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# Evaluation of Drought Tolerance in USDA Tomato Germplasm at Seedling Stage

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**Abstract:** Drought, a crucial abiotic stressor, markedly reduces the growth and yield of tomato crops (*Solanum lycopersicum* L.). Consequently, adopting drought-resistant cultivars and implementing breeding programs to enhance drought tolerance have emerged as enduring solutions to alleviate the adverse effects of drought in various tomato cultivation regions. In this study, 68 United States Department of Agriculture (USDA) tomato accessions were assessed in a controlled greenhouse experiment, encompassing both water deficit treatment and a control group subjected to standard watering conditions. The experiment was arranged in a randomized complete block design with three replications. The results of this study pinpointed four accessions, PI 365956, PI 584456, PI 390510, and PI 370091, as drought-tolerant accessions. Additionally, high broad-sense heritability was revealed for leaf wilting, leaf rolling, and SPAD chlorophyll content (total leaf chlorophyll). Furthermore, positive correlations were found among parameters associated with leaf wilting, leaf rolling, and SPAD chlorophyll content. The findings offer valuable insights for tomato breeding initiatives, especially those focused on enhancing drought tolerance in elite cultivars. Future studies will expand the evaluation to include a larger pool of tomato accessions and conduct a genome-wide association study to identify single nucleotide polymorphism (SNP) markers for molecular breeding in tomatoes.

**Keywords:** tomato; *Solanum lycopersicum*; drought; drought tolerance; leaf wilting; leaf rolling; plant height; total leaf chlorophyll

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## 1. Introduction

Global environmental shifts have become an undeniable reality [1], marked by an escalation in extreme aridity or drought, heat, and floodwaters [1–4]. These occurrences are growing in both frequency and intensity [5]. These factors bear significant and immediate consequences for the agricultural sector, resulting in diminished productivity [3,6], leading to reduced food supply, elevated food prices, and adverse effects on the livelihoods of many households [5–8].

Among all the factors adversely affecting sustainable crop production, drought, referred to as deficit irrigation or soil water deficit [9], has emerged as a pervasive global challenge, representing a substantial agricultural catastrophe. The crop and yield losses resulting from drought surpass the cumulative impact of other environmental factors [10,11]. Approximately 25% of the world's population is at risk due to drought, with the majority hailing from developing countries in Africa and Asia [10]. Thus, it is important to develop drought-tolerant cultivars through rigorous screening and selection processes [12,13].

Tomato (*Solanum lycopersicum* L.) is widely cultivated in the open field and greenhouse and is a widely consumed horticultural crop, with wild accessions demonstrating significant resilience to drought conditions, unlike the cultivated accessions [14,15]. Despite this resilience, the global cultivation of tomatoes is exposed to the prominent abiotic constraint of drought. For optimal growth and yield, tomatoes require a continuous water supply [16–18]. The impact of drought stress on tomato yield varies based on soil and climatic conditions [16,19]. Short periods of water deficit result in both qualitative and quantitative losses in fruit production [16]. Inadequate water availability negatively impacts multiple aspects of tomato plant growth and overall yield [16]. Additionally, irrigation costs contribute to more than 10% of the total expenses in tomato cultivation, posing a considerable financial risk to tomato production. Therefore, it is crucial to implement measures that can alleviate the substantial expenses associated with supplying water to tomato crops [20]. Addressing farmers' needs, the development of drought-tolerant tomato varieties is a key focus in contemporary breeding programs [21–24].

The occurrence of severe drought stress limits plant development by curtailing the photosynthetic rate, causing wilting, stomatal closure, reduced water content, and decreased growth and cell size [25,26]. However, crop varieties displaying strong drought tolerance tend to uphold an elevated photosynthetic rate, substantial growth, and slow plant wilting even in drought conditions, as described and cited by Cui et al. [10] and Ravelombola et al. (2020) [27]. The identification of such varieties relies on two principal dimensions: adaptability in crop structure and internal organization, and the adaptability of physiological and biochemical responses in plants. Commonly utilized indicators for this assessment encompass morphological characteristics, markers related to growth and development, as well as indicators associated with physiological and biochemical processes.

Ensuring global food security demands the strategic development of plants that can withstand stress and maintain stable yields in challenging environments [28]. Traditionally, breeders focused on increasing crop yields, resulting in a shortage of modern varieties with stress tolerance [29]. Contemporary plant breeding now prioritizes enhancing stress resilience by exploring ancestral varieties (landraces) and leveraging wild relatives of important crops known for their beneficial stress-tolerant traits [30]. Incorporating wild crop relatives provides a strong foundation for discovering new genes and understanding the mechanisms behind physiological adaptations [31]. Concerning tomatoes, undomesticated species inherently exhibit adaptability to diverse soil and climatic conditions. Studies by [32] illustrate these adaptive traits. Such adaptations play a pivotal role in the development of genetic constitutions that demonstrate increased tolerance to abiotic stresses as indicated by Gong et al. [25], Wang et al. [33], and Yang et al. [34].

Around the globe, there are over 62,800 varieties of cultivated (*S. lycopersicum*) and wild tomatoes (*S. pimpinellifolium* and other *Solanum* species), mostly belonging to the *S. lycopersicum* species, preserved in gene banks [14], including repositories such as the Asian Vegetable Research and Development Center (AVRDC) located in Tainan, Taiwan, China, the Plant Genetic Resources Unit at Geneva (PGRU) under the United States Department of Agriculture (USDA) in New York, USA, and the CM Rick Tomato Genetics Resource Center (TGRC) situated at the University of California, Davis, in California, USA. The gene banks are a basis of successful genetic improvements due to the preservation of genetic variation [13]. Considering the observed diversity and differing stress responses among various cultivated and wild tomato varieties, the existence of gene banks accommodating a wide range of tomato accessions presents a valuable resource for breeders. This enables the screening and selection of drought-tolerant cultivars. This study aimed to evaluate the effects of drought stress on 68 tomato accessions from the United States Department of Agriculture (USDA) at a seedling stage. The goal was to identify potential drought-tolerant accessions that can serve as parent plants in subsequent tomato breeding programs, with a focus on enhancing genetic resistance or tolerance to drought stress and providing information for genetic study.

This paper presents a comprehensive evaluation presented in the Master's thesis by Chiwina [35]. The research, conducted at the University of Arkansas, explored the complexities in understanding and enhancing drought tolerance in tomato.

## 2. Materials and Methods

### 2.1. Plant Material

Sixty-eight USDA tomato germplasm accessions were used for drought tolerance evaluation in this study. Out of 68 accessions, 14 (20.6%) were originally collected from the United States; 9 from Canada; 5 from Peru; and the remaining 40 from 24 other countries (Supplementary Table S1).

### 2.2. Evaluation for Drought Tolerance

Evaluation of tomato accessions was performed in a greenhouse with natural lighting at the Arkansas Agricultural Research and Extension Center, Fayetteville, AR (Figure 1A) between January 2023 and February 2023. During the experiment, the greenhouse temperature and relative humidity were kept at 21/18 °C in the day/night and 73%, respectively. The temperature and relative humidity variables in the greenhouse were monitored using a WADSWORTH Control System 1.800.821.5829. The screening procedure was followed as described in previous reports by [36–38], with slight modifications.

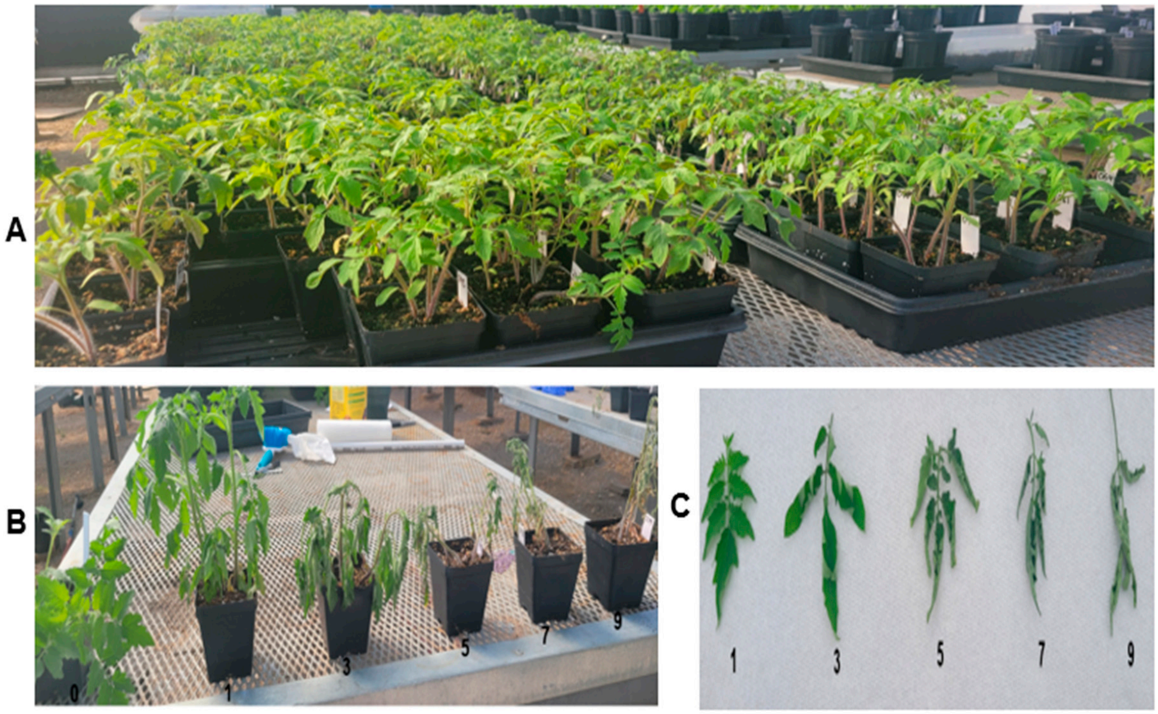


**Figure 1.** Drought tolerance treatment in a greenhouse and measurement: (**A**) tomato plants, (**B**) 0–9 scale for leaf wilting, and (**C**) 1–9 scale for leaf rolling.

