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Uncovering tomato candidate genes associated with drought tolerance using *Solanum pennellii* introgression lines

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Abstract

Tomato plants are sensitive to drought stress throughout their growth cycle. To be considered drought-tolerant, a cultivar should display tolerance at all developmental stages. This study aimed to evaluate whether *Solanum pennellii* introgression lines (ILs) previously selected as drought-tolerant during germination/seedling growth maintained this tolerance in the vegetative/reproductive stage. We then investigated these ILs to uncover candidate genes. The plants were subjected to two different environmental conditions: well-watered and drought-stressed (water withheld for $\leq$ 20 d after flowering). Phenotyping for morphological, physiological, fruit quality, and yield-related traits was performed, and the data was analyzed using a mixed-model approach. Using a multi-trait index that relies on factor analysis and genotype-ideotype distance (FAI-BLUP index), the genotypes were ordered based on how far they were from the drought-tolerant ideotype. Afterward, the tomato IL population map furnished by the SOL Genomics Network was utilized to identify introgressed segments of significance for the identification of candidate genes. Significant genotypic differences were found in the yield, water content, mean weight, length, and width of the fruit, the percentage of fruits displaying blossom-end rot, and titratable acidity. The drought-tolerance ideotype was built considering the maximum values for the fruit water content, number of fruits, mean fruit weight, and yield, minimum values for blossom-end rot, and mean values for titratable acidity. IL 1-4-18, IL 7-4-1, IL 7–1, IL 7-5-5, and IL 1–2 were ranked above M-82 and therefore considered drought-tolerant during the vegetative/reproductive stage. IL 1-4-18 and IL1-2 sustained drought tolerance displayed during germination/seedling growth into the vegetative/reproductive stage. The following candidate genes associated with drought tolerance were identified: *AHG2*, *At1g55840*, *PRXIIF*, *SAP5*, *REF4-RELATED 1*, *PRXQ*, *CFS1*, *LCD*, *CCD1*, and *SCS*. Because they are already associated with genetic markers, they can be transferred to elite tomato cultivars through marker-assisted technology after validation.

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Introduction

Solanum lycopersicum L., the domesticated tomato, is a popular vegetable crop produced and consumed worldwide and is one of the world's most economically important vegetables [1]. Tomato plants are sensitive to drought stress from seed germination to harvest [2] demanding a high water supply throughout the growing cycle [3, 4]. As in other crops, even short periods of drought might cause tomato growers qualitative and quantitative losses in fruit production [5, 6]. Overall, water shortages can adversely affect tomato plant growth as well as physiological traits, and yield [5–7]. Therefore, anticipating farmer needs, the development of tomatoes possessing drought tolerance has gained attention in tomato breeding programs [8–10].

One of the challenges delaying the release of drought-tolerant cultivars is that this trait seems to be a stage-specific phenomenon [11]. Nevertheless, an ideal drought-tolerant genotype must display drought resistance at all developmental stages of plant growth [12]. Therefore, specific stages throughout the ontogeny of the plant must be evaluated separately to assess drought tolerance and to identify its genetic components [13]. Also, drought tolerance seems to be controlled by many genes with different effects and is highly influenced by environmental variation [14, 15]. Since environmental conditions such as rainfall, temperature, salinity, and nutrient availability cannot be fully controlled [16], identifying candidate genes associated with drought tolerance, and implementing marker assisted selection can increase the precision of the selection of promising drought-tolerant genotypes.

In terms of drought tolerance, the primary genetic variation sources in tomatoes are the wild green-fruited relatives *Solanum chilense* L. and *Solanum pennellii* Corr [13, 17, 18]. However, several crossing barriers with cultivated tomatoes make it impractical to use *S. chilense* in breeding programs [19]. Unlike *S. chilense*, *S. pennellii* is a crossable wild relative, but direct crosses with cultivated tomatoes can result in the undesired linkage drag of traits associated with fruit quality and yield [12].

A viable way to overcome this problem is through the exploitation of the *S. pennellii* genome available with a background of domesticated tomatoes in the collection of introgression lines (ILs) [20]. In this collection, defined genomic segments of *S. pennellii* have replaced homologous regions in the commercial cultivar M-82, providing complete coverage of the wild species genome. ILs are a valuable genetic resource for studying drought tolerance and identifying the genomic regions associated with it because any phenotypic differences found between an IL and the recurrent parent M-82 when subjected to drought conditions can be solely attributed to the introgressed chromosomal segment of the drought-tolerant *S. pennellii* [21, 22].

