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Article

# 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 tomato accessions from the United States Department of Agriculture (USDA) 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 of this study provide valuable insights relevant to tomato breeding initiatives, particularly those aimed at fortifying drought tolerance within the elite cultivars of this essential crop.

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

## 1. Introduction

Global environmental shifts have become an undeniable reality (Fahad et al., 2017), marked by an escalation in extreme aridity or drought, heat, and floodwaters (Devi et al., 2009; Fahad et al., 2017; McCarthy et al., 2021; Warsame et al., 2023). These occurrences are growing in both frequency and intensity (Conti et al., 2023). These factors bear significant and immediate consequences for the agricultural sector, resulting in diminished productivity (Chandio et al., 2022; Warsame et al., 2023), leading to reduced food supply, elevated food prices, and adversely affecting the livelihoods of many households (Chandio et al., 2022; Conti et al., 2023; Wiebe et al., 2019).

Among all the factors adversely affecting sustainable crop production, drought, referred to as deficit irrigation or soil water deficit (Cui et al., 2020), 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 (Cui et al., 2020a; Placide et al., 2014). Approximately 25% of the world's population is at risk due to drought, with the majority hailing from developing countries in Africa and Asia (Cui et al., 2020a). Mitigating this challenge involves the creation of drought-tolerant cultivars through rigorous screening and selection processes (Seleiman et al., 2021; Shamim et al., 2014).

Tomato (*Solanum lycopersicum* L.) is a widely cultivated and consumed horticultural crop, with wild accessions demonstrating significant resilience to drought conditions unlike the cultivated

accessions (Foolad, 2007; Krishna et al., 2022). Despite this resilience, the global cultivation of tomatoes exposes to the prominent abiotic constraint of drought. For optimal growth and yield, tomatoes require a continuous water supply (Ayankojo et al., 2018; Foolad et al., 2007; Sharma et al., 2015). The impact of drought stress on tomato yield varies based on soil and climatic conditions (Ayankojo et al., 2018; Zegbe et al., 2007). Short periods of water deficit result in both qualitative and quantitative losses in fruit production (Ayankojo et al., 2018). Inadequate water availability negatively impacts multiple aspects of tomato plant growth and overall yield (Ayankojo et al., 2018). 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 (De Oliveira et al., 2021). Addressing farmers' needs, the development of drought-tolerant tomato varieties is a key focus in contemporary breeding programs (Borba et al., 2017; Dariva et al., 2021; Morales et al., 2015; Zhao et al., 2021).

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 (Gong et al., 2010a; Hussain et al., 2018). 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. (2020a) and Ravelombola et al. (2020). 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 (Godfray et al., 2010). Traditionally, breeders focused on increasing crop yields, resulting in a shortage of modern varieties with stress tolerance (Gilliham et al., 2017). 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 (Chen et al., 2018). Incorporating wild crop relatives provides a strong foundation for discovering new genes and understanding the mechanisms behind physiological adaptations (Isayenkov, 2019). Concerning tomatoes, undomesticated species inherently exhibit adaptability to diverse soil and climatic conditions. Studies by Egea et al. (2018), Dempewolf et al. (2017), and Olivieri et al. (2020) 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. (2010), Wang et al. (2015), and Yang et al. (2021).

Around the globe, there are over 62,800 varieties of cultivated and wild tomatoes, mostly belonging to the *L. esculentum* species, preserved in gene banks (Foolad, 2007), 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 (Shamim et al., 2014). 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 sixty-eight 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.

## 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 rest 40 from other 24 countries (Supplementary Table S1).

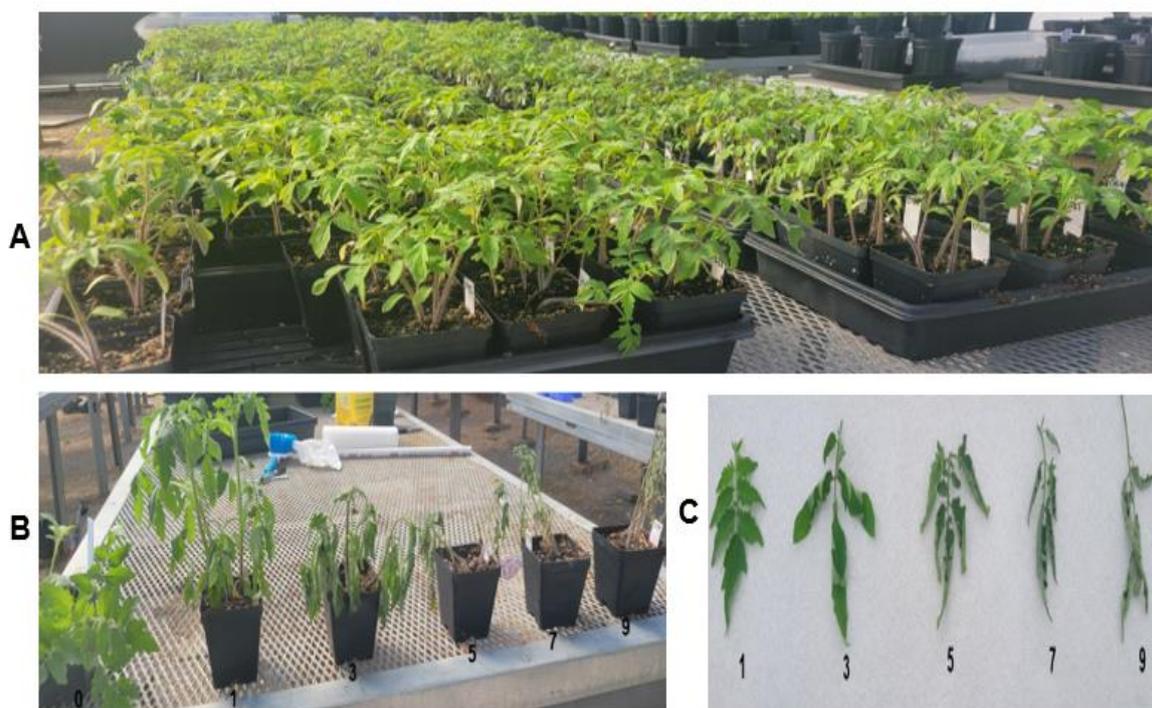
### 2.2. Evaluation for drought tolerance