Five seeds of each tomato accession were sown in pots (8.5 cm high, 8.5 cm top diameter, and 5.8 cm base diameter) placed in trays (52 cm long, 26 cm wide, and 6 cm high). Each tray contained 12 pots filled with commercial compost (Berger, berger.ca, BM 6) up to 8 cm in 1 day before seeds were sown. Soon after seed sowing, each pot and tray were filled with 300 mL and 2 L of water, respectively. After the initial irrigation, the pots and trays were left unirrigated for 6 days. After seed germination, each pot kept at most three plants, and extra plants were removed if the pot had more than three plants.

Subsequently, a consistent irrigation schedule of 180 mL per pot was implemented every 3 days for 28 days, preceding the initiation of the drought treatment.

During the study, 180 mL of liquid (0.5 teaspoon per gallon or 3.8 L) fertilizer (Miracle-Gro Water Soluble All Purpose Plant Food 24-8-16), containing ammoniacal nitrogen (N) (3.5%), urea nitrogen (N) (20.5%), available phosphate ($P_2O_5$) (8%), soluble potash ($K_2O$) (16%), boron (B) (0.02%), water-soluble copper (Cu) (0.07%), chelated iron (Fe) (0.15%), manganese (Mn) (0.05%), molybdenum (Mo) (0.0005), and water-soluble zinc (Zn) (0.06%), was applied in liquid form to each pot every 10 days (and every 14 days in subsequent applications following seed sowing), before the plants were exposed to drought treatment.

The experiment was a randomized complete block design (RCBD) with three blocks, organized in a split-plot manner under greenhouse conditions, with the drought treatment as the main plot and the tomato accessions as the subplot. During the experiment, thinning was performed 15 days after planting. Three plants per pot were kept for each tomato accession in a block. The drought treatment was applied to the tomato plants 35 days after seed sowing until susceptible genotypes were completely dead approximately 10 days without watering in this study, showing vulnerability to water scarcity conditions. The control treatment was constantly maintained with 180 mL of tap water every 3 days.

### *2.3. Measurements*

Measurements on plant height, leaf wilting, leaf rolling, and SPAD chlorophyll content (total leaf chlorophyll) were recorded. Plant height was measured from each plant per accession in each replicate for the drought-stressed and non-drought-stressed plants in 10 days after drought stress was initiated.

Visual assessment of leaf wilting and leaf rolling were performed based on a scale of 0 to 9 (Figure 1B) and 1 to 9 (Figure 1C), respectively (Table 1), with slight modifications based on the symptoms associated with leaf drying and folding [37–39]. The scores were recorded for each plant of the genotypes in the drought treatment, and the average score in each accession was calculated to determine the drought tolerance response under drought treatment.

**Table 1.** Visual assessment of leaf wilting and leaf rolling on a scale of 0 to 9 and 1 to 9, respectively, in 68 tomato accessions assessed for drought tolerance.

| Category * | Leaf Wilting | Leaf Rolling Stage |
|---|---|---|
| 0 | Normal (not wilted) | |
| 1 | Slightly wilted | No symptom of leaf rolling |
| 2 | Slight wilting—minimal signs of leaf wilting, but overall plant health was relatively unaffected | Minimal leaf rolling: Slight curling of a few leaves |
| 3 | Wilted leaves, with loss of turgidity, but the plant remains moderately healthy | Mild leaf rolling: Some curling and folding of a small number of leaves |
| 4 | Moderate wilting—significant wilting observed in several leaves, indicating a moderate level of stress | Moderate leaf rolling: Noticeable curling and folding of several leaves. |
| 5 | Moderate to severe wilting—a substantial number of leaves wilted, indicating a higher level of stress | Significant leaf rolling: Extensive curling and folding of a majority of leaves |
| 6 | Severe wilting—all leaves wilted, and the plant was under considerable stress | Significant leaf rolling: Extensive curling and folding of a majority of leaves. |
| 7 | Extreme wilting—all leaves wilted, and the plant is severely stressed | Significant leaf rolling: Further increase in curling and folding, affecting a significant portion of leaves |
| 8 | Critical wilting—all leaves and stem dried, and the plant almost dead | Severe leaf rolling: Intense curling and folding of almost all leaves, potentially impacting plant health |
| 9 | Dead | Leaves tightly rolled (Severe leaf rolling: Maximum intensity of curling and folding, with nearly all leaves affected) |

* 0–4 = drought tolerant; 5 = moderately tolerant; 7–9 = drought sensitive.

The SPAD chlorophyll content was measured from three regions of trifoliate leaves for all plants of each genotype per treatment (drought and without drought) using the

SPAD-502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL, USA). The measurements for each region in the leaf were recorded, separately.

The following data were collected and computed [27] (Table S1):

i. LW-d6: leaf wilting on day 7 after drought treatment based on a 0–9 scale;
ii. LW-d10: leaf wilting on day 10 after drought treatment based on a 0–9 scale;
iii. LR-d6: leaf rolling on day 7 after drought treatment based on a 1–9 scale;
iv. LR-d10: leaf rolling on day 10 after drought treatment based on a 1–9 scale;
v. SPAD_healthy: leaf chlorophyll content is healthy without drought stress, measured by the SPAD-502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL, USA);
vi. SPAD_stress: leaf chlorophyll content under drought conditions;
vii. SPAD_AD: absolute decrease in leaf chlorophyll content (SPAD_healthy—SPAD_stress);
viii. SPAD_II: inhibition Index in leaf chlorophyll content = [100 × (SPAD_healthy—SPAD_stress)/SPAD_healthy].
ix. SPAD_RDT: relative drought tolerance in leaf chlorophyll content measured = (100 × SPAD_stress/SPAD_healthy) = (100—SPAD_II);
x. PlHt_healthy: plant height under irrigation treatment;
xi. PlHt_stress: plant height under drought treatment;
xii. PlHt_AD: absolute decrease in plant height (PlHt_healthy—PlHt_stress)
xiii. PlHt_II: inhibition index in plant height = [100 × (PlHt_healthy—PlHt_stress)/PlHt_healthy];
xiv. PlHt_RDT = relative drought tolerance in plant height = [100 × (PlHt_stress/PlHt_healthy)] = (100—PlHt_II);
xv. Broad-sense heritability ($H^2$);
xvi. Pearson's correlation analysis.

### *2.4. Phenotypic Data Analysis*

#### 2.4.1. Statistical Model

The statistical model for analysis of variance (ANOVA) was the following: Yij = μ + Bi + Gj + Ɛij, where i = 1, 2, 3 and j = 1.. . .68, with μ representing the overall mean, Yij representing the response from the jth accession (Gj) (fixed effect) at the ith block (Bi) (random effect), and eij representing the random error associated with the ijth observation.

#### 2.4.2. ANOVA, Distribution, Descriptive Statistics, and Pearson's Correlation

The data were analyzed using JMP PRO 17. Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure. Mean separation was conducted using the Student T-test at alpha = 0.05. The distribution of the data was visualized using the 'Distribution'; descriptive statistics were estimated using the 'Tabulate'; and the Person's correlation coefficients and their *p*-values were calculated by 'Multivariate Methods' options of JMP PRO 17, respectively. The broad-sense heritability ($H^2$) was estimated, using the following formula [39]: $H^2 = 100 \times \sigma^2{}_G/[\sigma^2{}_G + (\sigma^2{}_{GE}/e) + (\sigma^2{}_E/re)]$, where $\sigma^2{}_G$ is the total genetic variance, $\sigma^2{}_{GE}$ is variance between genetic and environment (here: block) interaction; $\sigma^2{}_E$ is the residual variance; e is the number of the environment (block); and r is the number of replications. The estimates for $\sigma^2{}_G$, $\sigma^2{}_{GE}$, and $\sigma^2{}_E$ are $\sigma^2{}_E$ = MSE, $\sigma^2{}_{GE}$ = (MSGE—MSE)/r, and $\sigma^2{}_G$ = (MSG—MSGE)/re.

#### 2.4.3. Absolute Decrease, Inhibition Index, and Relative Drought Tolerance

To completely evaluate tomato accessions for tolerance to drought, absolute decrease (AD), inhibition index (II), and relative drought tolerance (RDT) were estimated for plant height and SPAD chlorophyll content (leaf chlorophyll) in Microsoft Excel. The AD was a measure of the absolute change (decrease) in the plant height or SPAD chlorophyll content. The AD in plant height and SPAD chlorophyll content was achieved by subtracting the plant height or SPAD chlorophyll content for the drought-stressed plants from those of well-irrigated plants (AD = the value in healthy plants without drought stress—the value under drought conditions). The greater the AD value, the more likely a tomato accession had its height or chlorophyll content decreased, showing high susceptibility of the accession to drought stress. Conversely, the lower the AD value, the more likely the accession had greater drought tolerance.

The inhibition index (II) was a measure of the inhibition percentage to drought tolerance and was calculated as II = [100 × (the value in healthy—the value under drought treatment)/the value in healthy under normal irrigation]. In this study, a greater percentage of II indicated a decrease in plant height and SPAD chlorophyll content, suggesting a greater susceptibility of the tomato accession to drought stress. Conversely, the lower the II value, the higher the drought tolerance; thus, an II decrease in plant height and SPAD chlorophyll content showed an increase in tolerance to drought stress.

On the other hand, RDT was a measure of the relative change (decrease) percentage in the plant height or SPAD chlorophyll content, estimated by dividing the value under drought conditions by healthy under proper irrigation. In this investigation, a greater RDT percentage for a particular tomato accession indicated a lesser decrease in plant height and SPAD chlorophyll content, signifying greater tolerance to drought, while a lower RDT percentage showed a greater decrease in plant height and SPAD chlorophyll content, showing high vulnerability of the tomato accession to dry conditions.

#### 2.4.4. Rank of Drought Tolerance in Tomato Accessions

The 68 tomato accessions were ranked from 1 to 68 for each of the 10 traits (LW-d6, LW-d10, LR-d6, LR-d10, SPAD_AD, SPAD_II, PlHt_AD, PlHt_II, PlHt_RDT, and SPAD_RDT), where 1 was the top drought tolerance and 68 was the most vulnerable. Because the value of II equals 100 minus ADT value (II = 100-ADT), the rank of ADT was the same as the rank order of II, and both PlHt_RDT and SPAD_RDT are not listed.