To aid and accelerate the development of drought-tolerant cultivars, we evaluated *S. pennellii* ILs that were previously selected based on their level of drought tolerance during their germination and seedling stages [23]. ILs were exposed to drought by withholding water during the vegetative and reproductive stages and were ranked according to their drought tolerance. Our main goal was to provide information on whether the previously selected drought-resistant genotypes can sustain their tolerance when drought stress is applied in the vegetative and reproductive stages and should, therefore, be incorporated in the next phases of tomato breeding programs. Moreover, the introgressed *S. pennellii* fragments of the genotypes selected as drought-tolerant were analyzed and candidate genes associated with this trait in the vegetative and reproductive stages were identified.

Materials and methods

Plant materials and greenhouse experiments

To evaluate the response of genotypes to drought stress two experiments with similar conditions, except for irrigation (environmental condition), were conducted simultaneously. On

environmental condition 1, plants were kept in non-stressed conditions throughout the growing season, with the soil water content maintained at field capacity. On environmental condition 2 plants were challenged with drought by withholding water for up to 20 d after flowering [24].

Plant materials were chosen based on a previous study [23] that evaluated drought tolerance of ILs from the *S. pennellii* population previously in developed [20] during germination and seedling stages. The five ILs previously ranked as the most drought-tolerant (IL 1-4-18, IL 2–3, IL 1–2, IL 9–2, and IL 10–1) and the five ranked as the most drought-sensitive (IL 8–3, IL 7-4-1, IL 7-5-5, IL 9–3, and IL 7–1) together with one of their parents, the commercial variety M-82, were evaluated in this study (Table 1).

The greenhouse experiment was conducted in the Research and Extension Farm Unit *Horta Velha* at the Universidade Federal de Viçosa, Viçosa, MG, Brazil (20˚ 45′ 14″ S; 42˚ 52′ 53″ W; 648.74 m altitude) from July to December 2019. Temperature (T, ˚C) and relative air humidity (RH, %) inside the greenhouse were monitored daily using the weather station E4000 (Irriplus ® Scientific Equipment).

Seeds were sown in polystyrene trays of 128 cells each, filled with Topstrato substrate (Vida Verde, BRA). Plants at the three to four true leaf stage were transplanted into 15-L pots (one plant per pot) containing a mixture of soil, sand, and composted cow manure (3:1:1). The soil texture was classified as sandy clay (sand, 52.9%; silt, 4.5%; clay, 42.6%). Fertilization and fungicide/insecticide applications were based on crop recommendations. The plants were tied to bamboo stakes placed inside each pot to prevent falling.

Each experiment was arranged in a randomized block design, with three replicates. Each replicate consisted of three plants arranged side-by-side (S1 Fig).

Irrigation management and calculation

Tomato plants are more sensitive to drought during flowering and fruit set, [25, 26], therefore, this was the stage when drought conditions were imposed on the plants in environmental condition 2.

Under environmental condition 1, the soil water content was maintained at 100% available soil water (ASW) during the entire growing season. Under environmental condition 2, after the flowering stage, plants were subjected to drought stress by withholding water until the soil matric potential approached the wilting point ~ 0% ASW (~ −1500 kPa).

Table 1. List of genotypes evaluated.

Drought tolerance at germination and seedling stages*	Genotype	Introgression line
Drought tolerant	1	IL 1-4-18
	2	IL 2–3
	3	IL 1–2
	4	IL 9–2
	5	IL 10–1
Drought sensitive	6	IL 8–3
	7	IL 7-4-1
	8	IL 7-5-5
	9	IL 9–3
	10	IL 7–1
Parent	**11**	**cv. M-82**

*Drought tolerance and/or sensitivity were determined in a previous study [23].

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Irrigation management and calculations followed the methodology described by Dariva [27]. A soil sample was taken to estimate the soil water retention curve parameters based on the Van Genuchten equation [28] using SWRC Fit software [29]. To estimate the water tension value corresponding to 100% ASW, we assumed that field capacity was reached at a soil matric potential of −33 kPa [30]. To estimate when to stop water deprivation under environmental condition 2, we assumed that the wilting point would be reached at a soil matric potential of −1500 kPa [30]. Therefore, the plants were re-watered when the soil matric potential was estimated to be approximately −1490 kPa. After the drought stress period, the plants under both environmental conditions were kept well-watered until the end of the growing season.

Before filling them with soil, all pots were weighed to determine their weight (W_{pot}). In addition, the bamboo stakes used in each pot were weighed to determine stake weight (W_{stake}). Subsequently, all pots were filled with the same dry soil weight (W_{ds}). The water weight (W_{water}) of the first irrigation was determined by multiplying W_{ds} by the soil water content (kg/kg) at a soil water potential of −33 kPa.

The soil water content was monitored daily by weighing each pot. For the daily irrigations, the applied W_{water} was determined as total pot weight ($W_{totalpot}$) minus the pot weight measured on that day. The total pot weight ($W_{totalpot}$) was calculated as follows:

$$Wtotalpot = Wpot + Wds + Wwater + Wplant + Wstake \quad (1)$$

where Wpot is the pot weight, Wds is the dry soil weight, Wwater is the water weight, Wplant is the plant weight, and Wstake is the bamboo stake weight. Wplant was determined by weighing same-aged spare plants grown within the experiment. One spare plant was harvested and weighed every 10 d to adjust Wplant value.