Evaluation of tomato accessions was performed in a greenhouse at the Arkansas Agricultural Research and Extension Center, Fayetteville, AR (Fig 1 A) between January 2023 and February 2023. During the experiment, the greenhouse temperature and humidity were kept at 21/18°C in day/night and 73%, respectively. The screening procedure was followed as described in previous reports by Cardoso et al. (2022), Cui et al. (2020), Engelbrecht et al. (2007), and Susanto et al. (2019), with slight modifications.

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

Immediately after seed sowing, 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%), were applied in liquid form per pot 10 days and every 14 days in subsequent applications 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, with the drought treatment as the main plot and the tomato accessions as the subplot. During the experiment, thinning was done in 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 in 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 maintained with 180 mL of tap water every 3 days constantly.



**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.

### 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.

**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.

Visual assessment of leaf wilting and leaf rolling were performed based on a scale of 0 to 9 (Fig 1 B) and 1 to 9 (Fig 1 C), respectively (Table 1), with slight modifications based on the symptoms associated with leaf drying and folding (Cui et al., 2020a; Engelbrecht et al., 2007; Susanto et al., 2019). 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.

The SPAD chlorophyll content was measured from three regions of trifoliolate 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). The measurements for each region in the leaf were recorded, separately.

The following data were collected and computed (Ravelombola., 2021) (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);
- 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. PIHt\_healthy: Plant height under irrigation treatment;
- xi. PIHt\_stress: Plant height under drought treatment;
- xii. PIHt\_AD: Absolute decrease in plant height (PIHt\_healthy - PIHt\_stress)
- xiii. PIHt\_II: Inhibition Index in plant height =  $[100 * (PIHt\_healthy - PIHt\_stress) / PIHt\_healthy]$ ; and
- xiv. PIHt\_RDT = Relative drought tolerance in plant height =  $[100 * (PIHt\_stress / PIHt\_healthy)] = (100 - PIHt\_II)$ .
- xv. Broad-sense Heritability ( $H^2$ )
- xvi. Pearson's correlation analysis

## 2.4. Data analysis

### 2.4.1. Statistical model

The statistical model for analysis of variance (ANOVA) was the following:  $Y_{ij} = \mu + B_i + G_j + \epsilon_{ij}$  where  $i = 1, 2, 3$  and  $j = 1, \dots, 68$ , with  $\mu$  representing the overall mean, and  $Y_{ij}$  representing the response from the  $j$ th accession ( $G_j$ ) (fixed effect) at the  $i$ th block ( $B_i$ ) (random effect), and  $\epsilon_{ij}$  representing the random error associated with the  $ij$ th 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 (Holland et al., 2003).  $H^2 = 100 * \sigma^2_G / [\sigma^2_G + (\sigma^2_{GE} / e) + (\sigma^2_E / re)]$ , 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  $\sigma^2_E = MSE$ ;  $\sigma^2_{GE} = (MSG - MSE)/r$ ; 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 chlorophyll content was achieved by subtracting the plant height or chlorophyll content for the drought-stressed plants from those of well-watered plants (AD = the value in healthy 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  $II = [100 * (\text{the value in healthy} - \text{the value under drought treatment}) / \text{the value in healthy under normal irrigation}]$ . As same the AD value, the greater the II percentage for accession, the plant height, and SPAD chlorophyll content decreased, showing the tomato accession had greater susceptibility under drought conditions. Conversely, the lower the AD value, the more tolerant the accession was.

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. Conversely as AD and II, 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. Contrarily, a lower RDT percentage suggested greater susceptibility in the accession under drought conditions.

#### 2.4.5. 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, PIHt\_AD, PIHt\_II, PIHt\_RDT, and SPAD\_RDT), where 1 as the top associated with drought tolerance and 68 as the most vulnerable one. 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 PIHt\_RDT and SPAD\_RDT were not listed.

#### 2.4.6. 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 (Elshire et al., 2011) in Pair-end sequencing libraries sequenced by Illumina NovaSeq in University of Wisconsin Biotechnology Center (UWBC) (<https://biotech.wisc.edu/>). 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](https://phytozome-next.jgi.doe.gov/info/Slycopersicum_ITAG4_0)) and SNPs were postulated in a pipeline using TASSE\_GBS (Glaubitz et al., 2014) and Stacks 2 (Rochette et al., 2019;

<https://catchenlab.life.illinois.edu/stacks/>). 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.

#### 2.4.7. Principal Component Analysis (PCA) and Genetic Diversity

Principal components were analyzed and the Dendrogram was drawn by hierarchical cluster method using JMP Pro 17 based on either trait (LW-d6, LW-d10, LR-d6, LR-d10, SPAD\_AD, SPAD\_II, PIHt\_AD, and PIHt\_II) among the 68 tomato accessions. Genetic diversity was analyzed, and a phylogenetic tree was generated using MEGA 11 based on 5,003 single nucleotide polymorphism (SNP) markers distributed on 12 chromosomes in 65 USDA GRIN tomato accessions except the three accessions, PI 365956, PI 438587, and PI 600901 out of the 68 accessions in Table S1. Because they had bad 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.

