0.97 $\pm$ 0.45	0.32	2.97	1.26 $\pm$ 0.56	−30.7
SFW	273553 ***	3291 ***	208 ***	208	21.3	34.4	42.7	160.3	100.4 $\pm$ 27.8	6.70	87.3	35.3 $\pm$ 25.0	64.9
SDW	568.8 ***	88.3 ***	24 ns	24.01	47.3	5.14	4.07	21.33	11.8 $\pm$ 4.04	3.30	18.9	8.31 $\pm$ 2.82	25.1
RFW	51949 ***	2636 ***	106 ***	106	15.3	24.5	38.3	130.3	81.7 $\pm$ 23.8	15.0	98.0	53.3 $\pm$ 22.5	34.8
RDW	3657.4 ***	99.3 ***	19.5 ***	19.5	36.1	10.5	6.00	41.3	17.5 $\pm$ 6.91	3.67	12.7	7.03 $\pm$ 2.27	55.2
Car	1.47 ***	0.13 ***	0.001 ***	0.001	4.8	0.07	0.45	1.37	0.74 $\pm$ 0.20	0.36	0.90	0.59 $\pm$ 0.12	20.5
Chla	10.05 ***	0.82 ***	0.003 ***	0.003	3.1	0.14	1.13	3.35	1.97 $\pm$ 0.51	0.94	2.50	1.58 $\pm$ 0.31	20.0
Chlb	1.61 ***	0.16 ***	0.01 ***	0.01	14.9	0.21	0.41	1.42	0.75 $\pm$ 0.23	0.35	1.04	0.59 $\pm$ 0.14	21.0
ChIT	19.7 ***	1.67 ***	0.021 ***	0.021	5.9	0.34	1.54	4.77	2.69 $\pm$ 0.70	1.30	3.38	2.17 $\pm$ 0.44	20.3
PC	23321.8 ***	1605.5 ***	70.2 ***	70.2	44.6	20.01	−1.09	45.00	9.27 $\pm$ 9.78	0.70	141.2	28.3 $\pm$ 27.7	−205.1

Keys: *** Significant at $p < 0.001$, ns: non-significant; DF: degree of freedom; PWC: plant water consumption; RWC: relative water content; gs: stomata conductance; RL: root length; SL: shoot length; RL:SL: root to shoot length ratio; SFW: shoot fresh weight, SDW: shoot dry weight, Car: carotenoids, Chla: chlorophyll a, Chlb: chlorophyll b, ChIT: total extracted chlorophyll (a + b), PC: proline content, MSerr: mean square error; Trt: treatment; G: genotypes; CV coefficient of variation (%); LSD: least significant digits (5%); Min: minimum; Max: maximum; SD: standard deviation; RC: relative change due to stress (%); the (−) sign on RC of RL:SL and PC indicated the mean value of the stress were higher than the control.

Figure 1. Graphical presentation of (A) plant water consumption (PWC, g plants^{−1}), (B) leaf relative water content (RWC, %) and (C) stomatal conductance (gs, mmol m^{−2}s^{−1}) of wheat seedlings grown under PEG mediated osmotic stress. G33 and G34 stands for standard Chinese dryland and G35 for irrigated land cultivars. Vertical lines on each bar showed standard error (n = 3).

The highest and lowest reduction in PWC was observed in G39 (95.5%) and G12 (47.8%) respectively as compared to the control. Likewise, the highest and lowest reduction in RWC was noted in the irrigated land reference line G35 (70.6%) and landrace variety G7 (−10.5%) respectively. On the other hand, the highest and lowest reduction in gs occurred in G27 and G42 respectively (Supplementary Table S2). Overall, G12 showed a better response to PEG induced osmotic stress as compared to other genotypes and corresponding reference Chinese spring wheat cultivars (Supplementary Table S2 and Figure 1A–C). The overall means across 43 bread and durum wheat genotypes decreased significantly by 83.0% (PWC), 21.6% (RWC) and 61.6% (gs) under stress conditions as compared to its control (Supplementary Table S2).

3.2. Root Length, Shoot Length, and Their Biomasses

Growth traits such as root length (RL), shoot length (SL), root length/shoot length (Rt/St), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW) were investigated in 43 bread and durum wheat genotypes after subsequent severe drought stress imposed by 20% (*w/v*) PEG-6000 and Hoagland nutrient solution (control). Our findings showed that the effect of drought treatment (Trt), genotypes (G), and their interaction (Trt × G) was highly significant ($p < 0.001$) except SDW (Table 1) but the genotypes respond differently for all the investigated traits (Figure 2A–F). PEG simulated drought stress had significantly declined RL, SL, SFW, SDW, RFW, and RDW by 30.4%, 42.2%, 64.9%, 25.1%, 34.8%, and 55.2%, respectively as compared to the control (Supplementary Table S2). The mean value of growth attribute traits ranged from 9.9–35.1 cm (RL), 7.3–33.2 cm (SL), 42.7–160.3 mg (SFW), 4.1–21.3 mg (SDW), 38.3–130.3 mg (RFW), and 6–41.3 mg (RDW) in control (Supplementary Table S2). A prominent effect of PEG stress on growth attribute traits was noted and ranged from 7.7–22.4 cm (RL), 6.3–27.6 cm (SL), 6.7–87.3 mg (SFW), 3.3–18.9 mg (SDW), 15–98 mg (RFW), 3.7–12.7 mg (RDW) (Supplementary Table S2). Under the water-deficit condition, a plant with deep rooting and reduced shoot length can better escape the stress than shallow root and long leave types. Based on this, the Chinese standard cultivars G34 and G35 showed both better RL performance with reduced SL. Whereas G30 and G32 showed better RL and SL than the standard Chinese cultivars (Supplementary Table S2 and Figure 2A,B) under PEG stress conditions.

3.3. Photosynthesis Pigments

To examine the response of photosynthesis pigments among wheat genotypes towards PEG mediated stress, healthy and fully extended leaves were collected and extracted for OD readings from both stress and non-stress treatments. In this study, we observed that drought stress treatment had significantly ($p < 0.001$) affected photosynthetic pigments (Table 1). In the control conditions, Car, Chla, Chlb and ChlT ranged from 0.45–1.37, 1.13–3.35, 0.41–1.42; 1.54–4.77 mg g^{−1}FW respectively, and G39 showed the highest mean performance in all photosynthetic pigments. In contrast, under drought stress conditions, wheat genotypes responded differently towards stress and showed a considerable change in their quantities (Supplementary Table S2 and Figure 3A–D). Mean values of Car, Chla, Chlb and ChlT ranged from 0.36–0.90; 0.94–2.50; 0.35–1.04 and 1.30–3.38 mg g^{−1}FW respectively and genotypes G39, G22, G8, and G27 showed the highest mean values in Car, Chla, Chlb and ChlT respectively (Supplementary Table S2 and Figure 3A–D). The overall means across 43 bread and durum wheat genotypes decreased significantly by 20.5% (Car), 20.0% (Chla), 21.1% (Chlb), and 19.4% (ChlT) under stress conditions as compared to its control (Supplementary Table S2).

Figure 2. Graphical presentation of (A) root length (RL, cm), (B) shoot length (SL, cm), (C) shoot fresh weight (SFW, mg plant⁻¹), (D) shoot dry weight (SDW, mg plant⁻¹), (E) root fresh weight (RFW, mg plant⁻¹), (F) root dry weight (RDW, mg plant⁻¹) of wheat seedlings grown under PEG mediated osmotic stress. G33 and G34 stands for standard Chinese dryland and G35 for irrigated land cultivars. Vertical lines on each bar showed standard error (n = 3).

Figure 3. Graphical presentation of (A) chlorophyll a (Chl a, $\text{mg g}^{-1}\text{FW}$), (B) chlorophyll b (Chl b, $\text{mg g}^{-1}\text{FW}$), (C) carotenoids (Car, $\text{mg g}^{-1}\text{FW}$), and (D) total chlorophyll content (ChlT, $\text{mg g}^{-1}\text{FW}$) of wheat seedlings grown under PEG mediated osmotic stress. G33 and G34 stands for standard Chinese dryland and G35 for irrigated land cultivars. Vertical lines on each bar showed standard error ($n = 3$).