Phenotyping

Physiological and morphological traits. Leaf water potential (LWP) was measured predawn (3:00–5:00) at the end of the stress period using a pressure chamber (model 3000; Soil Moisture, Santa Bárbara, CA, USA), according to the method described by [31]. LWP measurements were performed on young fully expanded leaves, the terminal leaflet and the one right bellow it, were used to take the measurements. The LWP value for each repetition was the mean value of two leaflets.

The leaf relative water content (LWC) was determined at the end of the drought stress period for three leaf samples taken from the top most fully expanded leaves. LWC was calculated according to [32] using the following formula:

$$LWC = \frac{LFW - LDW}{LSW - LDW} \quad (2)$$

where LFW is the fresh weight, LDW is the dry weight obtained after oven-drying for 48 h at 80˚C, and LSW is the saturation weight determined after 24 h of re-saturation in tap water.

Shoot dry matter (SDM) was determined for the whole shoot (leaves and stems) at the end of the experiment by oven-drying for 48 h at 80˚C. Shoot water content (SWC) was determined for the whole shoot (leaves and stems) at the end of the experiment using the following formula [32]:

$$Shoot\ water\ content = \frac{SFW - SDW}{SSW - SDW} \quad (3)$$

where SFW is the fresh weight, SDW is the dry weight obtained after oven-drying for 48 h at 80˚C and SSW is the saturation weight determined after 24 h of re-saturation in tap water.

Fruit water content (FWC) was determined for a sample of three to four fully ripened fruits per plant picked randomly at the end of the experiment, according to the following formula [32]:

$$FWC = \frac{FFW - FDW}{FSW - FDW} \quad (4)$$

where FFW is the fresh weight, FDW is the dry weight obtained after oven-drying for 48 h at 80˚C and FSW is the saturation weight determined after 24 h of re-saturation in tap water.

Yield. Tomato fruits were harvested when fully ripe during the course of the experiment. Fruits were considered fully ripe when they achieved stage red, meaning that more than 90% of the tomato surface, in the aggregate, was red. The fresh weight of all fruits from each plant was measured. The yield parameters consisted of total plant yield (Kg plant^{-1}), mean fruit weight (MFW, g), and number of fruits per plant (N_fruits).

Fruit size attributes. The visual attributes—fruit length (FL) and width (FW)—of all harvested fruits were measured using a digital caliper (Louisware® Stainless Steel Caliper 150mm/0-6 inch). Each fruit was visually evaluated to assess the presence of blossom-end rot (BER), scars, and cracking, and the results were expressed in terms of the percentage of fruits with defects.

Fruit quality attributes. Organoleptic quality measurements were performed randomly on four fully ripened fruits per plant. All four fruits per plant were homogeneous in size and color and were harvested on the same day. Fruit quality assessment began immediately after harvest.

Fruit firmness, described as the mean maximum penetration force required for pericarp rupture and expressed in Newtons (N), was measured using a digital penetrometer (model PDF-200, Soilcontrol, USA) fitted with a cylindrical stainless-steel probe with a round tip (Ø 8 mm). Two measurements, located 180º apart from one another, were taken in the equatorial region of each fruit.

After firmness measurement, the four selected fruits were macerated together in a blender to produce tomato juice, which was used to determine the total acidity (pH), total soluble solids (TSS), and titratable acidity (TA). The pH values of the juice samples were determined immediately using a benchtop pH meter (model pH 21, Hanna Instruments, Italy). TSS, expressed as ˚Brix, was determined using a digital refractometer (model HI 96801, Hanna Instruments, Italy). For TA measurements, samples of approximately 5 g of tomato juice were transferred to 100-mL volumetric flasks, which were then filled to capacity with distilled water. An aliquot of 10 mL of this solution was then titrated with a NaOH solution (0.005 mol L^{-1}) using 1% phenolphthalein as an indicator. TA values were expressed as % citric acid and were obtained using the following formula:

$$TA = \frac{[A \times B \times C \times D \times 100]}{grams\ of\ juice\ sample} \quad (5)$$

Where: A = (*mL NaOH*); B = (0.005 *N NaOH*); C = (0.064 *mEq acid citric factor*); D = (*correction – factor*).

Finally, the TSS/TA ratio was calculated as an indicator of flavor, as described by [33].

Statistical analyses

A mixed-model methodology was adopted for statistical analyses via REML/BLUP (restricted residual maximum likelihood/best linear unbiased prediction) [34, 35], using R software and the Sommer package.