### *2.5. DNA Extraction, Genotyping by Sequencing (GBS), and SNP Discovery*

The DNA (genome) was extracted from fresh leaves of tomato plants using the CTAB/SDS method. DNA sequencing was conducted using the genotyping-by-sequencing (GBS) approach [40] in Pair-end sequencing libraries sequenced by Illumina NovaSeq in University of Wisconsin Biotechnology Center (UWBC) (https://biotech.wisc.edu/, accessed on 14 February 2024). The short-read sequences data are aligned to tomato genome reference, *Solanum lycopersicum*, ITAG_4.0 (https://phytozome-next.jgi.doe.gov/info/Slycopersicum_ITAG4_0, accessed on 14 February 2024), and SNPs were postulated in a pipeline using TASSE_GBS [41] and Stacks 2 [42] (https://catchenlab.life.illinois.edu/stacks/, accessed on 14 February 2024). A total of 392,496 single nucleotide polymorphism (SNP) markers were discovered across 287 tomato genotypes distributed on 12 chromosomes of tomato and provided by UWBC.

### *2.6. Principal Component Analysis (PCA) and Genetic Diversity*

Principal components were analyzed and the Dendrogram was drawn by the hierarchical cluster method, using JMP Pro 17 based on either trait (LW-d6, LW-d10, LR-d6, LR-d10, SPAD_AD, SPAD_II, PlHt_AD, and PlHt_II) among the 68 tomato accessions. Genetic diversity was analyzed, and a phylogenetic tree was generated using MEGA 11, based on 5003 single-nucleotide polymorphism (SNP) markers distributed on 12 chromosomes in 65 USDA GRIN tomato accessions, except for 3 accessions, PI 365956, PI 438587, and PI 600901 out of the 68 accessions in Table S1. Because they had poor GBS (genotyping-by-sequencing) sequencing data, the three accessions were filtered out (removed) from the genetic diversity analysis. The SNP marker set consisted of 5,003 SNP markers across the 65 accessions, after filtering and keeping the SNP markers with minor allele frequency (MAF) >1.5%, missing allele <15%, and heterogeneous rate <=35% in this study.

## 3. Results

### *3.1. Parameters and Distributions of Drought-Related Traits*

#### 3.1.1. Leaf Wilting

The leaf wilting (LW) scale of 0–9 varied among the 68 tomato accessions under 6 and 10 days of drought stress (Supplementary Tables S1 and S2). A large range was observed: 7.0 for LW-d6 and 6.3 for LW-d10 (Table S2). The mean was 6.9 ± 0.18 for LW in 6 days of drought treatment (LW-d6), and 8.4 ± 0.17 under 10 days of drought treatment (LW-d10) (Supplementary Table S2). These leaf wilting data revealed significant variation in tolerance response to drought stress among the 68 tomato accessions.

The distributions of leaf wilting scores for either 6-day (LW-d6) or 10-day (LW-d10) drought treatment were right-skewed (Figure 2A,B), showing that most of the 68 tomato accessions were extreme susceptibility to drought stress, where the two accessions, PI 647531 and PI 634828, were the most susceptible, with 8.9 and 9 (highest scale defined) in either LW-d6 and LW-d10, respectively, indicating they can be used as susceptible control in drought evaluation experiment or as susceptible parents in breeding programs or in genetic study of QTL (genomic regions) mapping of drought tolerance in tomato. The accessions PI 365956, PI 584456, PI 390510, and PI 370091 had average leaf wilting scores of less than 4 in both treatments (Supplementary Tables S1 and S2), showing that they were the most drought tolerant and suggesting that the four accessions could be useful as parents in breeding elite cultivars of tomato for drought tolerance.

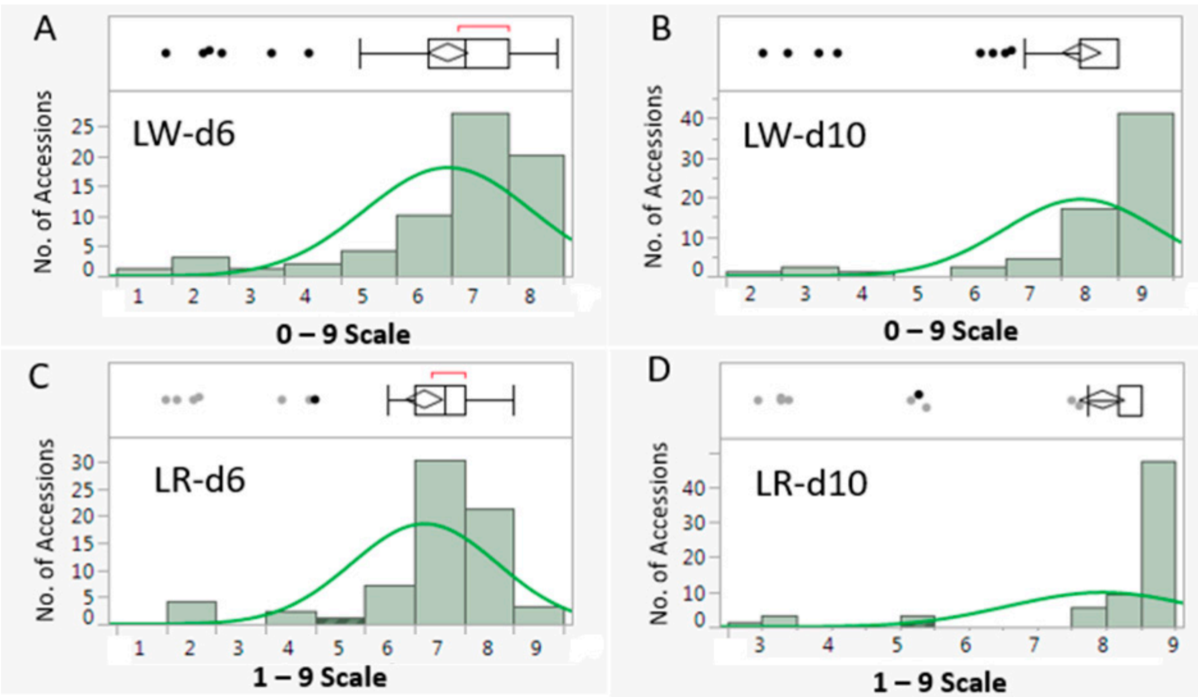


**Figure 2.** The four distributions of leaf wilting (LW) (**A**,**B**) and leaf rolling (LR) (**C**,**D**) in 68 tomato accessions: (**A**,**C**) in 6 days and (**B**,**D**) in 10 days after drought treatment. X-axis represents the 0–9 and 1–9 scale of leaf wilting for day 6 and day 10 (LW-d6 and LW-d10) and leaf rolling for day 6 and day 10 (LR-d6 and LR-d10); Y-axis is for number of accessions; the bracket represents the peak of the distribution; and the green line represents the theoretical normal distribution.

#### 3.1.2. Leaf Rolling

The average leaf rolling (LR) scores for 6-day (LR-d6) and 10-day (LR-D10) drought stress ranged from 2.0 to 9 and 3.4 to 9 (Supplementary Table S2), respectively, and the mean of LR was 7.2 ± 0.18 in 6 days of drought treatment (LR-d6) and 8.4 ± 0.17 under 10 days of drought treatment (LR-10d) (Supplementary Table S2), showing significant differences and a large range (7.0 for LR-d6 and 5.6 for LR-d10) in reaction to drought stress among the 68 tomato accessions.

Distribution of leaf rolling scores for either 6-day (LR-d6) or 10-day (LR-d10) drought treatment were right-skewed (Figure 2C,D), the same trend as those in leaf wilting, showing that most of the 68 tomato accessions were extremely susceptible to drought stress, where the three accessions PI 647531, PI 196297, and PI 634828 had the highest scale of 9 in both LR-d6 and LR-d10 (Table S1), indicating that the three accessions can be used as susceptible control in drought evaluation experiment or as susceptible parents in genetic study of QTL mapping of drought tolerance in tomato. The shown accessions PI 365956, PI 584456, PI 390510, and PI 370091 were found to have leaf rolling scores of less than 4, showing the lowest scales, as they had the lowest leaf wilting scale values (Table S1), indicating that they exhibited a greater level of tolerance to drought, suggesting that the accessions could be useful as parents in breeding elite cultivars of tomato for drought tolerance.

3.1.3. Plant Height

Plant height measurements were taken for 68 tomato accessions under both irrigated and drought conditions. For the well-watered plants, the average plant height (PlHt_healthy) ranged from 11.8 cm to 34.2 cm at 10 days, with a nearly normal distribution skewed right among the 68 accessions (Figure 3A), with a mean of 26.6 cm and a standard deviation (Std Dev) of 4.02 (Supplementary Table S2). The accession PI 584456 was the shortest at 11.8 cm and PI 433016 was the tallest at 34.2 cm (Table S1).

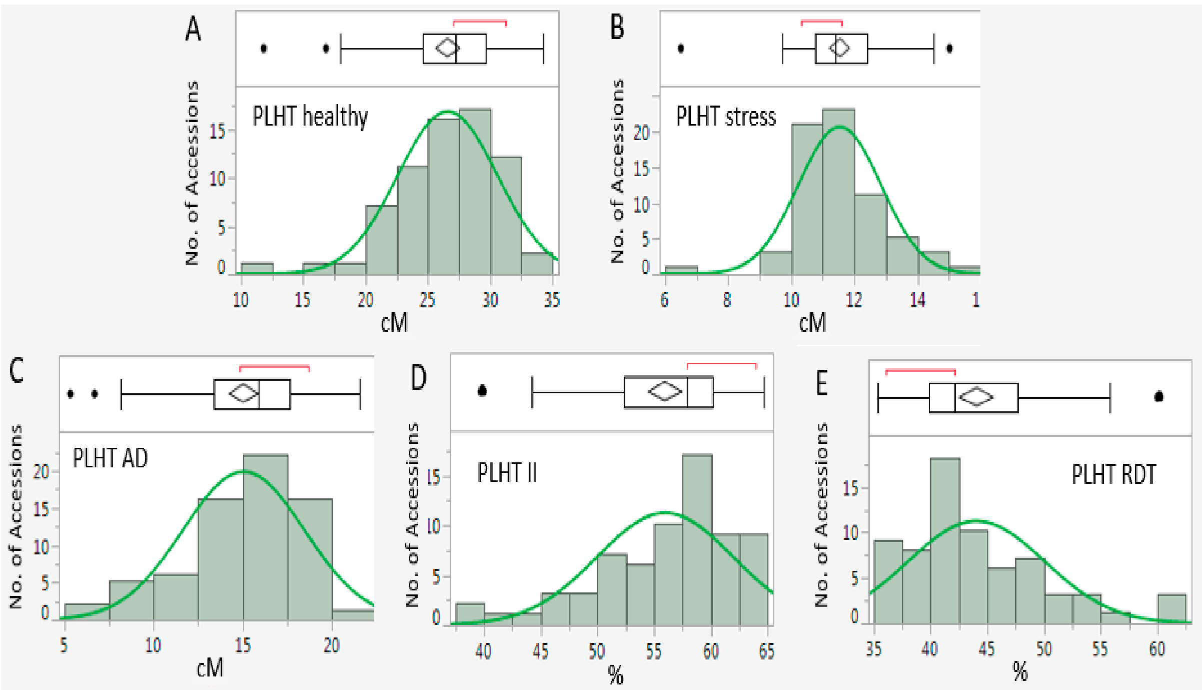


**Figure 3.** The 5 distributions of plant height (PLHT)-related traits for drought tolerance in 68 tomato accessions. * PlHt_healthy = Plant height under irrigation; PlHt_stress = Plant height under drought conditions; PlHt_AD = Absolute decrease in plant height = PlHt_healthy—PlHt_stress; PlHt_II = Inhibition Index in plant height = [100 × (PlHt_healthy—PlHt_stress)/PlHt_healthy]; and PlHt_RDT = Relative drought tolerance in plant height = [100 × (PlHt_stress/PlHt_healthy)] = (100—PlHt_II).