3.4. Proline Accumulation and Root Length to Shoot Length Ratio

An increase in root length to shoot length ratio (RL:SL) and proline accumulation in the leaf of the chloroplast is an indicator of drought stress. The increase in RL:SL ratio also showed a similar trend in proline accumulation in the leaf of wheat germplasms as shown in Figure 4. All wheat genotypes exhibited a low level of free proline under control conditions except G5 and G35, which showed a high level of proline as compared to other genotypes (Figure 4B). Under control condition, proline content (PC) ranged from -1.09 to 45.0 with an average mean of $9.27 \mu\text{mole g}^{-1}\text{FW}$. Whereas, under drought stress PC exhibited a significant increase in all genotypes except G13, G20 and G42 and ranged from 0.71 to 141.2 with an average mean of $28.3 \mu\text{mole g}^{-1}\text{FW}$ (Supplementary Table S2 and Figure 4B). As shown in Figure 5B, the standard Chinese dryland cultivars (G33 and G34) showed a small increase in PC while the irrigated land cultivar (G35) showed a considerable increase in PC accumulation under stress conditions.

Figure 4. Graphical presentation of (A) root and shoot length ratio (RL:SL), (B) proline content (PC, $\mu\text{mole g}^{-1}\text{FW}$) of wheat seedlings grown under PEG mediated osmotic stress. G33 and G34 stands for standard Chinese dryland and G35 for irrigated land cultivars. Vertical lines on each bar showed standard error ($n = 3$).

Figure 5. Loading plots of the PCA illustrates the relationship of variables for control (A), and stress (B). Abbreviations of variables are found in Table 1. STI: stress tolerance index.

Similarly, the RL:SL ratio showed a relative reduction in almost all genotypes ranged from 0.45 to 3.29 with an average of 0.97 (Supplementary Table S2) except G34, G19, G13, G25, and G3 which showed considerable mean performance under control conditions. Whereas, under drought stress conditions, RL:SL ratio ranged from 0.39 to 2.97 (Supplementary Table S2), and genotypes G35, G36, G33, G39, G8, G31, and G10 showed the highest mean values (Figure 4A and Supplementary Table S2). The overall mean value of RL to SL ratio and PC had increased by 30.7% and 205.1% respectively as compared to the control (Supplementary Table S2). The relative change due to drought stress revealed that the Rt/St length ratio had increased in almost all tested genotypes except G24(4%), G26(8.8%), G20(13.1%), G22(14.8%), G3(16.7%), G34(18%), G23(36.4%), G13(36.6%), G25(37.5%) and G19(43.6%) lower than the controls (Supplementary Table S2).

3.5. Multivariate Analysis

3.5.1. Principal Component Analysis (PCA)

To visualize the ratio of total variance explained by different components and variables, PCA was computed for control and stress treatments separately (Table 2). Results from PCA demonstrated that under control conditions, the first five PCs explained Eigenvalues more than unity and explained a cumulative variance of 79.58%. Among these, the first two PCs such as PC-1 and PC-2 explained 32.26% and 22.21% of phenotypic variation respectively. The major contributors (more than 30%) for PC-1 were PWC, Car, Chla, Chlb, and ChlT. Likewise, SL, SFW, and SDW were highly contributed to PC-2 in the control condition (Table 2). Similarly, under drought stress conditions, the first five PCs explained Eigenvalues more than unity and explained a cumulative variance of 80.86%. Among these, the first two PCs (PC-1 and PC-2) explained 32.12% and 22.76% of total variance respectively. The major contributors for PC-1 were PWC, gs, SL, SFW, and STI. While Car, Chla, Chlb, and ChlT were found to be the major contributors for PC-2 (Table 2).

Table 2. Principal component analysis of the measured physio-morphological and biochemical traits.

Traits	Stress					Traits	Control				
	PC-1	PC-2	PC-3	PC-4	PC-5		PC-1	PC-2	PC-3	PC-4	PC-5
PWC	0.323	−0.014	−0.219	0.356	−0.234	PWC	0.310	0.173	−0.118	0.052	0.397
RWC	0.227	−0.058	−0.356	0.222	0.529	RWC	0.143	0.086	0.440	−0.141	−0.282
gs	0.351	−0.030	−0.182	0.048	−0.168	gs	0.264	0.173	0.184	0.275	0.097
RL	−0.032	−0.135	0.435	0.500	0.089	RL	−0.005	−0.086	0.236	0.737	0.171
SL	0.369	−0.057	−0.019	−0.196	0.113	SL	0.142	0.306	−0.383	0.344	−0.056
RL: SL	−0.313	0.024	0.237	0.367	−0.158	RL: SL	−0.075	−0.351	0.477	0.150	0.121
SFW	0.366	−0.041	−0.006	0.193	0.056	SFW	0.252	0.368	0.047	−0.012	0.173
SDW	0.254	0.044	0.387	−0.211	−0.271	SDW	0.227	0.371	0.029	−0.136	0.058
RFW	0.166	−0.052	0.504	−0.241	0.150	RFW	0.175	0.184	0.453	−0.138	−0.206
RDW	0.157	−0.031	0.151	−0.324	0.390	RDW	0.212	−0.090	−0.072	0.381	−0.480
Car	0.009	0.483	0.073	−0.005	0.054	Car	0.341	−0.307	−0.138	−0.041	−0.003
Chla	0.056	0.486	0.009	0.033	0.117	Chla	0.341	−0.306	−0.104	−0.099	0.038
Chlb	−0.013	0.478	0.001	0.096	0.014	Chlb	0.350	−0.285	−0.091	−0.095	0.012
ChlT	0.035	0.500	0.007	0.056	0.089	ChlT	0.337	−0.317	−0.080	−0.044	−0.002
PC	−0.167	0.089	−0.265	−0.368	−0.309	PC	−0.206	−0.142	0.011	−0.016	0.555
STI	0.326	0.049	−0.012	0.032	−0.464						
E.value	5.460	3.869	1.991	1.403	1.023	E.value	5.162	3.554	1.542	1.384	1.092
Var. (%)	32.120	22.759	11.715	8.254	6.019	Var. (%)	32.260	22.211	9.637	8.648	6.827
Cum. (%)	32.120	54.878	66.593	74.846	80.866	Cum. (%)	32.260	54.471	64.107	72.755	79.582

Keys: PWC: Plant water consumption; RWC: relative water content; stomata conductance; RL: root length; SL: shoot length; RL:SL: root to shoot length ratio; SFW: shoot fresh weight, SDW: shoot dry weight, Car: carotenoids, Chla: chlorophyll a, Chlb: chlorophyll b, ChlT: total extracted chlorophyll (a + b), PC: proline content; STI: stress tolerance index; E.value: Eigenvalue; Var: explained variability (%); Cum.(%): cumulative variation (%).

To elucidate the relationship between variables, a PCA biplot based on correlation was performed for the control and stress treatments. Under normal conditions, PC-1 was positively associated with SL, SDW, SFW, RFW, Gs, PWC, RWC, SPAD, RDW, Chla, Chlb, Car, and ChlT. Whereas, PC-2 was negatively associated with RL, PC, and RL:SL (Figure 5A). Likewise, under PEG stress, PC-1 was positively associated with PWC, RWC, Gs, SL, SFW, SDW, RDW, RFW, and STI, Chla, and ChlT. Whereas PC-2 was negatively correlated with Car, Chlb, RL, RL:SL, and PC (Figure 5B).

A PCA biplot analysis of control and stress treatment presented in Figure 6A,B demonstrated the distribution of *T. aestivum* and *T. durum* based on the estimated value of the measured variables. To further elucidate the influence of PEG mediated drought stress on genotypes, the combined PCA biplot was constructed (Figure 6C). As a result, the high-value quantitative traits of control treatments (blue fonts) were distributed on the 1st and 4th quadrant whereas; the drought stress treatments (red fonts) were distributed on the 2nd and 3rd quadrants. Genotypes having the same performance appeared in the same square box/quadrant and genotypes with different performance also appeared in different quadrants as also discussed by Arteaga et al. [77] in common bean. Interestingly, G35, G11, and G12 appeared on 2nd, 3rd, and 4th quadrants respectively in both control and stress treatments (Figure 6C) which suggested that these genotypes have the same performance in both normal and drought stress conditions. Therefore, PCA in our study helped us to dis-

tinguish the correlation between variables and screening of potential genotypes extracted from the large dataset.