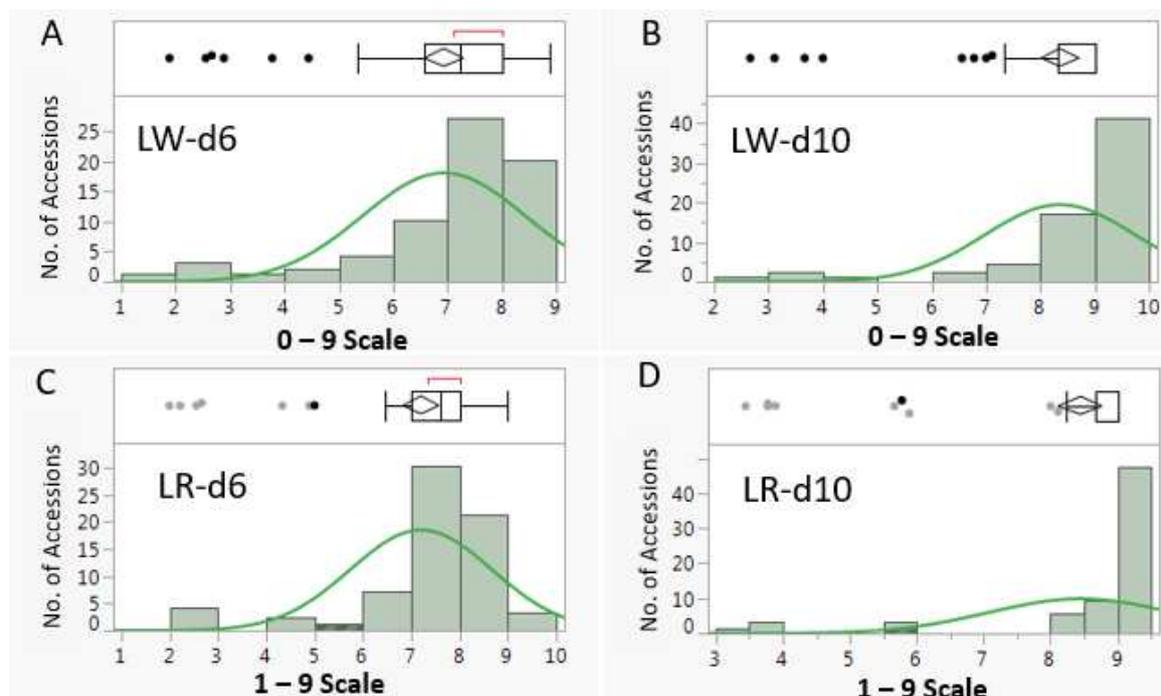
### 3. Results

#### 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 Table S1 & S2). A large range was observed: 7.0 for LW-d6 and 6.3 for LW-d10 (Table S2). The mean rate of 6.9, standard deviation (Std Dev) of 1.5, standard error (Std Err) of 0.18, and coefficient variation (CV) of 21.6% were observed for 6 days of drought treatment (LW-d6), while the mean rate of 8.4, Std Dev of 1.39, Std Err of 0.17, and CV of 16.6% were noted under 10 days 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 2 A&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 highest 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 Table S1 & 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 and B) and leaf rolling (LR) (C and D) in 68 tomato accessions: (A) and (C) in 6 days; and (B) and (D) in 10 days after drought treatment.