Under drought conditions, the average plant height (PlHt_stress) ranged from 6.5 cm to 15.0 cm at 10 days (Figure 3B), with a mean of 11.5 cm and a Std Dev of 1.31 (Supplementary Table S2). The accession PI 584456 was still the shortest with 6.5 cm and PI 258478 was the tallest with 15.0 cm (Table S1).

The absolute decrease in average plant height (PlHt_AD) had a large range of 16.2 cm and ranged from 5.3 cm to 21.5 cm with a mean of 15.1 ± 0.41 cm (Figure 3C, Supplementary Table S2), indicating that there was a large difference and variation in height decrease (AD) under drought stress among the 68 tomato accessions, whereas the accession PI 584456 showed the smallest AD of 5.3 cm plant height decrease (Table S2), indicating that the accession was somewhat drought tolerant. On the other hand, the accession PI 433016 showed the greatest AD of 21.5 cm (Table S2), indicating that the accession was the most susceptible to drought.

The inhibition index in plant height (PlHt_II), which represents the reduction in plant height of drought-stressed plants compared to well-watered plants, had a large range of 24.9% and ranged from 39.7% to 64.7% (Figure 3D; Supplementary Table S2), with a mean of 56.0 ± 0.73% (Supplementary Table S2), indicating that there was a large difference and variation in plant height inhibition (tolerance) to drought tolerance among the 68 tomato accessions. The accessions PI 600906, PI 330725, PI 499370, and PI 451970 had the lowest II% of 39.7, 39.8, 40.0, and 44.2%, respectively (Table S1), indicating that the accessions had the greatest drought tolerance in this study. The accessions PI 636277, PI 438859, PI 286255, PI 193399, and PI 644750 had the highest PlHt_II% of over 63% (Table S2), showing that they were the most drought-susceptible accessions.

Relative drought tolerance in plant height (PlHt_RDT), defined as the ability of a plant to maintain its height under drought compared to optimal irrigated conditions, had a large range of 24.9% and ranged from 35.3% to 60.3% (Figure 3E, Supplementary Table S2), with a mean of 44.0 ± 0.73% (Supplementary Table S4), indicating that there was a large range and difference among the 68 accessions. The three accessions PI 499370, PI 330725, and PI 600906 had the highest, with >60% of RDT (Table S1), and showed the greatest drought tolerance among the 68 accessions. The accessions PI 286255 (Moneymaker) and PI 644750 (Giant Tree) had the lowest RDT% with <36%, indicating that the two accessions were susceptible to drought.

### 3.1.4. SPAD Chlorophyll Content

The SPAD chlorophyll content (total leaf chlorophyll) for irrigated plants (SPAD_healthy) ranged from 29.5 to 34.6 with a range of 5.1 and showed a near-normal distribution among the 68 accessions (Figure 4A, Supplementary Table S2), with a mean of 32.1; Std Dev of 1.31; Std Err of 0.16; and CV of 4.1 (Supplementary Table S2). Accessions with the greatest SPAD chlorophyll content were PI 330342, PI 291337, and PI 258484 with 34.6, and the lowest were PI 451967, PI 127825, and PI 466917 with <30.0 (Supplementary Table S2).

For the plants under drought treatment, SPAD chlorophyll content (SPAD_stress) varied from 11.1 to 20.3, and the mean and standard deviation were 12.9 and 1.66, respectively (Supplementary Table S2). Distribution of SPAD chlorophyll data under drought stress among the 68 accessions was right-skewed (Figure 4B). Accessions with the highest SPAD chlorophyll content under stress were PI 365956 (LA 1373) (20.3), PI 584456 (19.5), PI 370091 (18.8), and PI 390510 (18.2) (Supplementary Table S1), indicating that these accessions were more tolerant to drought stress. PI 158760 and PI 438587 had the lowest SPAD chlorophyll values with less than 11.5 (Supplementary Table S2), showing high sensitivity of the accessions to drought stress.

The absolute decrease in average SPAD chlorophyll content (SPAD_AD) had a large range of 11.5 and ranged from 11.1 to 20.3 with a mean of 19.2, a Std Dev of 2.10, Std Err of 0.25, and CV of 10.9% (Figure 4C, Supplementary Table S2), indicating that there was a large difference and variability in chlorophyll content decrease (AD) under drought stress among the 68 tomato accessions. Accessions PI 584456 and PI 365956 exhibited the smallest chlorophyll content decrease values, measuring 11.1 and 11.6, respectively (Table S2), indicating that PI 584456 and PI 365956 displayed a relatively low reduction in chlorophyll content, indicating a degree of drought tolerance in these particular accessions. The accessions PI 645361 and PI 600906 showed the greatest SPAD_AD of 22.6% (Table S2), indicating that the two accessions were more drought-susceptible.

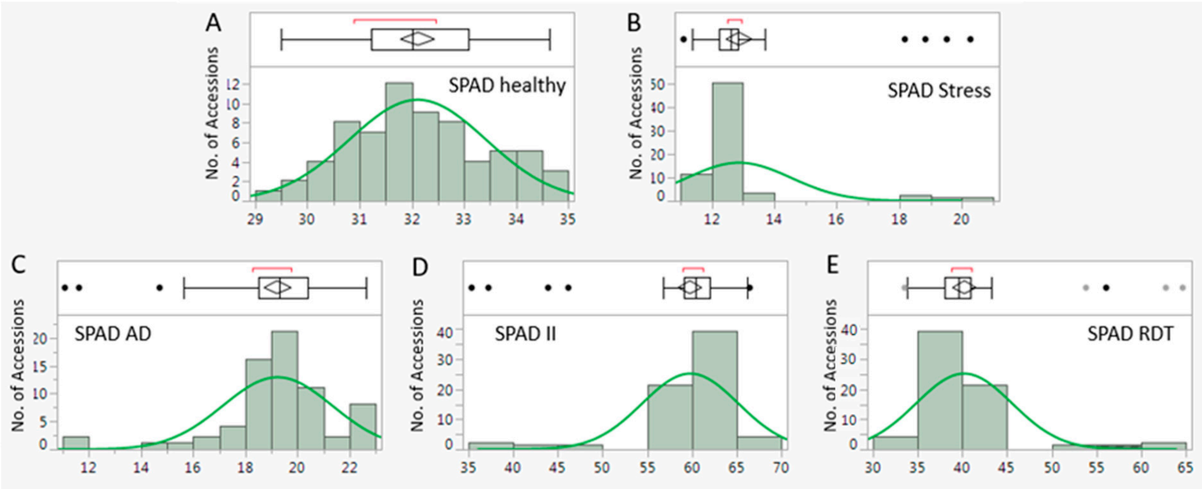


**Figure 4.** The 5 distributions of leaf chlorophyll content (SPAD leaf chlorophyll)-related traits for drought tolerance in 68 tomato accessions. * SPAD_healthy = leaf chlorophyll content in healthy without drought stress, measured by the SPAD-502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL, USA); SPAD_stress = leaf chlorophyll content under drought stress condition; SPAD_AD = Absolute decrease in leaf chlorophyll content (SPAD_healthy—SPAD_stress); SPAD_II = Inhibition index in leaf chlorophyll content = [100 × (SPAD_healthy—SPAD_stress)/SPAD_healthy]; and SPAD_RDT = Relative drought tolerance in leaf chlorophyll content measured = [100 × SPAD_stress/SPAD_healthy] = (100—SPAD_II).

The inhibition index in SPAD chlorophyll content (SPAD_II) had a large range of 31.1% and ranged from 35.4% to 66.4% (Figure 4D; Supplementary Table S2), with a mean of 59.8 ± 0.65% (Supplementary Table S2), indicating that there was a large difference and variability in SPAD chlorophyll content inhibition (tolerance) to drought tolerance among the 68 tomato accessions. The two accessions PI 365956 and PI 584456 had the lowest SPAD_II values of <37.5% (Table S1), displaying the greatest level of drought tolerance. The accessions PI 645361 and PI 158760 had the highest SPAD II% with over 66% (Table S2), being highly vulnerable to drought.

The relative drought tolerance in SPAD chlorophyll content (SPAD_RDT) varied from 33.6% to 64.6% with a large range of 31.1% (Figure 4E, Supplementary Table S2). The mean and standard deviation were 40.2% and 5.37%, respectively (Supplementary Table S4), showing significant differences in drought tolerance among the 68 tomato accessions. The largest SPAD_II values were observed in PI 365956 (64.6%), PI 584456 (62.8%), and PI 390510 (53.8%), indicating that the three accessions had the highest tolerance to drought stress based on SPAD chlorophyll content. In contrast, PI 158760 (33.6%) and PI 645361 (33.8%) showed the lowest relative drought tolerance values, indicating extreme vulnerability to drought stress (Supplementary Table S1). Overall, four tomato accessions, PI 365956 (LA1373), PI 584456 (Allure), PI 370091 (Vision), and PI 390510 (W-C 1050), are drought tolerant, with a scale of <4 in leaf wilting and leaf rolling, decreasing to <16 in absolute SPAD chlorophyll content and <47% in SPAD chlorophyll inhibition index (II), decreasing to <18 cm in absolute plant height and <62% in plant height inhibition index (II) (Table 2).