Figure 6. PCA biplot based distribution of 43 bread and durum wheat genotypes under (A) control (B) stress, and (C) control (blue) + stress (red). Blue and red font genotypes in (A,B) stand for *T. aestivum* and *T. durum* respectively. Genotypes encircled with green in B stand for best drought tolerant genotypes and yellow encircled genotypes for sensitive ones. Abbreviation of genotypes found in Supplementary Table S1.

3.5.2. Agglomerative Hierarchical Clustering (AHC)

Cluster analysis based on Euclidian distance groups similar data into clusters and allowed us to determine the inter-relationship between genotypes. To group the studied wheat genotypes according to their dissimilarity; AHC analysis was performed in XLSTAT add-in software. Based on standardized drought tolerant-related morpho-physiological data, all the studied wheat genotypes were clustered into four groups such as cluster II (C-II) and cluster III (C-III) accounted for the highest number (12) followed by cluster IV(C-IV) (11) (Figure 7). All drought tolerant genotypes were grouped in cluster III and sensitive genotypes in cluster II. The other two clusters (C-I and IV) containing moderately tolerant genotypes. Furthermore, the genotypes with similar characteristics were clustered in the same group and summarized in Supplementary Table S3.

Figure 7. Agglomerative hierarchical cluster analysis of 43 wheat genotypes based on Euclidean distance of the measured morphological and physio-biochemical traits under stress conditions; Abbreviations of genotypes are found in Supplementary Table S1. The highlighted red font genotype stands for dryland (G33 and 34) and light blue irrigated land (G35) Chinese spring wheat cultivars. Cluster III (blue) (drought tolerant), cluster IV (green) and cluster I (pink) (moderately tolerant) and cluster II (red) (drought-sensitive).

3.5.3. Correlation Analysis

Correlation is the measure of the degree of association between two variables or factors. The association of growth and physio-biochemical traits under control and stress conditions were computed separately by Pearson's correlation coefficient ($r = 1$) using XLSTAT add-in excel software (Table 3). A significant and positive correlation was found between PWC and Gs; PWC and SL; PWC and SFW; RWC and Gs; RWC and SL and RWC and SFW in both growing conditions. Moreover, a significant and positive correlation was also noted between PWC and RWC; RWC and SL; and RWC and SFW under stress conditions only. In contrast, PWC and RDW were positively correlated with all photosynthetic pigments (Car, Chla, Chlb, and ChIT) in control but did not show any correlation under stress conditions.

Table 3. Pearson's correlations among growth and physio-biochemical parameters of 43 bread and durum wheat genotypes evaluated in hydroponic solution under control (upper diagonal) and severe stressed (lower diagonal) conditions in the growth chamber.

Traits	PWC	RWC	Gs	RL	SL	RL:SL	SFW	SDW	RFW	RDW	Car	Chla	Chlb	ChIT	PC
PWC	1	0.120	0.588	−0.009	0.412	−0.350	0.636	0.595	0.232	0.165	0.373	0.374	0.392	0.331	−0.157
RWC	0.522	1	0.303	−0.038	0.011	−0.015	0.231	0.218	0.407	0.084	0.082	0.116	0.145	0.119	−0.197
Gs	0.766	0.479	1	0.159	0.319	−0.150	0.460	0.455	0.333	0.340	0.192	0.185	0.243	0.210	−0.267
RL	−0.014	−0.075	−0.157	1	0.157	0.447	−0.026	−0.234	−0.045	0.196	0.014	−0.018	−0.027	0.023	0.089
SL	0.476	0.457	0.709	−0.113	1	−0.698	0.541	0.380	0.029	0.190	0.010	−0.041	−0.042	−0.060	−0.340
RL:SL	−0.395	−0.506	−0.540	0.493	−0.827	1	−0.440	−0.461	−0.081	−0.019	0.140	0.158	0.119	0.186	0.193
SFW	0.712	0.537	0.703	0.099	0.697	−0.530	1	0.839	0.461	0.030	0.040	0.055	0.087	0.050	−0.403
SDW	0.164	−0.113	0.280	0.125	0.552	−0.365	0.453	1	0.425	0.121	−0.017	0.010	0.059	−0.035	−0.399
RFW	−0.039	−0.138	0.213	0.225	0.386	−0.157	0.288	0.515	1	0.143	0.052	0.061	0.095	0.049	−0.289
RDW	0.104	0.222	0.178	0.001	0.279	−0.265	0.206	0.251	0.375	1	0.430	0.362	0.399	0.446	−0.290
Car	−0.037	−0.111	−0.044	−0.172	−0.095	0.082	−0.054	0.139	−0.006	0.044	1	0.979	0.953	0.945	−0.211
Chla	0.038	0.028	0.007	−0.229	0.028	−0.066	0.061	0.144	−0.043	−0.027	0.897	1	0.949	0.953	−0.192
Chlb	0.007	−0.095	−0.049	−0.161	−0.142	0.109	−0.086	0.017	−0.109	−0.089	0.868	0.849	1	0.944	−0.205
ChIT	0.029	−0.009	−0.012	−0.212	−0.028	−0.009	0.015	0.107	−0.067	−0.047	0.919	0.985	0.928	1	−0.189
PC	−0.267	−0.257	−0.108	−0.333	−0.284	0.135	−0.366	−0.283	−0.313	−0.047	0.137	0.043	0.128	0.072	1
STI	0.757	0.215	0.618	−0.124	0.493	−0.444	0.530	0.608	0.132	0.253	0.080	0.128	0.066	0.112	−0.210

Values in bold are different from 0 with a significance level $\alpha = 0.05$; STI: stress tolerance index.

Photosynthetic pigments showed a strong and positive correlation with each other in both growth conditions. On the other hand, a significant and negative correlation was found between PWC and RL:SL; SL and RL:SL; SFW and PC in both growth conditions. Moreover, PC exhibited a negative correlation with RL under stress conditions and also showed a non-significant positive correlation in control. STI showed a significant and positive correlation with PWC, SL, SFW, SDW, and negative correlations with RL:SL under stress conditions.

3.6. Screening of Wheat Genotypes Using Stress Tolerance Index (STI)

Based on a dry matter (SDW), drought stress indices were computed using iPASTIC: an online toolkit developed by Pour et al. [76]. Based on the STI bar graph (Figure 8) genotypes such as G12, G25, G40, G20, G16, G38, and G18 are categorized as drought-tolerant genotypes whereas, G13 (0.91), G17(1.11), G19(0.85), G21(1.04), G26(1.07), G27(0.83), G43(0.93), G14(0.87), G23(1.01) and G39(0.86) were categorized as medium tolerant and the remaining as susceptible. Interestingly, the Chinese standard dryland G33(0.74), G34(0.17) and irrigated land G35(0.27) cultivars showed STI < 0.82 (median value). However, predicting the drought tolerance and sensitivity of genotypes using a single trait may not be easy. Therefore, average sum of rank (ASR) was computed using all the drought stress indices (Supplementary Table S2), and genotypes having low ASR values were regarded as drought tolerant and high ASR as sensitive. Based on ASR G12, G25 and G16 have low ASR values and are categorized as the best drought-tolerant, and G6, G42, G4, G11, and G9 as bottom 5 sensitive genotypes with high ASR value.

Figure 8. Stress tolerance index (STI) of 43 bread (*T. aestivum*) and durum (*T. durum*) wheat genotypes. The horizontal red line indicated the median value (0.82). The numbers labeled above the median line indicated STI > 0.82. The red font values above the vertical bar are the highest STI which indicated the most drought tolerant genotypes.

4. Discussion

Among environmental factors, drought causes huge damage to the plant's morphological, physiological, biochemical, and molecular processes at all growth stages [30,40]. Breeding for drought tolerance is a major objective of plant breeders to enhance grain yield and maintain productivity under environmental stress. Landraces, often called "farmer varieties", were evolved from their wild progenitor's since the dawn of agriculture, and were grown on farmers' own land with the potential sources of genetic diversity and resistance to biotic/abiotic stresses [66,78] useful in the breeding of climate change-resilient modern cultivars. In this regard, many researchers speculated that Ethiopian durum wheat landraces have a potential source of genetic diversity and also have useful traits that can confer resistance to biotic and abiotic stresses [13,14,79]. Therefore, the identification and selection of promising genotypes possessing useful morpho-agronomic and physiological

responses under water stress environment is one of the key steps of breeding [80]. The present study was conducted to identify the best performing drought-tolerant genotypes by complementing morphological and physio-biochemical traits under stress and non-stress conditions at the seedling stage.