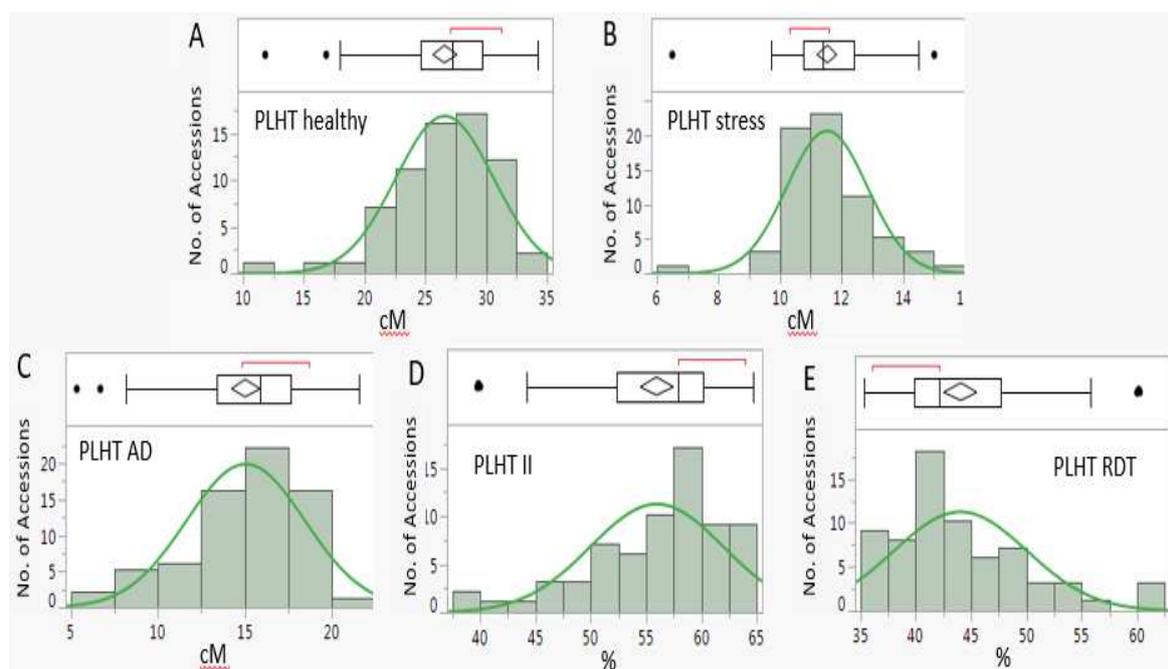
### 3.1.2. Leaf rolling

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, with a mean rate of 7.2, standard deviation (Std Dev) of 1.47, standard error (Std Err) of 0.18, and coefficient variation (CV) of 20.4% for 6 days drought treatment (LR-d6) and the mean rate of 8.4, standard Std Dev of 1.37, Std Err of 0.17, and CV of 16.3% under 10 days 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 2 C&D), as same trend as those in leaf wilting, showing that most of the 68 tomato accessions were extreme susceptibility 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 (PIHt\_healthy) ranged from 11.8 cm to 34.2 cm at 10 days with a nearly normal distribution skewed right side among the 68 accessions (Fig. 3 A), 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 five distributions of plant height (PLHT)-related traits for drought tolerance in 68 tomato accessions. \*PIHt\_healthy = Plant height under irrigation; PIHt\_stress = Plant height under drought conditions; PIHt\_AD = Absolute decrease in plant height = PIHt\_healthy - PIHt\_stress; PIHt\_II = Inhibition Index in plant height =  $[100 * (\text{PIHt\_healthy} - \text{PIHt\_stress}) / \text{PIHt\_healthy}]$ ; and PIHt\_RDT = Relative drought tolerance in plant height =  $[100 * (\text{PIHt\_stress} / \text{PIHt\_healthy})] = (100 - \text{PIHt\_II})$ .

Under drought conditions, the average plant height (PIHt\_stress) ranged from 6.5 cm to 15.0 cm at 10 days (Fig 3 B), 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 (PIHt\_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 cm, an Std Dev of 3.41 cm, Std Err of 0.41 cm, and CV of 22.6 % (Fig 3 C, Supplementary Table S2), indicating that there was a large difference and variable of 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.

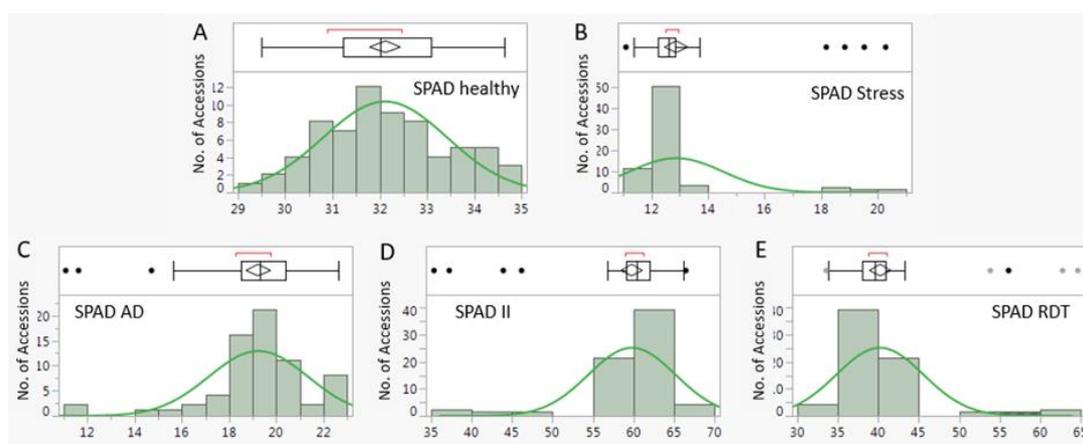
The inhibition index in plant height (PIHt\_II), which represents the reduction in plant height of drought-stressed plants compared to well-watered plants, had a large range of 24.9% and ranged from 39.7% to 64.7% (Fig 3 D; Supplementary Table S2), with a mean of 56.0%, a Std Dev of 6.02%, Std Err of 0.73, and CV of 10.8% (Supplementary Table S2), indicating that there was a large difference and variable of 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 PIHt\_II% with over 63% (Table S2), showing that they were the most drought susceptible accessions.

Relative drought tolerance in plant height (PIHt\_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% (Fig 3 E, Supplementary Table S2), with a mean of 44.0%, a Std Dev of 6.02%, Std Err of 0.73, and CV of 13.7 (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 (Fig 4 A, 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).



**Figure 4.** The five 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); 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 =  $[100 * SPAD\_stress / SPAD\_healthy] = (100 - SPAD\_II)$ .

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 (Fig 4 B). 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. The lowest SPAD chlorophyll values were recorded for PI 158760 and PI 438587 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% (Fig 4 C, Supplementary Table S2), indicating that there was a large difference and variable of chlorophyll content decreased (AD) under drought stress among the 68 tomato accessions. The accessions PI 584456 and PI 365956 showed the smallest with 11.1 and 11.6, respectively in chlorophyll content decreased (Table S2), indicating that PI 584456 and PI 365956 were somewhat drought tolerant. The accessions PI 645361 and PI 600906 were the greatest with a 22.6% decrease (Table S2), indicating that the two accessions were more drought-susceptible.

The inhibition index in SPAD chlorophyll content (SPAD\_II) had a large range of 31.1% and ranged from 35.4% to 66.4% (Fig 4 D; Supplementary Table S2), with a mean of 59.8%, an Std dev of 5.37%, Std Err of 0.65, and CV of 9.0% (Supplementary Table S2) indicating that there was a large difference and variable of 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% (Fig 4 E, Supplementary Table S2). The mean and standard deviation shown were 40.2% and 5.37%, respectively (Supplementary Table S4), showing significant differences in drought tolerance among the 69 tomato accessions. The largest SPAD\_II were observed in PI 365956 (64.6 %), PI 584456 (62.8%), and PI 390510 (53.8%), indicating that the four 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). Overview, 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 <16 in absolute SPAD chlorophyll content and <47% in SPAD chlorophyll inhibition index (II), decreasing < 18 cm in absolute plant height and <62% in plant height inhibition index (II).