**Table 2.** Top four tomato accessions with the highest drought tolerance based on eight traits.

| Accession | Name | Taxonomy | Origin | LW-d6 * | LW-d10 | LR-d6 | LR-d10 | SPAD_AD | SPAD_II | PlHt_AD | PlHt_II |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PI 365956 | LA1373 | *Solanum peruvianum* L. | Lima, Peru | 1.9 | 2.7 | 2.0 | 3.9 | 11.1 | 35.4 | 13.7 | 50.3 |
| PI 584456 | Allure | *Solanum lycopersicum* L. | United States | 2.7 | 3.1 | 2.2 | 3.8 | 11.6 | 37.2 | 5.3 | 45.1 |
| PI 370091 | Vision | *Solanum lycopersicum* L. | Canada | 2.6 | 4.0 | 2.6 | 3.4 | 14.7 | 43.9 | 17.7 | 61.7 |

**Table 2.** *Cont.*

| Accession | Name | Taxonomy | Origin | LW-d6 * | LW-d10 | LR-d6 | LR-d10 | SPAD_AD | SPAD_II | PlHt_AD | PlHt_II |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PI 390510 | W-C 1050 | *Solanum lycopersicum* L. var. *cerasiforme* (Alef.) Voss | Ecuador | 2.9 | 3.7 | 2.7 | 3.8 | 15.6 | 46.2 | 16.1 | 56.4 |

* LW-d6 = leaf wilting-d6, LW-d10 = leaf wilting-d10, LR-d6 = leaf rolling-d6, LR-d10 = leaf rolling-d10, SPAD_AD = SPAD chlorophyll absolute decrease (SPAD_healthy—SPAD_stress), SPAD_II = SPAD chlorophyll inhibition index [100 × (SPAD_healthy—SPAD_stress)/SPAD_healthy], PlHt_AD = Absolute decrease in plant height (PlHt_healthy—PlHt_stress), and PlHt_II = Inhibition index in plant height [100 × (PlHt_healthy—PlHt_stress)/PlHt_healthy].

### 3.1.5. Pearson's Correlation Analysis

The correlation coefficients (r-value) among the eight drought-tolerance-related traits in 68 tomato accessions were also shown in Figure 5. A clear linear regression line was observed in each pair among the six traits (leaf wilting-d6 (LW-d6), leaf wilting-10 (LW-d10), leaf rolling-d6 (LR-d6), leaf rolling-d10 (LR-d10), SPAD absolute decrease (SPAD_AD), and SPAD inhibition index (SPAD_II), and between plant height absolute decrease (PlHt_AD) and plant height inhibition index (PlHt_II)) with a high r-value (Table 3), indicating high correlations.

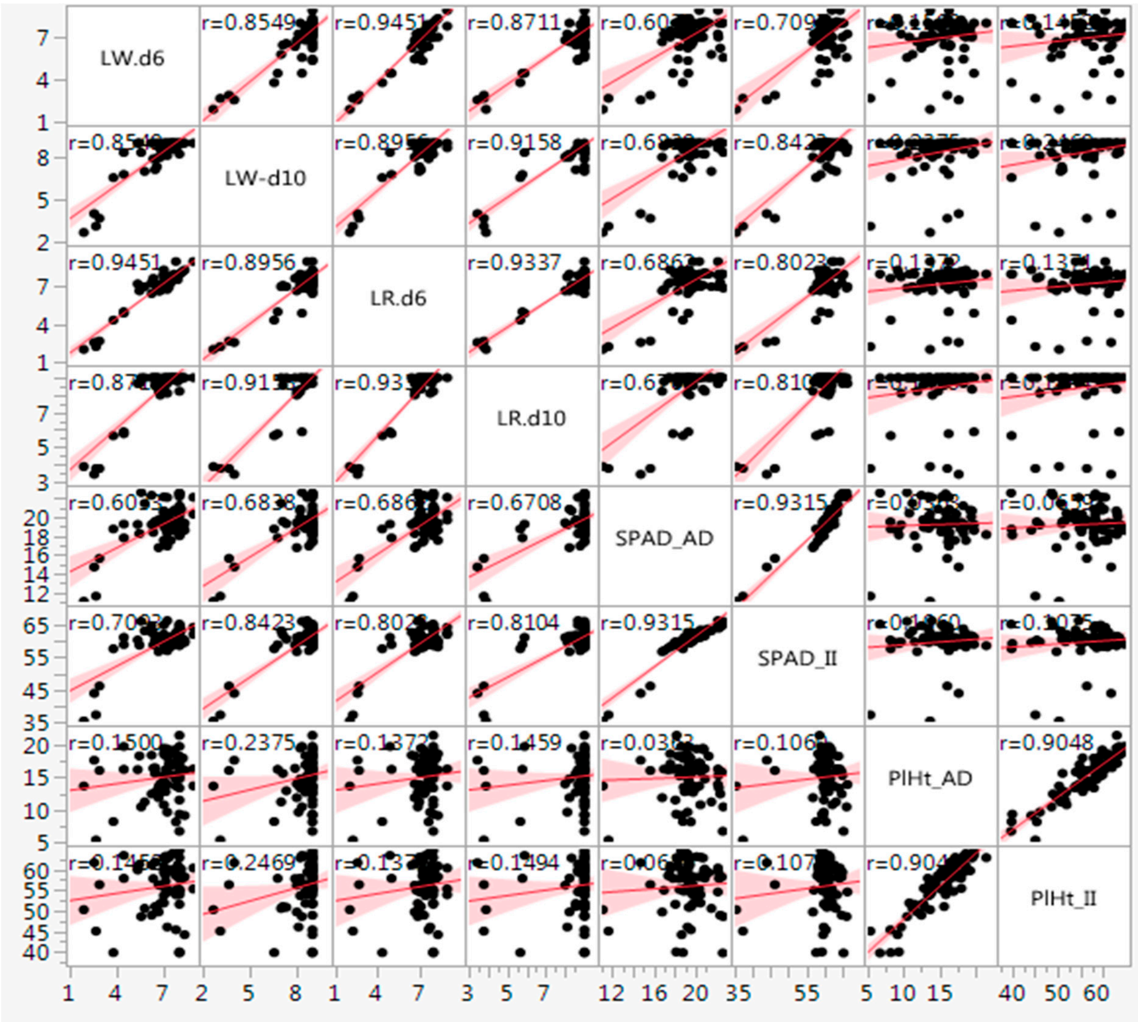


**Figure 5.** Correlation coefficients (r-value) among the eight drought-tolerance-related traits in 68 tomato accessions. * LW-d6 = leaf wilting-d6, LW-d10 = leaf wilting-d10, LR-d6 = leaf rolling-d6, LR-d10 = leaf

rolling-d10, SPAD_AD = SPAD chlorophyll absolute decrease content (SPAD_healthy—SPAD_stress), SPAD_II = SPAD chlorophyll inhibition index [100 × (SPAD_healthy—SPAD_stress)/SPAD_healthy], PlHt_AD = Absolute decrease in plant height (PlHt_healthy—PlHt_stress), and PlHt_II = Inhibition index in plant height [100 × (PlHt_healthy—PlHt_stress)/PlHt_healthy].

**Table 3.** Correlation coefficients (r-value) and their probability (*p*-value) among eight drought tolerance-related traits in 68 tomato accessions.

| Correlation Coefficients (r-Value) | LW-d6 | LW-d10 | LR-d6 | LR-d10 | SPAD_AD | SPAD_II | PlHt_AD |
|---|---|---|---|---|---|---|---|
| **LW-d10** | 0.85 | | | | | | |
| **LR.d6** | 0.95 | 0.90 | | | | | |
| **LR.d10** | 0.87 | 0.92 | 0.93 | | | | |
| **SPAD_AD** | 0.60 | 0.68 | 0.69 | 0.67 | | | |
| **SPAD_II** | 0.71 | 0.84 | 0.80 | 0.81 | 0.93 | | |
| **PlHt_AD** | 0.15 | 0.24 | 0.14 | 0.15 | 0.04 | 0.11 | |
| **PlHt_II** | 0.15 | 0.25 | 0.14 | 0.15 | 0.07 | 0.11 | 0.90 |
| **Probability (*p*-Value)** | **LW-d6** | **LW-d10** | **LR-d6** | **LR-d10** | **SPAD_AD** | **SPAD_II** | **PlHt_AD** |
| **LW-d10** | 1.75E-20 | | | | | | |
| **LR-d6** | 8.99E-34 | 6.54E-25 | | | | | |
| **LR-d10** | 4.64E-22 | 7.61E-28 | 3.85E-31 | | | | |
| **SPAD_AD** | 5.16E-08 | 1.30E-10 | 1.06E-10 | 3.87E-10 | | | |
| **SPAD_II** | 1.29E-11 | 2.22E-19 | 1.95E-16 | 5.68E-17 | 1.09E-30 | | |
| **PlHt_AD** | 0.22 | 0.05 | 0.26 | 0.24 | 0.77 | 0.39 | |
| **PlHt_II** | 0.24 | 0.04 | 0.27 | 0.22 | 0.59 | 0.38 | 3.68E-26 |

* LW-d6 = leaf wilting-d6, LW-d10 = leaf wilting-d10, LR-d6 = leaf rolling-d6, LR-d10 = leaf rolling-d10, SPAD_AD = SPAD chlorophyll absolute decrease content (SPAD_healthy—SPAD_stress), SPAD_II = SPAD chlorophyll inhibition index [100 × (SPAD_healthy—SPAD_stress)/SPAD_healthy], PlHt_AD = Absolute decrease in plant height (PlHt_healthy—PlHt_stress), and PlHt_II = Inhibition index in plant height [100 × (PlHt_healthy—PlHt_stress)/PlHt_healthy].

### 3.1.6. ANOVA and Broad-Sense Heritability

Analysis of variance (ANOVA) for the parameters of drought tolerance and board-sense heritability was estimated for all 14 traits (LW-d6, LW-d10, LR-d6, LR-d10, SPAD_healthy, SPAD_stress, SPAD_AD, SPAD_II, PlHt_healthy, PlHt_stress, PlHt_AD, PlHt_II, PlHt_RDT, and SPAD_RDT) in the 68 tomato accessions (Table S4). The Genotype (accession) had a significant effect at $p = 0.05$ level for all the 14 traits except SPAD_healthy. The significant effect of interaction between genotype (accession) and the block was also observed for PlHt_healthy, PlHt_stress, PlHt_AD, PlHt_II, PlHt_RDT, SPAD_stress, SPAD_II, and SPAD_RDT at $p = 0.05$ level, but not for LW-d6, LW-d10, LR-d6, LR-d10, SPAD_healthy, and SPAD_AD (Table S4), indicating the stability of LW-d6, LW-d10, LR-d6, and LR-d10.