It is well known that introducing PEG solution in the growth medium simulates drought stress similar to the natural condition without entering the plant cells [61]. Polle et al. [31] reported that drought-tolerant wheat genotypes condition themselves after imposed drought stress. Several researchers also documented that plant growth parameters such as shoot and root length and their biomasses were significantly affected by drought stress created by PEG osmoticum (plural: osmotica) and their significance for drought stress screening in wheat [24,49,61,63,81–85] and barley [57] have been extensively studied. In the present study, the effect of genotypes (G), treatment (Trt), and their interaction (Trt $\times$ G) was highly significant ($p < 0.001$) for the studied growth attribute traits except for SDW (Table 1) which is also in agreement with previous reports in wheat [31,43,81,86] and rice [87].

The root is the main organ of the plant for water and mineral acquisition from the soil. Deprivation of water in the soil causes a root system plasticity [84] which further reduces root proliferation and ultimately inhibits the upstream water and mineral transportation and largely reduces grain yield [56]. More recently, Ahmed et al. [58] reported that wheat plants with a deeper root growth and suppressed shoot system are an indicator of drought tolerance, as they have increased root to shoot ratios. In the present study, though drought stress had a significant effect ($p < 0.001$) on the shoot and root length and their fresh and dry biomasses, some of the wheat genotypes exhibited better root and shoot length under stress conditions (Supplementary Table S2), which might suggest that a smaller root to shoot ratio increased the transpiration rate as the water is not adequately transported via the root and the higher shoot may in turn, facilitate the transpiration rate [88,89] and eventually lead to wilting and senescence. Interestingly SFW was highly affected by PEG mediated drought stress (64.9%) followed by g_s (61.6%) and RDW (55.2%) (Table 1) as compared to the control and other growth attribute traits. In contrast, root to shoot length ratio showed an increment (30.7%) in most of the genotypes which are in agreement with Agathaokleous [90] findings who found a considerable increase in root to shoot ratio and concluded that the increment of root:shoot ratio during stress condition is an indicator of plant health status.

Plants need sufficient water during their life cycle [91]. Estimation of water status in the plant is therefore important to determine the drought tolerance and sensitivity of the plant under drought stress conditions. Previous studies indicated that physiological processes such as relative water content (RWC), transpiration rate, stomatal conductance (g_s), leaf water potential, and canopy temperature are the key determinant of plant water relations [89]. In the current study, we measured PWC, RWC, and g_s and the effect of PEG stress on these parameters was significant (Table 1). Total water consumption by the plant (PWC) is an important trait that signifies the amount of water consumed by the plant under normal and drought stress conditions [92]. Our data showed that drought-tolerant genotypes utilized more water under stress conditions as compared to sensitive types. In this regard, G12 was efficiently utilized more water ($4.73 \text{ g plant}^{-1}$) under stress conditions as compared to the Chinese irrigated land spring wheat cultivar G35 ($0.53 \text{ g plant}^{-1}$) and dry land cultivars G33 ($0.67 \text{ g plant}^{-1}$) and G34 ($0.87 \text{ g plant}^{-1}$) showed the least water usage by the plant. Moreover, a narrow range of water consumption ($0.43\text{--}4.73 \text{ g plant}^{-1}$) was observed under stress condition than plants grown in the normal condition ($2.13\text{--}11.57 \text{ g plant}^{-1}$) (Supplementary Table S2, Figure 1A). The wide range of variation pronounced in the plant water consumption under drought stress might be due to the osmotic stress created by PEG which strongly influence root hair traits and gradually inhibits the water and mineral absorption. It has been claimed that severe osmotic stress reduced root growth and root apical meristem (RAM) [93] and therefore the efficiency of water uptake and plants adaptation under drought stress condition depends on the root architecture [93], root length density [94] and osmotic adjustment of the plants [95,96].

In another study, Nakhforoosh et al. [97] identified four water use strategies (area type, spender type, conductance type and water saver types) as revealed by morphological (early stage shoot phenotyping) and physiological (stomatal conductance) techniques in the studied 12 world-wide durum wheat collections. According to this study, most of the landraces fall under area type that is due to the extensive leaf area, while modern cultivars showed high stomatal conductance which is the basis for high dry matter per unit leaf area. On the other hand, cuticular transpiration is a type transpiration under circumstance of maximum stomata closure and acting as a predictor of drought resistance [98,99]. Though it was not considered in this study, further investigations are needed to identify the effect of cuticular transpiration per unit of leaf area on plants water consumption in the future.

Leaf RWC is the most common physiological method used in drought screening to assess the sensitivity of the plant tissue and cell dehydration tolerance [37,100,101]. Several researchers also documented that drought-resistant genotypes retain more water in their leaf as compared to the sensitive genotypes [30,58,61,86]. Our results revealed that drought stress significantly declined RWC by 21.6% which is consistent with Pour et al. [37] findings who reported that drought stress decreased RWC in wheat. To reveal the drought sensitivity of Ethiopian wheat genotypes, a comparative assessment was done with the known drought resistant and sensitive Chinese spring wheat cultivars. Accordingly, the irrigated land Chinese spring wheat cultivar (G35) showed a maximum reduction of RWC (70.6%) as compared to dry land cultivars G33 (11.7%), and G34 (20.1%) and other investigated genotypes such as G12 (−0.30%), G1 (−0.60%) and G7 (−10.5%) which showed considerable water retention in their leaf under stress condition (Supplementary Table S2 and Figure 1B). This suggested that RWC is species-dependent [102] and different genotypes may have different threshold levels to maintain cell dehydration under stress and normal growth conditions. Nonetheless, plants regulate gas exchange (i.e., uptake of carbon dioxide) and loss of water via transpiration through sophisticated tiny pores located on the leaf epidermis called stomata [103]. Stomatal conductance (g_s) is a measure of the degree of stomata opening and can be used as an indicator of plant water status [103,104]. Ratzmann et al. [89] reported that stomatal closure is induced by reduction of leaf water potential which in turn reduced transpiration and ultimately leading to a lowered g_s . A strong and positive correlation found between RWC and g_s (Table 2) revealed that an increase in RWC opens stomata and thereby increases g_s which is in agreement with previous studies [105]. In the present study, a profound effect of PEG stress on g_s (61.6%) (Table 1) was observed consistent with previous works in wheat [37] and maize [106] findings, which found a considerable reduction of g_s under drought conditions. Moreover, the wide range of variation that existed in the normal (32.8–214.6 $\text{mmol m}^{-2}\text{s}^{-1}$) and drought stress (16.1–112.0 $\text{mmol m}^{-2}\text{s}^{-1}$) (Supplementary Table S2 and Figure 1C) conditions may be due to the high genetic variability among wheat genotypes.

Chlorophyll and carotenoids are the main pigments for photosynthesis in plants which are sensitive to drought stress [107], and therefore their decrease during stress is an indicator of plant health status. In the present study, the effect of drought stress on the chlorophyll and carotenoid content among genotypes behaved differently within a narrow range, and the concentration of Chla was found higher than Chlb (Supplementary Table S2 and Figure 3A–D). These results are in agreement with Ahmad et al. [105] who reported that the effect of drought stress on Chla was 3 times higher than Chlb. As a result, the significant degradation of Chla over Chlb ultimately impaired photosynthesis which might also be attributed to the lowering of ribulose-1,5-bisphosphate (RuBP) activity [102]. Desiccation tolerant plants maintain their metabolic process by employing several strategies such as substantial accumulation of proline in their leaves. Proline is an osmolyte acting also as an osmotic regulator and has an antioxidant activity by scavenging reactive oxygen species (ROS) and maintaining plants from further oxidative damage and cell death [108]. Several studies reported that the higher free proline accumulation is associated with drought tolerance and the lower for sensitivity [42,108]. Although proline was increased when exposed to drought stress in all considered wheat genotypes, the degree of proline accu-

mulation varied, and the increment was noticed on sensitive genotypes including Chinese drought-sensitive cultivar G35 (Supplementary Table S2 and Figure 4B). Similar studies from Rampino et al. [30] in wheat and Ibarra et al. [109] in maize reported that the level of free proline increased as RWC decreased in all sensitive wheat genotypes. A positive and non-significant correlation between proline and other chlorophyll pigments (Chla, Chlb, Car, and ChlT) (Table 3) highlights the increase in proline content in the leaf has little effect on the amount of chlorophyll as also indicated on Figures 3A–D and 4B. Though proline increases during drought stress, the role of proline accumulation in conferring toward drought stress tolerance remained controversial. Therefore, further study is required to avoid the discrepancies regarding whether or not the increase in proline levels can help plants cope during drought stress.