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

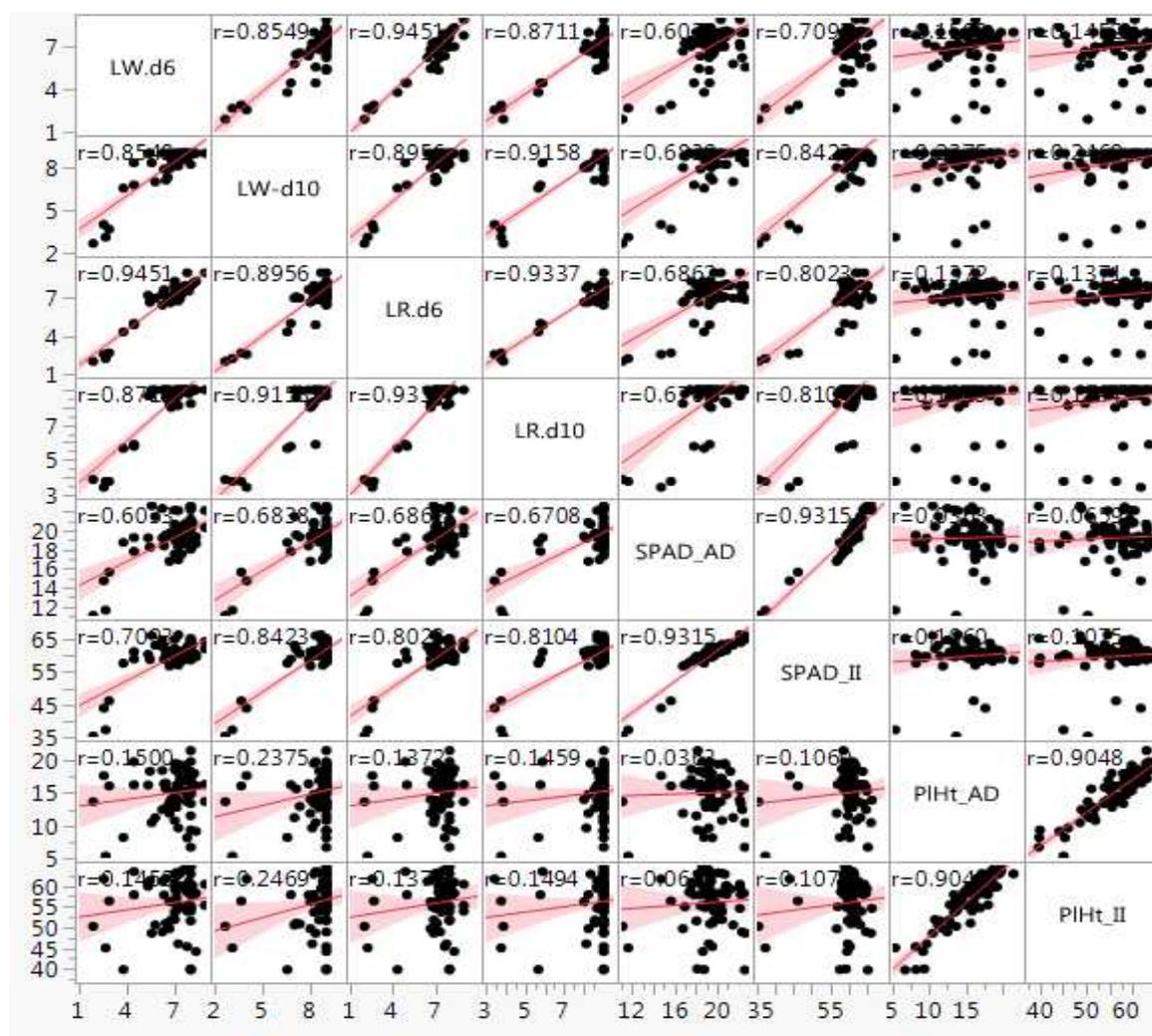
Accession	NA ME	TAXONOMY	ORIGIN	LW-d6	LW-d10	LR-d6	LR-d10	SPAD_AD	SPAD_II	PIHt_AD	PIHt_II
PI 365956	LA1373	Solanum peruvianum L.	Lima, Peru	1.9	2.7	2.0	3.9	11.1	35.4	13.7	50.3
PI 584456	Allure	Solanum lycopersicum L.	United States	2.7	3.1	2.2	3.8	11.6	37.2	5.3	45.1
PI 370091	Vision	Solanum lycopersicum L.	Canada	2.6	4.0	2.6	3.4	14.7	43.9	17.7	61.7
PI 390510	W-C 1050	Solanum lycopersicum var. cerasiforme (Alef.) Voss	Ecuador	2.9	3.7	2.7	3.8	15.6	46.2	16.1	56.4

### 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 (PIHt\_AD) and plant height inhibition index (PIHt\_II) with a high r-value, indicating high correlations.

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

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



**Figure 5.** Correlation coefficients (r-value) among the eight-drought tolerance related traits in 68 tomato accessions.

### 3.1.6. ANOVA and broad-sense heritability

Analysis of variance (ANOVA) for the parameters of drought tolerance and broad-sense heritability was estimated for all 14 traits (LW-d6, LW-d10, LR-d6, LR-d10, SPAD\_healthy, SPAD\_stress, SPAD\_AD, SPAD\_II, PIHt\_healthy, PIHt\_stress, PIHt\_AD, PIHt\_II, PIHt\_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 PIHt\_healthy, PIHt\_stress, PIHt\_AD, PIHt\_II, PIHt\_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 the traits.

The broad-sense heritability ( $H^2$ ) 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, PIHt\_healthy, PIHt\_stress, PIHt\_AD, PIHt\_II, PIHt\_RDT, SPAD\_healthy, SPAD\_stress, SPAD\_AD, SPAD\_II, and SPAD\_RDT, respectively (Table S4), showing that all the 14 traits had high heritability ( $H^2$ ) > 60% up to 94.1% except LW-d6 (52.2%), SPAD\_healthy (25.4%) and SPAD\_AD (48.5%) and implying that drought tolerance could be inherited.