The broad-sense heritability (H%) was calculated for each of the 14 traits, and they were 52.2, 89.2, 69.3, 90.4, 64.1, 62.4, 94.1, 70.3, 70.3, 25.4, 72.2, 48.5, 73.5, and 73.5% for LW-d6, LW-d10, LR-d6, LR-d10, PlHt_healthy, PlHt_stress, PlHt_AD, PlHt_II, PlHt_RDT, SPAD_healthy, SPAD_stress, SPAD_AD, SPAD_II, and SPAD_RDT, respectively (Table S4), showing that all the 14 traits had high heritability (H%), >60% up to 94.1% except LW-d6 (52.2%), SPAD_healthy (25.4%), and SPAD_AD (48.5%), implying that drought tolerance could be inherited.

### 3.1.7. Ranking of Accessions

In this study, the 68 tomato accessions were ranked 1 to 68 in terms of drought tolerance, where 1 denotes the highest level of drought tolerance and 68 represents the most susceptible. The values of PlHt_healthy, PlHt_stress, SPAD_healthy, and SPAD_stress in each tomato accession were determined by the genetic background of the tomato genotypes (accessions) themselves and their interaction with the environment, but were not directly associated with drought tolerance; therefore, their values were excluded from the ranking for drought tolerance. Each of the 10 traits, LW-d6, LW-d10, LR-d6, LR-d10, PlHt_AD, PlHt_II, PlHt_RDT, SPAD_AD, SPAD_II, and SPAD_RDT, was ranked from 1 to 68 (Table S5). In addition, two overall rankings of drought tolerance were used to rank the 68 accessions for their drought tolerance. Due to PlHt_RDT = 100—PlHt_II, PlHt_RDT had the same ranking order as PlHt_II and was removed from the overall ranking. The SPAD_RDT was also removed because it had the same ranking order as the SPAD_II. The first overall ranking was created for the eight traits LW-d6, LW-d10, LR-d6, LR-d10, PlHt_AD, PlHt_II, SPAD_AD, and SPAD_II, defined as Rank (8) (Table S5). Based on the correlation analysis, the plant height related to drought tolerance may have different mechanisms due to the low r-value between plant height-related traits and others (Tables 3 and S3, Figure 5); therefore, the second overall ranking was formed using the six traits LW-d6, LW-d10, LR-d6, LR-d10, SPAD_AD, and SPAD_II (Table S5). The four accessions with drought tolerance in Table 2, PI 365956, PI 584456, PI 370091, and PI 390510, were also listed as the top four drought tolerances, ranked based on Rank (6) and each of the six traits LW-d6, LW-d10, LR-d6, LR-d10, SPAD_AD, and SPAD_II individually (Table S5), indicating that the four accessions were the most drought tolerant from this study. They can be used in tomato breeding programs as parents to develop drought-tolerant cultivars. In addition to the four drought-tolerant accessions, the three accessions PI 330725, PI 193400, and PI 127825 were ranked highly (Table S5), suggesting an intermediate level of drought tolerance.

#### 3.1.8. PCA and Genetic Diversity

Two-way phylogenetic trees were created for (1) among the 68 accessions and (2) for the 8 traits LW-d6, LW-d10, LR-d6, LR-d10, PlHt_AD, PlHt_II, SPAD_AD, and SPAD_II (Figure 6). (1) For the 68 accessions, four clusters (groups) were formed (Figure 6). The four accessions with top drought tolerance in Table 2 and Table S5, PI 365956, PI 584456, PI 370091, and PI 390510, were grouped into the same cluster I based on hierarchical clustering analysis (Figure 6), indicating that they had similar drought tolerance. Two out of three accessions in cluster II, PI 330725 and PI 193400 (Figure 6), were also tolerant to drought stress (Table S1) and ranked among the top six (Table S5), indicating they had similar responses to drought stress at an intermediate level. The tomato accessions in clusters III and IV are drought susceptible (Figure 6), showing that susceptible accessions were merged together. This study has indicated that the eight traits LW-d6, LW-d10, LR-d6, LR-d10, PlHt_AD, PlHt_II, SPAD_AD, and SPAD_II can be used to distinguish drought tolerant and susceptible tomato accessions. (2) For the eight traits, there were two clusters: PlHt_AD and PlHt_II were clustered together as one group, and the other six as another cluster. This was further divided into two groups: SPAD_AD and SPAD_II in the same group and the other four as another group, where LW-d6 and LW-d10 were together, and LR-d6 and LR-d10 were a pair (Figure 6 bottom), indicating that leaf wilting and leaf rolling had similar results for drought tolerance, close to the results for SPAD chlorophyll content, but a little different from those for plant height.

The bioplot revealed a consistent pattern among LW-d6, LW-d10, LR-d6, LR-d10, SPAD_AD, and SPAD_II, indicating a close association with each other. In contrast, PlHt_AD and PlHt_II demonstrated proximity to each other but were notably distinct from the mentioned variables (Figure 7A). This suggests a strong correlation among LW-d6, LW-d10, LR-d6, LR-d10, SPAD_AD, and SPAD_II, while PlHt_AD and PlHt_II showed a distinct pattern. The screen plot (Figure 7B) and PCA plot (Figure 7C) further illustrated the presence of two or four distinct clusters within the 68 accessions.

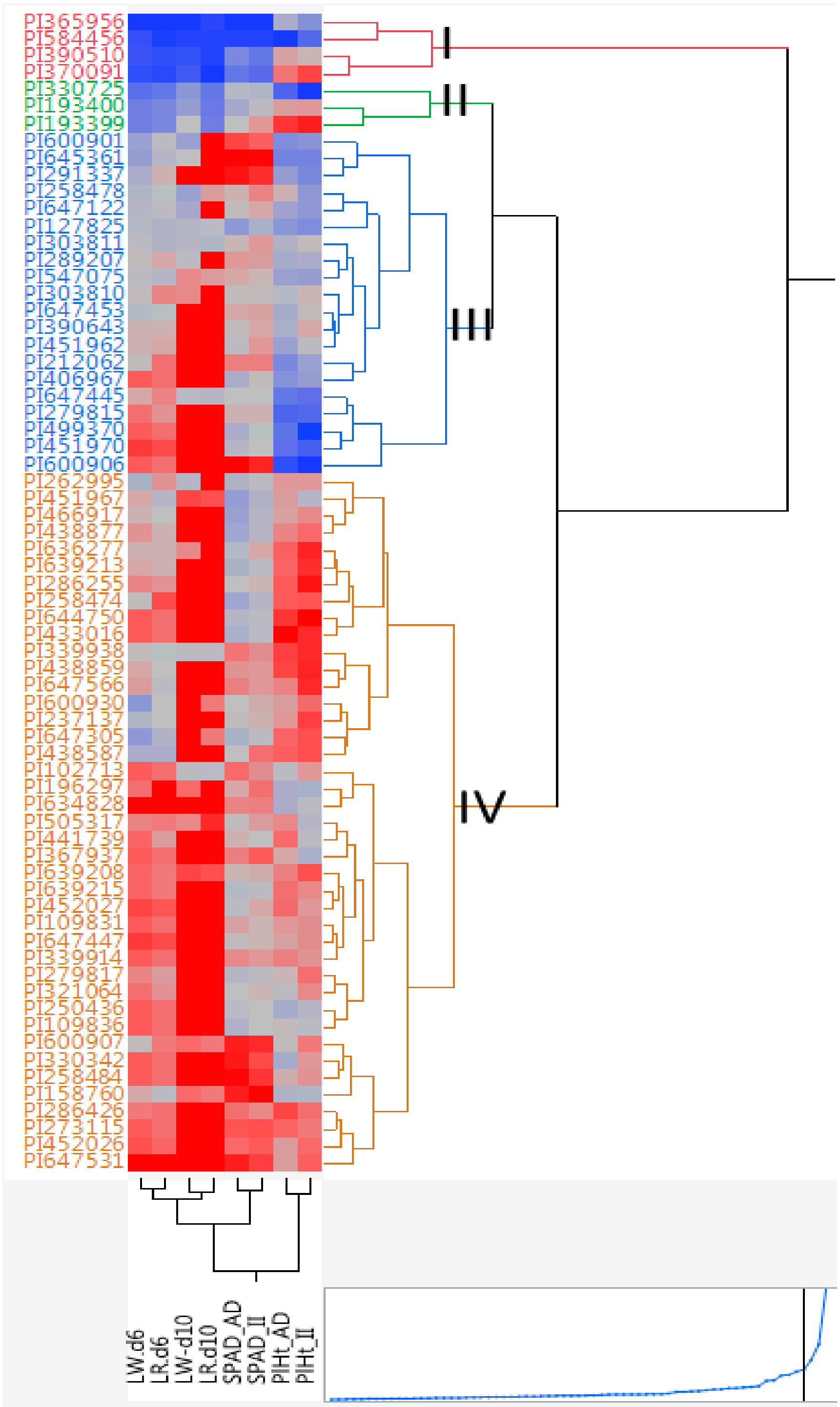


**Figure 6.** The two-way dendrogram in 68 tomato accessions by hierarchical cluster analysis in JMP Pro 17 based on 8 drought-tolerance-related traits: leaf wilting-d6 (LW-d6), leaf wilting-d10 (LW-d10), leaf rolling-d6 (LR-d6), leaf rolling-d10 (LR-d10), SPAD chlorophyll absolute decrease (SPAD_AD), SPAD chlorophyll inhibition index (SPAD_II), plant height absolute decrease (PlHt_AD), and plant height inhibition index (PlHt_II), where the top four drought tolerant accessions were grouped into cluster I (top). * LW-d6 = leaf wilting-d6, LW-d10 = leaf wilting-d10, LR-d6 = leaf rolling-d6, LR-d10 = leaf rolling-d10, SPAD_AD = SPAD chlorophyll absolute decrease content (SPAD_healthy—SPAD_stress), SPAD_II = SPAD chlorophyll inhibition index [100 × (SPAD_healthy—SPAD_stress)/SPAD_healthy], PlHt_AD = Absolute decrease in plant height (PlHt_healthy—PlHt_stress), and PlHt_II = Inhibition index in plant height [100 × (PlHt_healthy—PlHt_stress)/PlHt_healthy].