Among several drought stress indices, STI is the most widely used index to evaluate drought tolerance genotypes since it discriminates drought-tolerant and sensitive genotypes [86] having better biomass, stress tolerance potential [37,85,110], and its strong correlation with other indices. In this study, STI was computed based on the dry matter to identify the best drought-tolerant wheat genotypes under PEG simulated drought stress conditions (Supplementary Table S2 and Figure 8). Our result showed a wide range of STI values, that ranged from 0.17–4.28 in all 43 genotypes. Pour et al. [37] reported that STI is the best method to identify drought tolerance genotypes and found STI values (0.08–0.75) that were less than our finding. The difference in the value of STI might be due to the genetic variability of the study materials used, the stage and duration of drought stress application. Based on STI, our result revealed that Ethiopian wheat genotypes showed a better performance towards drought stress as compared to standard Chinese wheat cultivars (Figure 8). Several researchers also reported that genotypes having the maximum STI value are considered drought tolerant [76,111]. Accordingly, G12 and G25 were selected as the best drought-tolerant wheat genotypes having the highest STI value and G6, G4, G9, and G11 as the most drought-sensitive types (Figure 8) which is in agreement with previous reports.

A wide range of coefficient of variation (CV) was found in this study, ranging from 3.23% to 47.3%; the highest value being in SDW (47.3%) followed by PC (44.63%) and RDW (36.03%) (Table 1). Our result revealed that there was high genetic variability between these traits. A similar study from Pour et al. [37] found that the genetic variation found on SDW and other traits in the studied species of *Ae. tauschii* and *Ae. crassa* were genetically determined and therefore, drought stress breeding based on these traits on the identified drought-tolerant genotypes might be plausible.

Plant physio-morphological traits are very important and widely used for selection of better performed genotypes [112]. However, drought tolerance is a complex trait which is controlled by polygenes. Therefore, the identification of key genes that encode for drought stress in the identified wheat genotypes is very important for marker assisted breeding (MAB) program [113].

5. Conclusions

In conclusion, severe drought stress imposed at the early growth stage significantly affected the growth and physio-biochemical traits of wheat seedlings. Among morpho-physiological and biochemical traits, plant water consumption (PWC) was highly affected by drought stress followed by stomatal conductance (gs) and shoot fresh weight (SFW). The combined PCA biplot and ASR analysis of morpho-physiological and biochemical traits demonstrated that G12, G25, and G16 showed better performance under normal and drought stress conditions as compared to standard Chinese wheat cultivars and other studied genotypes. Furthermore, the agglomerative hierarchical clustering dendrogram showed that the identified wheat genotypes were clustered together with standard Chinese drought-tolerant cultivars which supported our conclusion. Therefore, to determine the underlying molecular mechanisms for drought tolerance in these genotypes, further studies are necessary at the molecular and genomic level to enhance them for breeding.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/su13094605/s1>, Supplementary Table S1 (Doc): Genotypes, codes, species, origin, and pedigree of 43 spring wheat genotypes used in this study. Supplementary Table S2 (Xlsx): Mean values of 43 bread and durum wheat genotypes evaluated hydroponically in the growth chamber using 16 seedling traits under drought stress and non-stress conditions (Sheet 1); Drought stress indices computed by iPASTIC: an online toolkit on shoot dry matter basis (Sheet 2); Ranking of wheat genotypes based on the estimated drought stress indices and average sum of ranks (ASR) (Sheet 3); Correlations between drought stress indices (Sheet 4). Supplementary Table S3 (Doc): Clustering of 43 bread and durum wheat genotypes using Euclidean distance under stress conditions.

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Abbreviations

ASR, Average sum of ranks; iPASTIC, Plant Abiotic Stress Index Calculator; OD, Optical density; PWC, plant water consumption; RCBD, randomized complete block design; STI, stress tolerance index; w/v , weight by volume ratio.

References

- Lev-Yadun, S.; Gopher, A.; Abbo, S. The cradle of agriculture. *Science* **2000**, *288*, 1602–1603. [\[CrossRef\]](#)
- Shiferaw, B.; Smale, M.; Braun, H.J.; Duveiller, E.; Reynolds, M.; Muricho, G. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Secur.* **2013**, *5*, 291–317. [\[CrossRef\]](#)
- Shewry, P.R.; Hey, S.J. The contribution of wheat to human diet and health. *Food Energy Secur.* **2015**, *4*, 178–202. [\[CrossRef\]](#) [\[PubMed\]](#)
- Lobell, D.B.; Gourdji, S.M. The influence of climate change on global crop productivity. *Plant. Physiol.* **2012**, *160*, 1686–1697. [\[CrossRef\]](#)
- FAO. *Crop Prospects and Food Situation-Quarterly Global Report No. 2*; FAO: Quebec City, QC, Canada, 2020. Available online: <http://www.fao.org/publications/card/en/c/b223a9f4-e425-4924-8478-6cf9e74647f7/> (accessed on 19 August 2020).
- Huaxia. *China Evaluates 24,000 Files of Drought-Resistant Wheat Resources*; Xuaxia: Beijing, China, 2020. Available online: http://www.xinhuanet.com/english/2020-06/10/c_139129191.html (accessed on 13 February 2021).
- He, Z.H. *A History of Wheat Breeding in China*; Cimmyt: Mexico City, Mexico, 2001.
- Li, H.; Zhou, Y.; Xin, W.; Wei, Y.; Zhang, J.; Guo, L. Wheat breeding in northern China: Achievements and technical advances. *Crop J.* **2019**, *7*, 718–729. [\[CrossRef\]](#)
- Hailu, G.-M.; Tanner, D.G.; Mengistu, H. *Wheat Research in Ethiopia: A Historical Perspective*; FAO: Quebec City, QC, Canada, 1991.
- Esterhuizen, D.; Bonsu, K. *Grain and Feed Annual Report*; USDA: Washington, DC, USA, 2020; pp. 1–19.
- Chesley, M.; Tefera, A. *Ethiopia Grain and Feed*; USDA: Washington, DC, USA, 2012.
- Tadesse, W.; Bishaw, Z.; Assefa, S. Wheat production and breeding in Sub-Saharan Africa: Challenges and opportunities in the face of climate change. *Int. J. Clim. Chang. Strateg. Manag.* **2019**, *11*, 696–715. [\[CrossRef\]](#)