### 3.1.7. Ranking of accessions

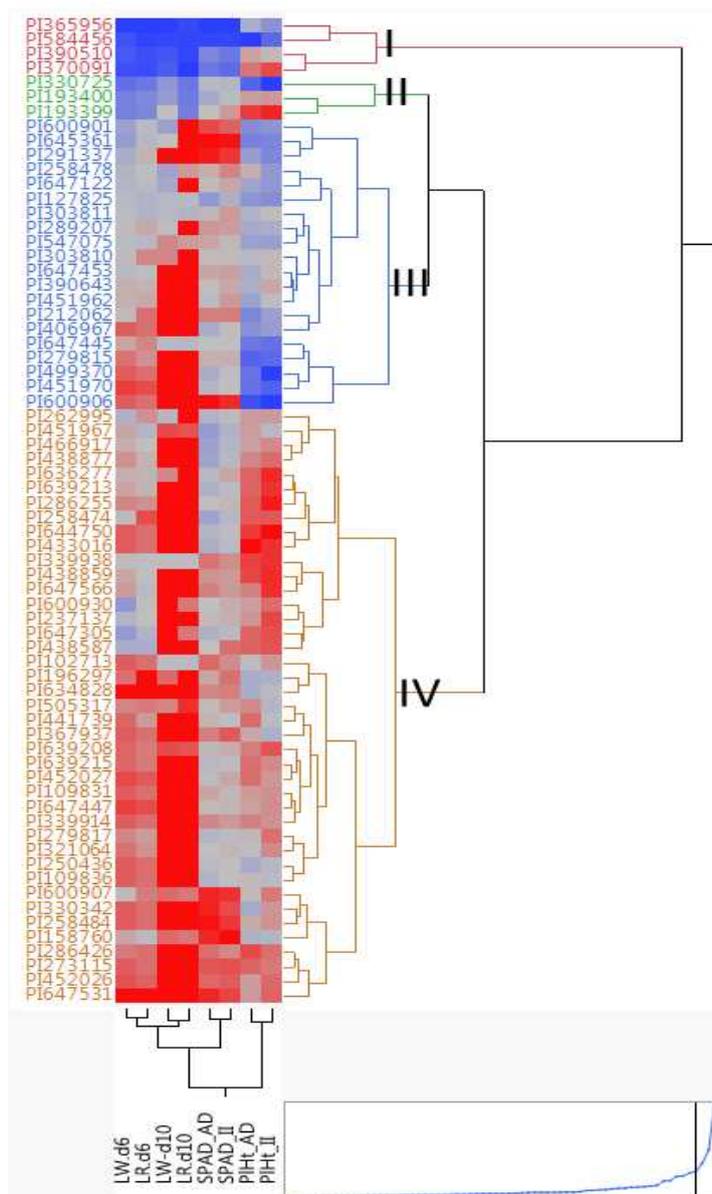
In this study, tomato accessions were ranked based on multiple parameters used for assessing drought tolerance using 1 to 68 for each of the 68 tomato accessions where 1 as the best drought

tolerance and 68 as the most susceptible. The values of PIHt\_healthy, PIHt\_stress, SPAD\_healthy, and SPAD\_stress in each tomato accessions 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 ten traits, LW-d6, LW-d10, LR-d6, LR-d10, PIHt\_AD, PIHt\_II, PIHt\_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  $PIHt\_RDT = 100 - PIHt\_II$ , PIHt\_RDT had the same ranking order as PIHt\_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, PIHt\_AD, PIHt\_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 (Table 3 & S3, Fig 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 tolerance ranked based on Rank (6), and each of 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 greatest drought tolerant from this study and they can be used in tomato breeding program as parents to develop drought-tolerant cultivars. Besides 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.