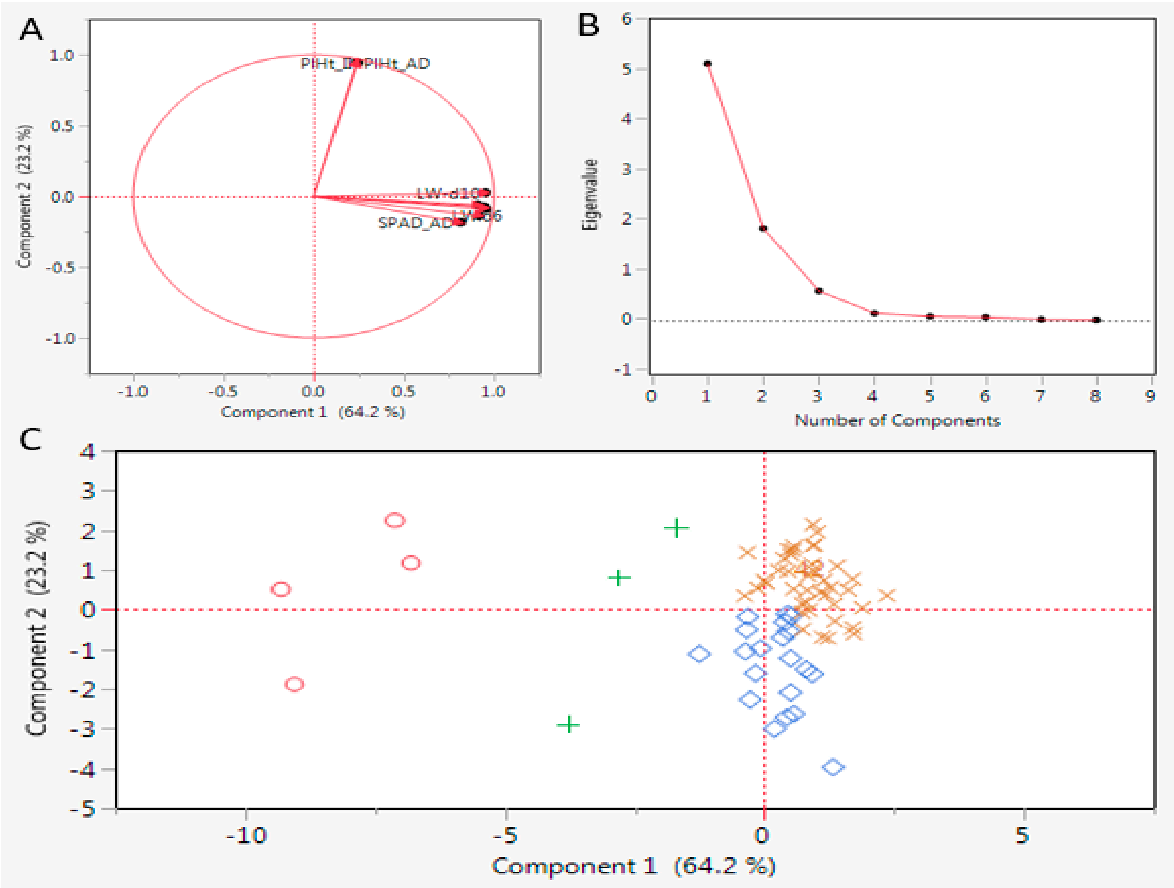


**Figure 7.** Principal component analysis (PCA) in 68 tomato accessions by JMP Genomics based on 8 drought-tolerance-related traits, leaf wilting-d6 (LW-d6), leaf wilting-d10 (LW-d10), leaf rolling-d6 (LR-d6), leaf rolling-d10 (LR-d10), SPAD chlorophyll absolute decrease (SPAD_AD), SPAD chlorophyll inhibition index (SPAD_II), plant height absolute decrease (PlHt_AD), and plant height inhibition index (PlHt_II): (**A**) Bioplot, (**B**) Screen plot, and (**C**) PCA with 4 clusters. ** LW-d6 = leaf wilting-d6, LW-d10 = leaf wilting-d10, LR-d6 = leaf rolling-d6, LR-d10 = leaf rolling-d10, SPAD_AD = SPAD chlorophyll absolute decrease content (SPAD_healthy—SPAD_stress), SPAD_II = SPAD chlorophyll inhibition index [100 × (SPAD_healthy—SPAD_stress)/SPAD_healthy], PlHt_AD = Absolute decrease in plant height (PlHt_healthy—PlHt_stress), and PlHt_II = Inhibition index in plant height [100 × (PlHt_healthy—PlHt_stress)/PlHt_healthy].

From the phylogenetic tree, among 65 tomato accessions, which did not include the 3 accessions PI 365956, PI 438587, and PI 600901 out of the 68 accessions in Table S1 since the 3 accessions did not have good DNA sequencing data, these 65 accessions were divided into 2 clusters (sub-populations), Q1 and Q2, based on 5,003 SNPs distributed on 12 chromosomes by MEGA 11, where Q1 has 9 accessions and Q2 has 56 accessions (Figure 8). The six drought-tolerant accessions, PI 584456, PI 370091, PI 390510, PI 330725, PI 193400, and PI 127825, were arranged into different locations (parts) in the phylogenetic tree; the PI 584456 was grouped to cluster Q1 and the other five to Q2 (Figure 8), indicating that the six accessions had different genetic bases and that PI 584456 is different from others, suggesting how to select these valuable drought-tolerance resources as parents in tomato breeding.

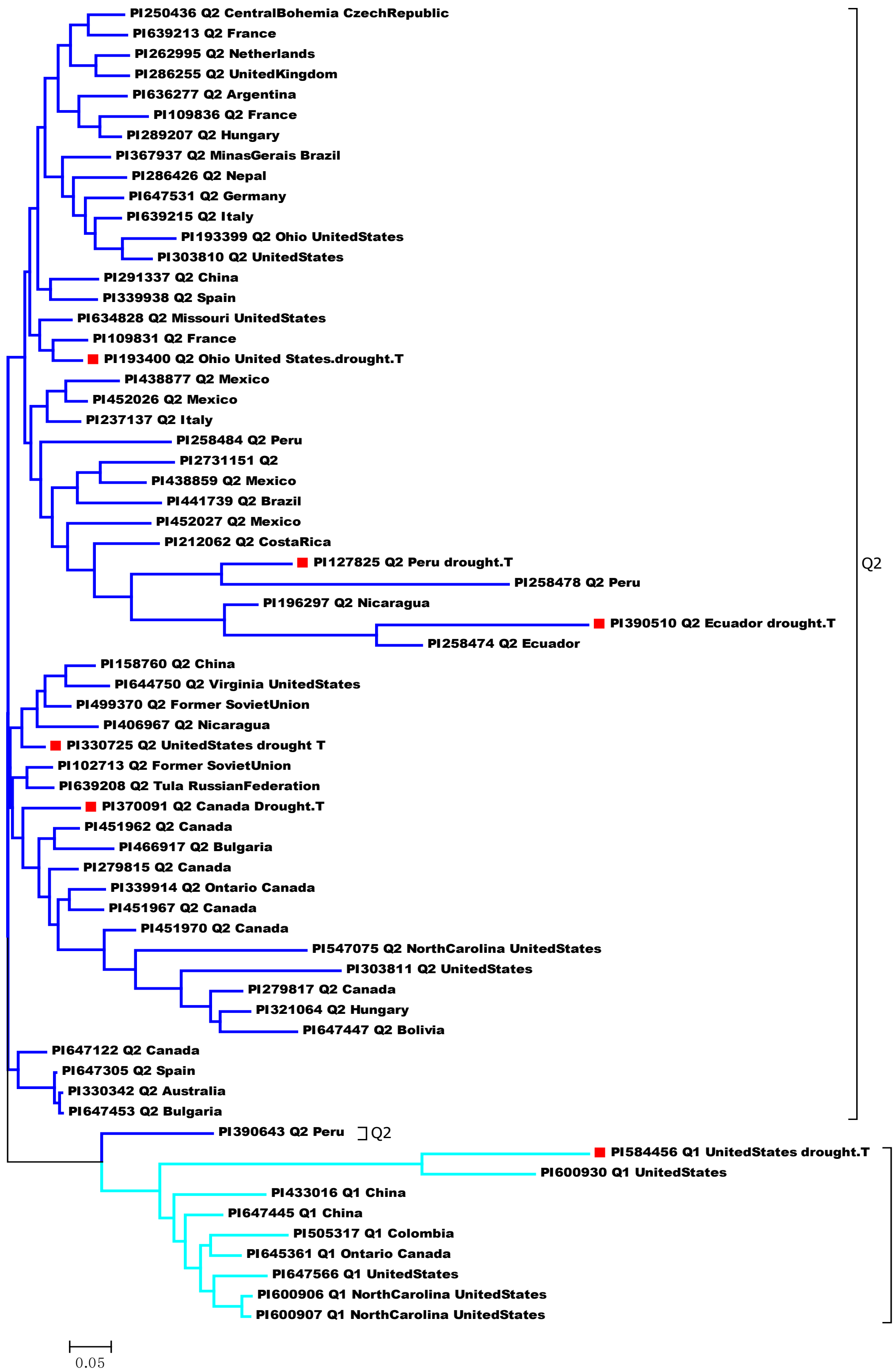


**Figure 8.** Phylogenetic tree created by MEGA 11 using the maximum likelihood (ML) method based on 5005 SNPs distributed on 12 chromosomes, where the accession number (PI), origin, and cluster (Q1, Q2) are merged as each taxon name in the tree. The red square shapes are the 6 greatest and intermediate drought-tolerant accessions with leaf wilting and leaf rolling rate of less than 4.0; 2 clusters (sub-populations), Q1 and Q2, were observed among the 65 USDA GRIN tomato accessions, where Q1 has 9 accessions and Q2 has 56 accessions.