13. Alemu, A.; Feyissa, T.; Letta, T.; Abeyo, B. Genetic diversity and population structure analysis based on the high density SNP markers in Ethiopian durum wheat (*Triticum turgidum* ssp. durum). *BMC Genet.* **2020**, *21*, 1–12. [\[CrossRef\]](#)
14. Mengistu, D.K.; Kiros, A.Y.; Pè, M.E. Phenotypic diversity in Ethiopian durum wheat (*Triticum turgidum* var. durum) landraces. *Crop J.* **2015**, *3*, 190–199. [\[CrossRef\]](#)
15. Harlan, J.R. Ethiopia: A center of diversity. *Econ. Bot.* **1969**, *23*, 309–314. [\[CrossRef\]](#)
16. Mengistu, D.K.; Kidane, Y.G.; Catellani, M.; Frascaroli, E.; Fadda, C.; Pè, M.E.; Dell’Acqua, M. High-density molecular characterization and association mapping in Ethiopian durum wheat landraces reveals high diversity and potential for wheat breeding. *Plant Biotechnol. J.* **2016**, *14*, 1800–1812. [\[CrossRef\]](#)
17. Maccaferri, M.; Harris, N.S.; Twardziok, S.O.; Pasam, R.K.; Gundlach, H.; Spannagl, M.; Ormanbekova, D.; Lux, T.; Prade, V.M.; Milner, S.G.; et al. Durum wheat genome highlights past domestication signatures and future improvement targets. *Nat. Genet.* **2019**, *51*, 885–895. [\[CrossRef\]](#)
18. Rao, I.U.; Pandey, B.K. Origin and introduction of crop plants, cereals, and pulses. *Econ. Bot.* **2007**, 1–34.
19. Hedberg, I.; Edwards, S. Flora of Ethiopia and Eritrea Poaceae. *Natl. Herb. Ethiop.* **1995**, *7*, 1–420.
20. Kabbaj, H.; Sall, A.T.; Al-Abdallat, A.; Geleta, M.; Amri, A.; Filali-Maltouf, A.; Belkadi, B.; Ortiz, R.; Bassi, F.M. Genetic diversity within a global panel of durum wheat (*Triticum durum*) landraces and modern germplasm reveals the history of alleles exchange. *Front. Plant. Sci.* **2017**, *8*, 1–13. [\[CrossRef\]](#)
21. Vavilov, N.I. Phylogeographic basis of plant breeding. The origin, variation, immunity, and breeding of cultivated plants. *Agron. J.* **1952**, *44*, 102.
22. Adhikari, U.; Nejadhashemi, A.P.; Woznicki, S.A. Climate change and eastern Africa: A review of impact on major crops. *Food Energy Secur.* **2015**, *4*, 110–132. [\[CrossRef\]](#)
23. Al-Othman, Z.A.; Ali, R.; Al-Othman, A.M.; Ali, J.A.; Habila, M. Assessment of toxic metals in wheat crops grown on selected soils, irrigated by different water sources. *Arab. J. Chem.* **2016**, *9*, S1555–S1562. [\[CrossRef\]](#)
24. Duan, H.; Zhu, Y.; Li, J.; Ding, W.; Wang, H.; Jiang, L.; Zhou, Y. Effects of drought stress on growth and development of wheat seedlings. *Int. J. Environ. Res. Public Health* **2017**, *19*, 1119–1124.
25. Abhinandan, K.; Skori, L.; Stanic, M.; Hickerson, N.M.N.; Jamshed, M.; Samuel, M.A. Abiotic stress signaling in wheat—an inclusive overview of hormonal interactions during abiotic stress responses in wheat. *Front. Plant Sci.* **2018**, *9*, 1–25. [\[CrossRef\]](#)
26. Lamaoui, M.; Jemo, M.; Datla, R.; Bekkaoui, F. Heat and drought stresses in crops and approaches for their mitigation. *Front. Chem.* **2018**, *6*, 1–14. [\[CrossRef\]](#)
27. DalCorso, G.; Fasani, E.; Manara, A.; Visioli, G.; Furini, A. Heavy metal pollutions: State of the art and innovation in phytoremediation. *Int. J. Mol. Sci.* **2019**, *20*, 3412. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Belay, A.; Recha, J.W.; Woldeamanuel, T.; Morton, J.F. Smallholder farmers’ adaptation to climate change and determinants of their adaptation decisions in the Central Rift Valley of Ethiopia. *Agric. Food Secur.* **2017**, *6*, 1–14. [\[CrossRef\]](#)
29. Sultan, M.A.R.F.; Hui, L.; Yang, L.J.; Xian, Z.H. Assessment of drought tolerance of some triticum l. species through physiological indices. *Czech J. Genet. Plant. Breed.* **2012**, *48*, 178–184. [\[CrossRef\]](#)
30. Rampino, P.; Pataleo, S.; Gerardi, C.; Mita, G.; Perrotta, C. Drought stress response in wheat: Physiological and molecular analysis of resistant and sensitive genotypes. *Plant. Cell. Environ.* **2006**, *29*, 2143–2152. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Polle, T.M.A.E.; Konzak, C.F. Screening spring wheat for drought tolerance. In *Developments in Plant and Soil Sciences*; Springer: Cham, Switzerland, 1987; pp. 79–87.
32. Sharifi, P.; Mohammadkhani, N. Effects of drought stress on photosynthesis factors in wheat genotypes during anthesis. *Cereal Res. Commun.* **2016**, *44*, 229–239. [\[CrossRef\]](#)
33. Lawlor, D.W.; Cornic, G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ.* **2002**, *25*, 275–294. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Szegletes, Z.; Erdei, L.; Tari, I.; Cseuz, L. Accumulation of osmoprotectants in wheat cultivars of different drought tolerance. *Cereal Res. Commun.* **2000**, *28*, 403–410. [\[CrossRef\]](#)
35. Zhu, J.-K. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant. Biol.* **2002**, *53*, 247–273. [\[CrossRef\]](#)
36. Zivcak, M.; Brestic, M.; Balatova, Z.; Drevenakova, P.; Olsovska, K.; Kalaji, H.M.; Yang, X.; Allakhverdiev, S.I. Photosynthetic electron transport and specific photoprotective responses in wheat leaves under drought stress. *Photosynth. Res.* **2013**, *117*, 529–546. [\[CrossRef\]](#)
37. Pour-Aboughadareh, A.; Omid, M.; Naghavi, M.R.; Etminan, A.; Mehrabi, A.A.; Pocza, 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. [\[CrossRef\]](#)
38. Botyanszka, L.; Zivcak, M.; Chovancek, E.; Sytar, O.; Barek, V.; Hauptvogel, P.; Halabul, A.; Brestic, M. Chlorophyll fluorescence kinetics may be useful to identify early drought and irrigation effects on photosynthetic apparatus in field-grown wheat. *Agronomy* **2020**, *10*, 1275. [\[CrossRef\]](#)
39. Sharma, D.K.; Andersen, S.B.; Ottosen, C.O.; Rosenqvist, E. Wheat cultivars selected for high Fv/Fm under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. *Physiol. Plant.* **2015**, *153*, 284–298. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Gonfa, A.B.; Tesfaye, K. Response of Ethiopian durum wheat genotypes to water deficit induced at various growth stages. *Afr. J. Plant Sci.* **2011**, *5*, 855–861.