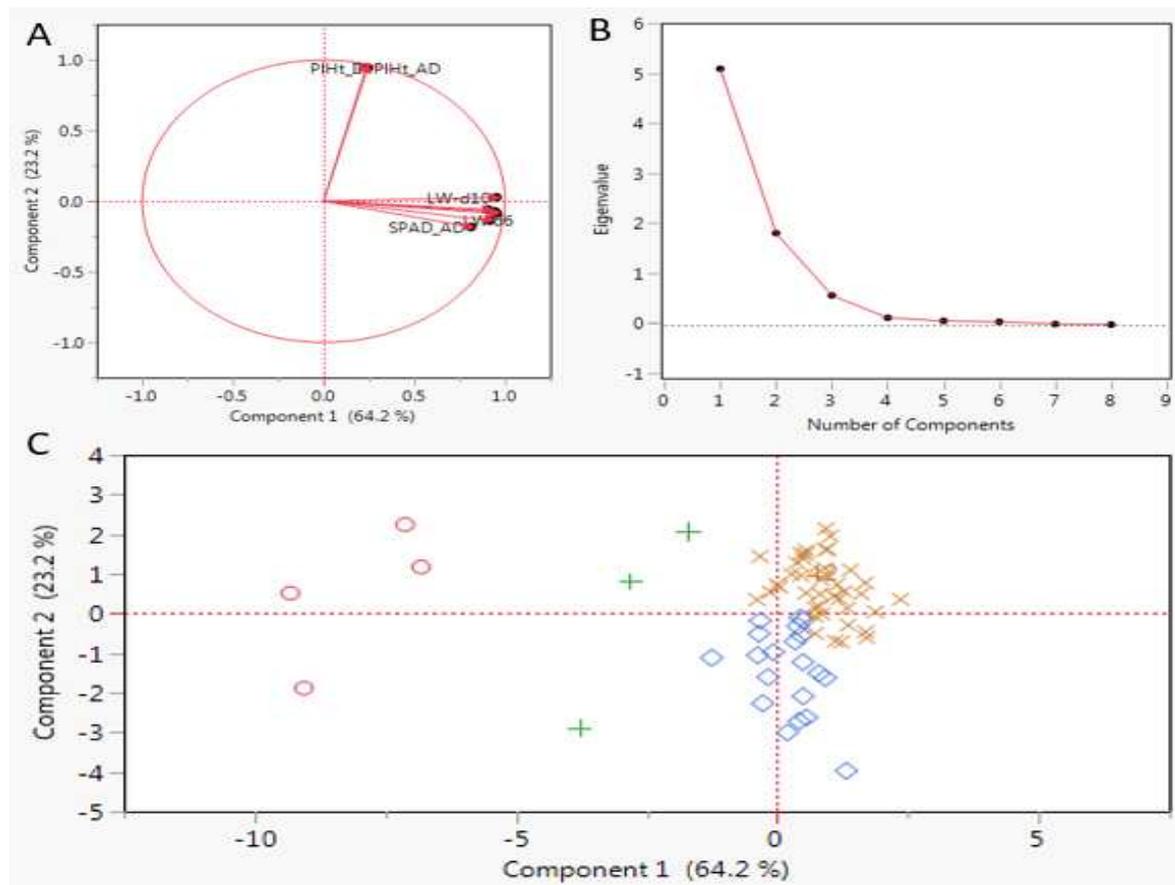
### 3.1.8. PCA and genetic diversity

The two-way phylogenetic tree were created for (1) among the 68 accessions and (2) for the eight traits, LW-d6, LW-d10, LR-d6, LR-d10, PIHt\_AD, PIHt\_II, SPAD\_AD, and SPAD\_II (Fig. 6). (1) For the 68 accessions, four clusters (groups) were formed (Fig. 6). The four accessions with top drought tolerance in Table 2 and S5, PI 365956, PI 584456, PI 370091, and PI 390510 were grouped into the same cluster I based on hierarchical clustering analysis (Fig. 6), indicating that they had similar drought tolerance. Two out of three accessions in cluster II, PI 330725, and PI 193400 (Fig. 6) were also tolerant to drought stress (Table S1) and ranked among the top 6 (Table S5), indicating they had similar responses to drought stress in mediate level. The tomato accessions in cluster III and IV are drought susceptible (Fig. 6), showing susceptible accessions were merged together. This study has indicated that the eight traits, LW-d6, LW-d10, LR-d6, LR-d10, PIHt\_AD, PIHt\_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: PIHt\_AD and PIHt\_II were clustered together as one group, and other six as another cluster, which 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 together, and LR-d6 and LR-d10 as a pair (Fig. 6 bottom), indicating that leaf wilting and leaf rolling had similar results for drought tolerance, close to the results of SPAD chlorophyll content, but little different from those of plant height related.

The biplot 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, PIHt\_AD and PIHt\_II demonstrated proximity to each other but were notably distinct from the mentioned variables (Fig. 7 A). This suggests a strong correlation among LW-d6, LW-d10, LR-d6, LR-d10, SPAD\_AD, and SPAD\_II, while PIHt\_AD and PIHt\_II showed a distinct pattern. The screen plot (Fig. 7 B) and PCA plot (Fig. 7 C) 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 eight 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 (PIHt\_AD), and plant height inhibition index (PIHt\_II), where the top four drought tolerant accessions were grouped into cluster I (top).



**Figure 7.** Principal component analysis (PCA) in 68 tomato accessions by JMP Genomics based on eight 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 (PIHt\_AD), and plant height inhibition index (PIHt\_II): (A) Biplot, (B) Screen plot, and (C) PCA with 4-clusters.

From the phylogenetic tree, among 65 tomato accessions which did not include the three accessions, PI 365956, PI 438587, and PI 600901 out of the 68 accessions in Table S1, 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 Q2 and other five to Q1 (Fig. 8), indicating that the six accessions have different genetic base and PI 584456 is more different from others, and suggesting how to select these valuable drought tolerance resources as parents in tomato breeding.