## 4. Discussion

Drought tolerance in crops is related to many factors that may contribute to tolerance, making studies on drought tolerance difficult. Numerous mechanisms of drought tolerance in several crops depend on the conditions, crop variety, and growth stages. As a result, many researchers have used multiple indicators to assess drought tolerance in a comprehensive and integrated manner, which can provide more accurate and realistic information on drought tolerance in crops and can help researchers discover and select drought-tolerant cultivars for cultivation and breeding. Inadequate information on drought tolerance in tomatoes has compromised the development of drought-tolerant cultivars. This study screened the germplasm collection of tomatoes using multiple parameters and generated valuable information on drought tolerance in tomatoes by supplying reactions of various tomato accessions to drought stress.

### *4.1. Drought-Associated Parameters*

#### 4.1.1. Leaf Wilting

The results of the drought tolerance study based on leaf wilting showed significant genetic variation among tomato accessions for tolerance to water stress. The results were consistent with past studies that have reported genetic variation in tomatoes for drought tolerance based on leaf wilting traits [43]. Finding genetic variation and developing highly drought-tolerant cultivars is critical for sustainable agriculture, as drought is a major environmental stress that affects crop productivity and quality worldwide. This study identified tomato accessions PI 365956, PI 584456, PI 390510, and PI 370091 imported from Peru, United States, Ecuador, and Canada (Supplementary Table S1; Table 2), respectively, to exhibit slow wilting under dry conditions, showing greater tolerance to drought stress, as the similar reports by Cardoso et al. (2022) [38], Abdellatif et al. (2023) [43], and Pathan et al. (2014) [44] They concluded that plant genotypes of tomato and soybean that maintained slow-wilting traits and less yield loss were drought-tolerant. The drought-tolerant accessions identified based on leaf wilting characteristics in this study could serve as valuable parental lines. These accessions exhibited reduced wilting traits, showing their usefulness in breeding for enhanced drought tolerance in high-yield but drought-susceptible tomato cultivars.

#### 4.1.2. Leaf Rolling

Leaf rolling is caused by dehydration of various sections across the leaf, which minimizes the leaf surface area for sunlight penetration and transpiration, leading to stomatal closure and reduced photosynthesis [45]. Leaf rolling is a significant indicator of drought tolerance in plants, as described by Baret et al. (2018) [46], Chandra et al. (2009) [47], and Merrium et al. [48]. The use of leaf rolling as an indicator of drought tolerance has recently been explored to facilitate the selection of more drought-tolerant cultivars of crops [46,49]. Baret et al. [46] recently phenotyped maize genotypes in the field and reported the occurrence of leaf rolling in water-stressed plants at the flowering stage even during the first day of exposure to drought. Another recent study by Yang et al. [49] aimed to compare rice varieties AK58 and ZM36 subjected to dry conditions based on the degree of leaf rolling at the seedling stage and they found rice variety AK58 to have its leaves slightly rolled, showing considerable tolerance to drought, unlike rice variety ZM36. Regarding tomatoes, Medyouni et al. [50] assessed tomato plants in an arid environment and noted a reduction in leaf size (a reduction in the number, width, and length of the leaves, as well as the leaf surface area). The results of this study showed significant variation in leaf rolling or folding among the 68 tomato accessions, indicating the importance of this trait to the overall drought tolerance level in tomato plants. Tomato accessions PI 370091, PI 390510, PI 584456, and PI 365956 were identified to exhibit great tolerance to drought based on leaf rolling scores (score of less than 4) (Supplementary Table S1; Table 2), showing that they could be utilized for selection as parental lines for successful breeding with a focus on developing more drought-tolerant tomato cultivars.

#### 4.1.3. Plant Height

This study was conducted to investigate the effects of drought stress on tomato plants. The assessment involved 68 tomato accessions, with particular attention paid to plant height as an indicator of drought tolerance. Drought stress is recognized for its role in inhibiting plant growth, attributed to compromised mitosis and the loss of turgor [51]. Ahmadikhah and Marufinia [52] observed a reduced plant height in rice cultivars exposed to water deficit conditions. Another recent study by Su et al. (2019) [53] showed that even drought-tolerant genotypes of maize reduced plant height under drought stress conditions. This study showed significant variations in plant height among the accessions under both well-irrigated and drought-stressed conditions, with a mean absolute decrease in plant height of 19.2 cm (Supplementary Table S2; Table 2) across the accessions under drought stress. The decrease in plant height is directly associated with the restriction of cell expansion, leading to the development of plants with diminished growth and reduced yield [54]. The inhibition index and relative drought tolerance were also calculated, recognizing accessions PI 365956, PI 584456, PI 370091, and PI 390510 to have a large inhibition index and relative drought tolerance, indicating that these accessions were drought tolerant. These drought-tolerant accessions were noted to be better adapted to water-deprived conditions than the others and could be suitable parental lines for utilization in breeding to enhance drought tolerance in tomatoes.

#### 4.1.4. SPAD Chlorophyll Content

Drought stress hinders plant growth by reducing photosynthesis [55], the mechanism through which plants transform light into energy [56]. Chlorophyll, a green pigment [57], is essential for photosynthesis [58], and drought-induced chlorophyll breakdown can affect a plant's ability to carry out photosynthesis efficiently, making the plant fail to complete its growth cycle. Several previous studies reported decreased chlorophyll content for plants exposed to extremely dry conditions, depending on the period of drought [59]. Leaf chlorophyll content is shown to increase during an early stage of water stress and decrease gradually with increasing periods of drought [60]. The results of this study showed that leaf chlorophyll content was greatly reduced in drought-stressed tomato plants, indicating that water stress negatively affected chlorophyll synthesis, as illustrated in many previous studies. Interestingly, some tomato accessions were shown to maintain slightly greater levels of chlorophyll content under drought stress as compared to others, indicating potential differences in drought tolerance among the accessions. Alidu et al. and Cardoso et al. [38,61] also reported moderately greater leaf chlorophyll content in drought-tolerant cowpea recombinant inbred line and tomato genotypes, respectively, subjected to dry conditions. Furthermore, the report by Monteoliva et al. [58] indicates that plants exhibiting more elevated chlorophyll levels than their counterparts under optimal water availability conditions are anticipated to exhibit greater tolerance. This hypothesis postulates a positive correlation between increased chlorophyll levels and enhanced rates of photosynthesis, consequently leading to elevated crop yields. In this investigation, the accessions with the highest relative drought tolerance based on chlorophyll content were PI 365956, PI 584456, PI 370091, and PI 390510 (Supplementary Table S1; Table 2), which all showed over 50% retention of chlorophyll content under drought stress. These results imply that chlorophyll content could be a suitable trait for detecting tomato accessions with greater drought tolerance and for breeding programs aimed at improving water stress tolerance in tomato plants.

#### 4.1.5. Pearson's Correlation Analysis

This study revealed robust positive correlations among leaf wilting, leaf rolling, and SPAD chlorophyll content parameters (Supplementary Table S3). O'Toole and Moya [62] established a strong association between leaf rolling, leaf tip drying, and the preservation of leaf water potential. Baret et al. [46] emphasized that prolonged drought conditions may lead to leaf rolling, potentially linked to a decline in chlorophyll content due to reduced leaf surface area exposed to sunlight. In a cowpea drought-tolerance study, Pungulani et al. (2013) [63] demonstrated a significant correlation between leaf wilting and relative water content. Conversely, weak correlations were observed between plant-height-related parameters and other traits (Supplementary Table S3; Table 3; Figure 5). Ahmadikhah and Marufinia, [52] also reported weak correlations between plant height and leaf chlorophyll content (chl. *a*) in a drought-tolerance study on rice.

The results of this study carry substantial implications for crop breeding initiatives aimed at enhancing drought tolerance. By prioritizing traits that exhibit strong correlations, such as leaf wilting, leaf rolling, and SPAD chlorophyll content, as demonstrated in this study, breeders can effectively work towards developing crops better suited for dry environments. Moreover, the observed weak correlations between plant-height-related parameters and other traits suggest that breeders may need to consider diverse sets of traits when targeting improved plant height in conditions with limited water availability.

## 5. Conclusions

In summary, this study effectively identified highly drought-tolerant tomato accessions, classifying them into three groups: drought tolerant, moderately tolerant, and drought sensitive. The outstanding performance of accession PI 365956 stands out, followed closely by PI 584456, PI 370091, and PI 390510, all demonstrating considerable drought tolerance. The potential presence of genes associated with drought tolerance, as noted in previous studies, highlights the importance of exploring these genetic resources in molecular and physiological investigations. These valuable tomato accessions could be key to understanding and improving the mechanisms that drive yields in water-scarce environments. As we move forward, the application of these findings could significantly contribute to crop adaptation to climate change and sustainable water resource management in agriculture. In future research, we plan to evaluate additional traits related to drought tolerance in a broader range of tomato germplasm accessions, conduct a genome-wide association study to identify molecular markers and candidate genes for drought tolerance, and implement genomic prediction for genomic breeding in tomato.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14020380/s1, Table S1: The 68 tomato accession ID, name, taxonomy, origin, country, and 14 traits for measuring drought tolerance; Table S2: Parameters of 14 traits for drought tolerance in 68 tomato accessions; Table S3: Correlation coefficients (r-value) and their probability (*p*-value) among 14 drought tolerance related traits in 68 tomato accessions; Table S4: ANOVA for the parameters of drought tolerance and board sense heritability estimation in 68 tomato accessions; Table S5: The ranks of the 68 tomato accessions for their drought torance in each of the eight traits plus the averages of six and eight traits, respectively.

**Author Contributions:** Conceptualization, G.B., H.X., N.K.J. and R.W.D.; methodology, K.E.C., G.B., H.X. and I.A.; software, A.S.; validation, K.E.C., T.M.P., G.B., H.X., I.A. and Y.C.; formal analysis, A.S. and K.E.C.; investigation, K.E.C., T.M.P., G.B., H.X., I.A. and Y.C.; resources, A.S., Z.S. and K.-S.L.; data curation, K.E.C., G.B., T.M.P., H.X. and I.A.; writing—original draft preparation, K.E.C.; writing—review and editing, A.S., K.E.C., G.B. and H.X.; visualization, K.E.C., A.S., G.B., T.M.P. and H.X.; supervision, A.S., N.K.J., R.W.D., K.-S.L. and G.B.; project administration, A.S., G.B., K.-S.L., N.K.J. and R.W.D.; funding acquisition, A.S., K.-S.L., Z.S., R.W.D. and N.K.J. All authors have read and agreed to the published version of the manuscript.

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