41. Mwadzingeni, L.; Shimelis, H.; Tesfay, S.; Tsilo, T.J. Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. *Front. Plant Sci.* **2016**, *7*, 1276. [\[CrossRef\]](#)
42. Torabi, M.; Mokhtarzadeh, A.; Mahlooji, M. The role of hydroponics technique as a standard methodology in various aspects of plant biology researches. In *Hydroponics a Standard Methodology for Plant Biological Researches*; IntechOpen: London, UK, 2009.
43. Ayalew, H.; Ma, X.; Yan, G. Screening wheat (*triticum* spp.) genotypes for root length under contrasting water regimes: Potential sources of variability for drought resistance breeding. *J. Agron. Crop Sci.* **2014**, *201*, 189–194. [\[CrossRef\]](#)
44. Michel, B.E.; Kaufmann, M.R. The osmotic potential of polyethylene glycol 6000. *Plant Physiol.* **1973**, *51*, 914–916. [\[CrossRef\]](#)
45. Mdululi, S.Y.; Shimelis, H.; Mashilo, J. Screening for pre- and post-anthesis drought responses in selected bread wheat (*Triticum aestivum* L.) genotypes. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2020**, *70*, 272–284. [\[CrossRef\]](#)
46. Liwani, U.; Magwaza, L.S.; Odindo, A.O.; Sithole, N.J. Growth, morphological and yield responses of irrigated wheat (*Triticum aestivum* L.) genotypes to water stress. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2019**, *69*, 369–376. [\[CrossRef\]](#)
47. Faisal, S.; Mujtaba, S.M.; Asma; Mahboob, W. Polyethylene glycol mediated osmotic stress impacts on growth and biochemical aspects of wheat (*Triticum aestivum* L.). *J. Crop Sci. Biotechnol.* **2019**, *22*, 213–223. [\[CrossRef\]](#)
48. Ahmad, N.S.; Kareem, S.H.S.; Mustafa, K.M.; Ahmad, D.A. Early screening of some Kurdistan wheat (*Triticum aestivum* L.) cultivars under drought stress. *J. Agric. Sci.* **2017**, *9*, 88–103. [\[CrossRef\]](#)
49. Bansal, R.; Pradheep, K.; Kumari, J.; Kumar, S.; Yadav, M.C.; Gurung, B.; Kumari, N.K.P.; Rana, J.C. Physiological and biochemical evaluation for drought tolerance in wheat germplasm collected from arid western plains of India. *Indian J. Biochem. Biophys.* **2016**, *53*, 212–217.
50. Sallam, A.; Mourad, A.M.I.; Hussain, W.; Baenziger, P.S. Genetic variation in drought tolerance at seedling stage and grain yield in low rainfall environments in wheat (*Triticum aestivum* L.). *Euphytica* **2018**, *214*. [\[CrossRef\]](#)
51. Khan, A.S.; Allah, S.U.; Sadique, S. Genetic variability and correlation among seedling traits of wheat (*Triticum aestivum*) under water stress. *Int. J. Agric. Biol.* **2010**, *12*, 247–250.
52. Tripathi, P.; Rushton, P.J. Understanding water-stress responses in soybean using hydroponics system—A systems biology perspective. *Front. Plant Sci.* **2015**, *6*, 1–5. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Toit, D.; Adriaan, A.G. Hydroponics as a Tool in Wheat Breeding. 2005. Available online: <http://etd.uovs.ac.za/ETD-db/theses/available/etd-02142006-081835/unrestricted/DUTOITAGA.pdf> (accessed on 30 November 2020).
54. Shavrukov, Y.; Genc, Y.; Hayes, J. The use of hydroponics in abiotic stress tolerance research. hydroponics-a stand. In *Hydroponics. A Standard Methodology for Plant Biological Researches*; IntechOpen: London, UK, 2012.
55. Liu, H.; Able, A.J.; Able, J.A. Genotypic water-deficit stress responses in durum wheat: Association between physiological traits, microRNA regulatory modules and field components. *Funct. Plant Biol.* **2017**, *44*, 538–551. [\[CrossRef\]](#)
56. Figueroa-Bustos, V.; Palta, J.A.; Chen, Y.; Siddique, K.H.M. Early season drought largely reduces grain yield in wheat cultivars with smaller root systems. *Plants* **2019**, *8*, 305. [\[CrossRef\]](#)
57. Szira, F.; Bálint, A.F.; Börner, A.; Galiba, G. Evaluation of drought-related traits and screening methods at different developmental stages in spring barley. *J. Agron. Crop Sci.* **2008**, *194*, 334–342. [\[CrossRef\]](#)
58. Ahmed, H.G.M.D.; Khan, A.S.; LI, M.-ju.; Khan, S.H.; Kashif, M. Early selection of bread wheat genotypes using morphological and photosynthetic attributes conferring drought tolerance. *J. Integr. Agric.* **2019**, *18*, 2483–2491. [\[CrossRef\]](#)
59. Ayalew, H.; Dessalegn, T.; Liu, H.; Yan, G.; Markos, D. Performance of Ethiopian bread wheat (*Triticum aestivum* L.) genotypes under contrasting water regimes: Potential sources of variability for drought resistance breeding. *Aust. J. Crop Sci.* **2016**, *10*, 370–376. [\[CrossRef\]](#)
60. Sewak, R.; Tomar, S.; Tiwari, S.; Naik, B.K. Molecular and morpho-agronomical characterization of root architecture at seedling and reproductive stages for drought tolerance in wheat. *PLoS ONE* **2016**, *11*, e0156528. [\[CrossRef\]](#)
61. Ahmed, H.G.M.D.; Zeng, Y.; Yang, X.; Anwaar, H.A.; Mansha, M.Z.; Hanif, C.M.S.; Ikram, K.; Ullah, A.; Alghanem, S.M.S. Conferring drought-tolerant wheat genotypes through morpho-physiological and chlorophyll indices at seedling stage. *Saudi J. Biol. Sci.* **2020**, *27*, 2116–2123. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Ahmed, K.; Shabbir, G.; Ahmed, M.; Nawaz, K. Phenotyping for drought resistance in bread wheat using physiological and biochemical traits. *Sci. Total Environ.* **2020**, *729*, 139082. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Pour, A.; Alireza, A.; Mostafa, E.; Siddique, K.H.M. Assessment of biochemical and physiological parameters of durum wheat genotypes at the seedling stage during polyethylene glycol- induced water stress. *Plant Growth Regul.* **2020**, *92*, 81–93. [\[CrossRef\]](#)
64. Liu, H.; Searle, I.R.; Mather, D.E.; Able, A.J.; Able, J.A. Morphological, physiological and yield responses of durum wheat to pre-anthesis water-deficit stress are genotype-dependent. *Crop Pasture Sci.* **2015**, *66*, 1024–1038. [\[CrossRef\]](#)
65. Lin, Y.; Yi, X.; Tang, S.; Chen, W.; Wu, F.; Yang, X.; Jiang, X.; Shi, H.; Ma, J.; Chen, G.; et al. Dissection of phenotypic and genetic variation of drought-related traits in diverse Chinese wheat landraces. *Plant Genome* **2019**, *12*, 190025. [\[CrossRef\]](#)
66. Lopes, M.S.; El-Basyoni, I.; Baenziger, P.S.; Singh, S.; Royo, C.; Ozbek, K.; Aktas, H.; Ozer, E.; Ozdemir, F.; Manickavelu, A.; et al. Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *J. Ex. Bot.* **2015**, *66*, 3477–3486. [\[CrossRef\]](#)
67. Hoagland, D.R.; Arnon, D.I. The water-culture method for growing plants without soil. *Circ. Calif. Agric. Exp. Stn.* **1950**, 347.
68. Cao, H.X.; Zhang, Z.B.; Sun, C.X.; Shao, H.B.; Song, W.Y.; Xu, P. Chromosomal location of traits associated with wheat seedling water and phosphorus use efficiency under different water and phosphorus stresses. *Int. J. Mol. Sci.* **2009**, *10*, 4116–4136. [\[CrossRef\]](#)