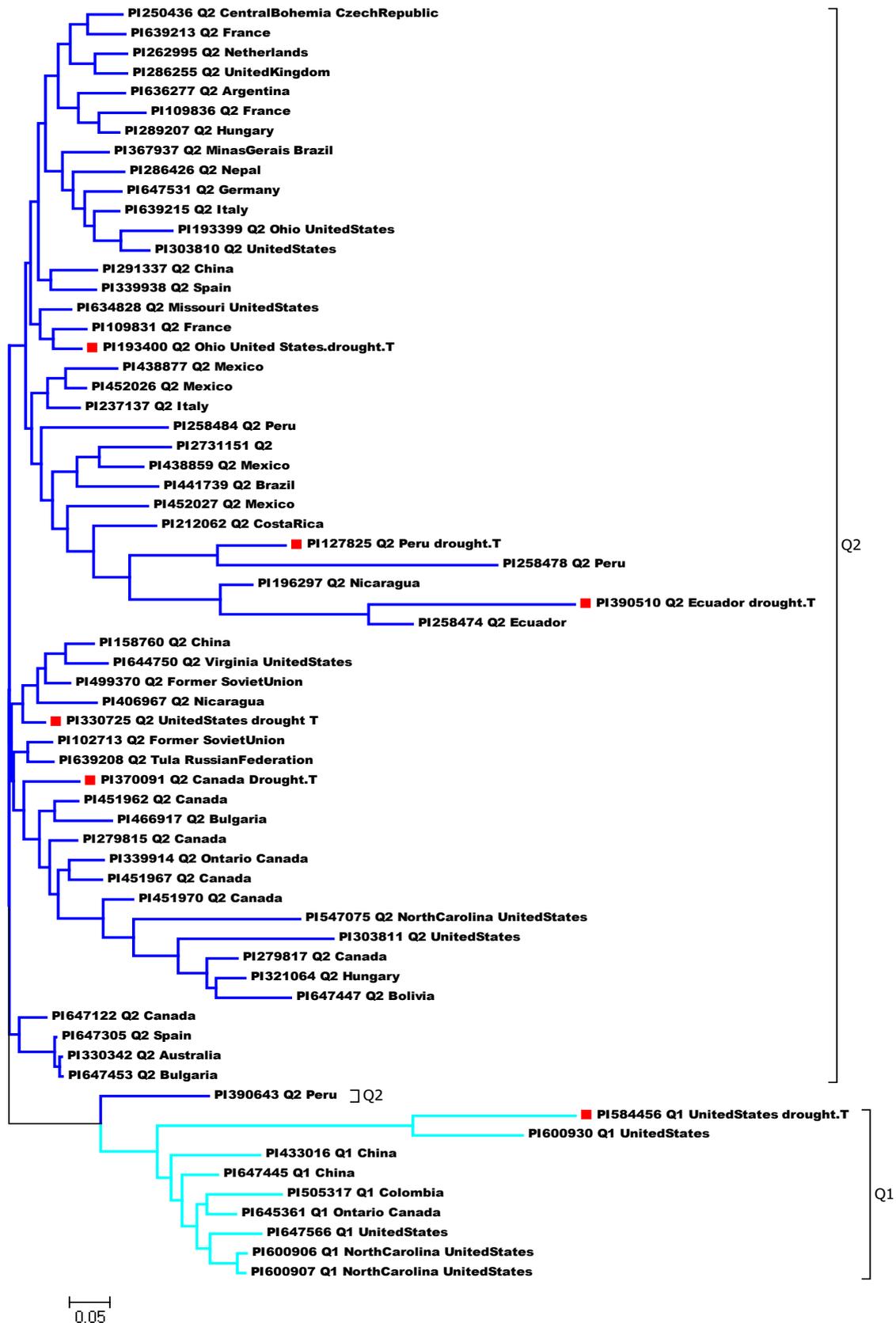


Figure 8. Phylogenetic tree created by MEGA 11 based on 5,003 single nucleotide polymorphism (SNP) markers distributed on 12 chromosomes in 65 USDA GRIN tomato accessions.

## 4. Discussion

Drought tolerance of 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 (Abdellatif et al., 2023). Finding genetic variation and developing high 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. Abdellatif et al. (2023), Cardoso et al. (2022), Pathan et al. (2014), and Zhou et al. (2020) conducted a similar experiment. 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-yielding 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 (Kadioglu et al., 2012; Kadioglu & Terzi, 2007; Baret et al., 2018). Leaf rolling is a significant indicator of drought tolerance in plants, as described by Baret et al. (2018), Chandra & Dubey, (2009), G. Zhang et al. (2021), and Merrium et al. (2022). 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 (Baret et al., 2018; Yang et al., 2022). Baret et al. (2018) 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. (2022) 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. (2021) 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, respectively, and the leaf surface). 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 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 (Farooq et al., 2009). Ahmadikhah & Marufinia, (2016) observed a reduced plant height in rice cultivars exposed to water deficit conditions. Another recent study by (Su et al., 2019) who evaluated drought stress tolerance in maize genotypes showed that even drought-tolerant genotypes of maize had lower plant height. 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 (Ribeiro et al., 2019). 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 (Zhang et al., 2018), the mechanism through which plants transform light into energy (Johnson, 2016). Chlorophyll, a green pigment (Ebrahimi et al., 2023), is essential for photosynthesis (Monteoliva et al., 2021) 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 (Cui et al., 2020; Hu et al., 2023; Mafakheri et al., 2010; Ravelombola et al., 2020). Leaf chlorophyll content is shown to increase during an early stage of water stress and decrease gradually with increasing periods of drought (Abdelhaleim et al., 2022). 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. (2019) and Cardoso et al. (2022) 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. (2021) indicates that those plants exhibiting 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 Correlations Analysis

This study revealed robust positive correlations among leaf wilting, leaf rolling, and SPAD chlorophyll content parameters (Supplementary Table S3). O'Toole & Moya, (1978) established a strong association between leaf rolling, leaf tip drying, and the preservation of leaf water potential. Baret et al. (2018) 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) 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 & Marufinia, (2016) 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

This study aimed to identify drought-tolerant tomato accessions to improve crop yields in drought-prone regions. Using a rapid screening method, we classified 68 USDA tomato accessions into three groups: drought-tolerant, moderately drought-tolerant, and drought-sensitive. Notably, PI 365956 emerged as the top-performing drought-tolerant accession, followed by PI 584456, PI 370091, and PI 390510, which also showed high tolerance. The identification of these highly drought-tolerant tomato accessions suggests the possible presence of genes associated with drought tolerance, as described by Egea et al. (2018), Pessoa et al. (2023b), and Wang et al. (2015). Exploring these genetic resources in molecular and physiological studies could provide valuable insights into mechanisms that enhance yield in water-scarce environments.

**Author Contributions:** KEC, GB, HX, and TMP conducted a phenotypic evaluation of drought tolerance in tomato accessions. AS performed the DNA sequencing and provided the SNP data. KEC, HX, GB, and AS performed phenotypic and genotypic data analysis. KEC, GB, HX, and AS are the Project Investigators. TMP, YC, IA, RD, and NJ collaborated in the study. KEC, GB, and HX wrote the draft of the manuscript. AS, GB, HX, and RM revised the manuscript. All authors reviewed and approved the manuscript.

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**Data availability statement:** The original information presented in the study is available in the article/Supplementary Material.

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