69. Qaseem, M.F.; Qureshi, R.; Shaheen, H. Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (*triticum aestivum* L.) genotypes varying in sensitivity to heat and drought stress. *Sci. Rep.* **2019**, *9*, 1–12. [CrossRef]
70. Turner, N.C. Crop water deficits: A decade of progress. *Adv. Agron.* **1986**, *39*, C1–C51.
71. Barrs, H.D.; Weatherley, P.E. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* **1962**, *15*, 413–428. [CrossRef]
72. Decagon Device Inc. *Operator's Manual*; Decagon Devices Inc.: Pullman, WA, USA, 2002. Available online: www.decagon.com (accessed on 30 July 2020).
73. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* **1987**, *148*, C350–C382.
74. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil.* **1973**, *39*, 205–207. [CrossRef]
75. de Mendiburu, F. CRAN-Package Agricolae. Available online: <https://cran.r-project.org/web/packages/agricolae/index.html> (accessed on 17 February 2021).
76. Pour-Aboughadareh, A.; Yousefian, M.; Moradkhani, H.; Moghaddam Vahed, M.; Pocza, P.; Siddique, K.H.M. iPASTIC: An online toolkit to estimate plant abiotic stress indices. *Appl. Plant Sci.* **2019**, *7*, 1–6. [CrossRef] [PubMed]
77. Arteaga, S.; Yabor, L.; Díez, M.J.; Prohens, J.; Boscaiu, M.; Vicente, O. The use of proline in screening for tolerance to drought and salinity in common bean (*Phaseolus vulgaris* L.) genotypes. *Agronomy* **2020**, *10*, 817. [CrossRef]
78. De Carvalho, M.A.A.P.; Bebeli, P.J.; Bettencourt, E.; Costa, G.; Dias, S.; Santos, T.M.M.D.; Slaski, J. Cereal landraces genetic resources in worldwide GeneBanks. A review. *Agron. Sustain. Dev.* **2013**, *33*, 177–203. [CrossRef]
79. Sall, A.T.; Chiari, T.; Legesse, W.; Seid-Ahmed, K.; Ortiz, R.; van Ginkel, M.; Bassi, F.M. Durum wheat (*Triticum durum* Desf.): Origin, cultivation and potential expansion in Sub-Saharan Africa. *Agronomy* **2019**, *9*, 263. [CrossRef]
80. Tshikunde, N.M.; Mashilo, J.; Shimelis, H.; Odindo, A. Agronomic and Physiological Traits, and Associated Quantitative Trait Loci (QTL) Affecting Yield Response in Wheat (*Triticum aestivum* L.): A Review. *Front. Plant Sci.* **2019**, *10*, 1428. [CrossRef]
81. Ahmed, H.G.M.; Sajjad, M.; Li, M.; Azmat, M.A.; Rizwan, M.; Maqsood, R.H. Selection criteria for drought-tolerant bread wheat genotypes at seedling stage. *Sustainability* **2019**, *11*, 2584. [CrossRef]
82. Baloch, M.J.; Dunwell, J.; Khan, N.U.; Jatoti, W.A.; Khakhwani, A.A.; Vessar, N.F.; Gul, S. Morpho-physiological characterization of spring wheat genotypes under drought stress. *Int. J. Agric. Biol.* **2013**, *15*, 945–950.
83. Djanaguiraman, M.; Prasad, P.V.V.; Kumari, J.; Rengel, Z. Root length and root lipid composition contribute to drought tolerance of winter and spring wheat. *Plant Soil* **2019**, *439*, 57–73. [CrossRef]
84. Jain, N.; Singh, G.P.; Yadav, R.; Pandey, R.; Ramya, P.; Shine, M.B.; Prabhu, K.V. Root trait characteristics and genotypic response in wheat under different water regimes. *Cereal Res. Commun.* **2014**, *42*, 426–438. [CrossRef]
85. Pour-Aboughadareh, A.; Mohammadi, R.; Etmiran, A.; Shooshtari, L.; Maleki-Tabrizi, N.; Pocza, P. Effects of drought stress on some agronomic and morpho-physiological traits in durum wheat genotypes. *Sustainability* **2020**, *12*, 5610. [CrossRef]
86. Pour-aboughadareh, A.; Ahmadi, J.; Mehrabi, A.A.; Etmiran, A. Physiological responses to drought stress in wild relatives of wheat: Implications for wheat improvement Physiological responses to drought stress in wild relatives of wheat: Implications for wheat improvement. *Acta Physiol. Plant.* **2017**, *39*, 106. [CrossRef]
87. Xu, W.; Cui, K.; Xu, A.; Nie, L.; Huang, J.; Peng, S. Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. *Acta Physiol. Plant.* **2015**, *37*, 9. [CrossRef]
88. Grzesiak, M.T.; Hordyńska, N.; Maksymowicz, A.; Grzesiak, S.; Szechyńska-Hebda, M. Variation among spring wheat (*Triticum aestivum* L.) genotypes in response to the drought stress. II—Root system structure. *Plants* **2019**, *8*, 584. [CrossRef] [PubMed]
89. Ratzmann, G.; Zakharova, L.; Tietjen, B. Optimal leaf water status regulation of plants in drylands. *Sci. Rep.* **2019**, *9*, 1–9. [CrossRef]
90. Agathokleous, E.; Belz, R.G.; Kitao, M.; Koike, T.; Calabrese, E.J. Does the root to shoot ratio show a hormetic response to stress? An ecological and environmental perspective. *J. For. Res.* **2019**, *30*, 1569–1580. [CrossRef]
91. Dryden, G. Water use and requirements. *Anim. Nutr. Sci.* **2015**, 115–129.
92. Wang, L.; Wang, S.; Chen, W.; Li, H.; Deng, X. Physiological mechanisms contributing to increased water-use efficiency in winter wheat under organic fertilization. *PLoS ONE* **2017**, *12*, e0180205. [CrossRef]
93. Cajero-Sanchez, W.; Aceves-Garcia, P.; Fernández-Marcos, M.; Gutiérrez, C.; Rosas, U.; García-Ponce, B.; Alvarez-Buylla, E.; de la Paz Sanchez, M.; Garay-Arroyo, A. Natural root cellular variation in responses to osmotic stress in arabidopsis thaliana accessions. *Genes* **2019**, *10*, 983. [CrossRef]
94. Nakhforoosh, A.; Grausgruber, H.; Kaul, H.P.; Bodner, G. Dissection of drought response of modern and underutilized wheat varieties according to passoura's yield-water framework. *Front. Plant Sci.* **2015**, *6*, 1–13. [CrossRef]
95. Blum, A. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant Cell Environ.* **2017**, *40*, 4–10. [CrossRef]
96. Kim, Y.; Chung, Y.S.; Lee, E.; Tripathi, P.; Heo, S.; Kim, K.H. Root response to drought stress in rice (*Oryza sativa* L.). *Int. J. Mol. Sci.* **2020**, *21*, 1513. [CrossRef]
97. Nakhforoosh, A.; Bodewein, T.; Fiorani, F.; Bodner, G. Identification of water use strategies at early growth stages in durum wheat from shoot phenotyping and physiological measurements. *Front. Plant Sci.* **2016**, *7*, 1–13. [CrossRef]

98. Kerstiens, G. Cuticular water permeability and its physiological significance. *J. Exp. Bot.* **1996**, *47*, 1813–1832. [[CrossRef](#)]
99. Gašparovič, K.; Živčák, M.; Brestič, M.; Hauptvogel, P. Diversity of leaf cuticular transpiration and growth traits in field-grown wheat and aegilops genetic resources. *Agronomy* **2021**, *11*, 522. [[CrossRef](#)]
100. Boyer, J.S.; James, R.A.; Munns, R.; Condon, T.; Passioura, J.B. Osmotic adjustment leads to anomalously low estimates of relative water content in wheat and barley. *Funct. Plant Biol.* **2008**, *35*, 1172–1182. [[CrossRef](#)] [[PubMed](#)]
101. Shivakrishna, P.; Reddy, K.A.; Rao, D.M. Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. *Saudi J. Biol. Sci.* **2018**, *25*, 285–289. [[CrossRef](#)]
102. Bota, J.; Medrano, H.; Flexas, J. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytol.* **2004**, *162*, 671–681. [[CrossRef](#)]
103. Use, W.; Zhao, W.; Liu, L.; Shen, Q.; Yang, J.; Han, X. Effects of water stress on photosynthesis, yield, and water use efficiency in winter wheat. *Water* **2020**, *12*, 2127.
104. Laffray, D.; Louguet, P. Stomatal responses and drought resistance. *Bull. la Soc. Bot. Fr. Actual. Bot.* **1990**, *137*, 47–60. [[CrossRef](#)]
105. Ahmad, Z.; Waraich, E.A.; Akhtar, S.; Anjum, S.; Ahmad, T.; Mahboob, W.; Hafeez, O.B.A.; Tapera, T.; Labuschagne, M.; Rizwan, M. Physiological responses of wheat to drought stress and its mitigation approaches. *Acta Physiol. Plant* **2018**, *40*. [[CrossRef](#)]
106. Hussain, H.A.; Men, S.; Saddam, H.; Chen, Y.; Ali, S.; Zhang, S.; Zhang, K.; Li, Y.; Xu, Q.; Liao, C.; et al. Interactive effects of drought and heat stresses on morpho- physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. *Sci. Rep.* **2019**, *9*, 3890. [[CrossRef](#)]
107. Faisal, S.; Mujtaba, S.M.; Khan, M.A.; Mahboob, W. Morpho-physiological assessment of wheat (*Triticum aestivum* L.) genotypes for drought stress tolerance at seedling stage. *Pak. J. Bot.* **2017**, *49*, 445–452.
108. Upadhyay, D.; Budhlakoti, N.; Kumar, A.S.; Bansal, R.; Kumari, J.; Chaudhary, N.; Padaria, J.C.; Sareen, S.; Kumar, S. Drought tolerance in *Triticum aestivum* L. genotypes associated with enhanced antioxidative protection and declined lipid peroxidation. *3 Biotech.* **2020**, *10*, 1–17. [[CrossRef](#)]
109. Ibarra-Caballero, J.; Villanueva-Verduzco, C.; Molina-Galán, J.; Sánchez-de-Jiménez, E. Proline accumulation as a symptom of drought stress in maize: A tissue differentiation requirement. *J. Exp. Bot.* **1988**, *39*, 889–897. [[CrossRef](#)]
110. Geravandi, M.; Farshadfar, E.; Kahrizi, D. Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. *Russ. J. Plant Physiol.* **2011**, *58*, 69–75. [[CrossRef](#)]
111. Semahegn, Y.; Shimelis, H.; Laing, M.; Mathew, I. Evaluation of bread wheat (*Triticum aestivum* L.) genotypes for yield and related traits under drought stress conditions. *Acta Agric. Scand. Sect. B Soil Plant. Sci.* **2020**, *70*, 474–484. [[CrossRef](#)]
112. Sallam, A.; Alqudah, A.M.; Dawood, M.F.A.; Baenziger, P.S.; Börner, A. drought stress tolerance in wheat and barley: Advances in physiology, breeding and genetics research. *Int. J. Mol. Sci.* **2019**, *20*, 3137. [[CrossRef](#)]
113. De la Rosa, L.; Zambrana, E.; Ramirez-Parra, E. Molecular bases for drought tolerance in common vetch: Designing new molecular breeding tools. *BMC Plant Biol.* **2020**, *20*, 71. [[CrossRef](#)]