The statistical model was denoted by:

$$y = X\tau + Z_e\mu_e + Z_g\mu_g + Z_i\mu_i + e \quad (6)$$

where Y is the phenotypic data vector; τ is the fixed effects vector (overall mean and blocks); μ_g is the genotype effects vector (random), with $\mu_g \sim N\left(0, \sigma_g^2\right)$; μ_e is the environment effects vector (random), with $\mu_e \sim N\left(0, \sigma_e^2\right)$, μ_i is the genotype × experiment interaction (random), with $\mu_i \sim N\left(0, \sigma_i^2\right)$; e is the residual effects vector (random), with $e \sim N(0,R)$; and X, Z_g, Z_e and Z_i are incidence matrices for τ, μ_g, μ_e, and μ_i, respectively. R is a diagonal matrix with residual variances, that is, $R = \overset{i=1}{\overset{t}{\otimes}} \sigma_{e_i}^2$ where $\sigma_{e_i}^2$ is the residual variance in experiment i and t is the number of environmental conditions (in this case = 2).

For the random effects of the model, the significance of the likelihood ratio test was assessed using the chi-square statistic with one degree of freedom. Genetic values (BLUP means) were predicted for each of the 11 genotypes based on the 37 evaluated traits and two environmental conditions. For traits where significant genotype effects were detected, the BLUP means were used to estimate Pearson's linear correlation coefficients.

Single-trait and multi-trait IL ranking. The genetic values (BLUP means) of traits that showed significant genotype effects ($P < 0.05$) were submitted to single-trait ranking, according to the designed ideotype: maximum values for FWC, N_Fruit, MFW, and yield; minimum values for BER; and mean values for TA. For each trait, the percentage difference of each IL was calculated relative to the commercial cultivar M-82. Subsequently, a multi-trait index based on factor analysis and genotype–ideotype design, known as the FAI-BLUP index, was used to rank the genotypes. Principal component analysis, factor analysis, ideotype determination, and genotype–ideotype distance calculation were performed in R software using the FAI-BLUP index routine developed by Rocha [36].

Principal component analysis was used to extract the genetic correlation matrix factorial loads obtained by the genetic values. The varimax criterion [37] was used for analytic rotation, and the weighted least squares method [38] was used to calculate the factor scores. The number of ideotypes was defined based on a combination of desirable and undesirable factors for the selection objective. The following algorithm provides the number of ideotypes:

$$NI = 2^n \quad (7)$$

in which, *NI* is the number of ideotypes and n is the number of factors.

After ideotype determination, genotype–ideotype distances were estimated and converted into spatial probabilities, enabling genotype ranking. The following algorithm was used.

$$P_{ij} = \frac{\frac{1}{d_{ij}}}{\sum_{i=1;j=1}^{i=n;j=m} \frac{1}{d_{ij}}} \quad (8)$$

where P_{ij} is the probability that the i^{th} genotype (i = 1, 2,. . ., n) is similar to the j^{th} ideotype (j = 1, 2,. . ., m) and d_{ij} is the genotype–ideotype distance from the i^{th} genotype to the j^{th} ideotype, based on the standardized mean Euclidean distance.

Candidate gene identification

The genomic segments of *S. pennellii* introgressed into the ILs selected by the FAI-BLUP index were further analyzed to identify candidate genes for drought tolerance, adapting the methodology described previously [39]. The tomato IL population map provided in [40] was utilized,

which delineates individual chromosomes with restriction sites for introgressed segments and all identified marker genes.

To automatically identify all marker genes from each chosen IL in the HTML code, we modified the script created by Toubiana [39]. We then utilized information on the corresponding orthologs in the *Arabidopsis thaliana* genome [41] to infer the functionality of each gene that was identified. The identification of candidate genes for drought tolerance was based on the relevance of their functionality to this process.

Results

Analysis of deviance

The effect of environment was significant for LWP (p-value > 0.05), meaning that the mean LWP of the two environmental conditions were different. Fig 1 shows a boxplot of LWP under environmental conditions 1 and 2. As expected, the values observed for environmental condition 1, in which the plants were well-watered throughout the growing cycle, were higher than those observed for environmental condition 2, in which the plants were subjected to drought stress by water deprivation. Even the outliers observed in environmental conditions 1 and 2 follow a similar pattern, in environmental condition 1 genotypes displayed higher LWP than in environmental condition 2.

Among the 18 evaluated traits, significant genotypic differences were found for five yield and fruit quality attributes: FWC, MFW, FL, FW, BER, and TA. The effect of the interaction between genotype and environmental conditions was not significant for any of the evaluated traits.

Pearson's correlation

Our study on the associations between the yield and fruit quality attributes revealed 19 significant linear correlations, of which 13 were positive and six negative ($p < 0.05$, t-test) (Fig 2).

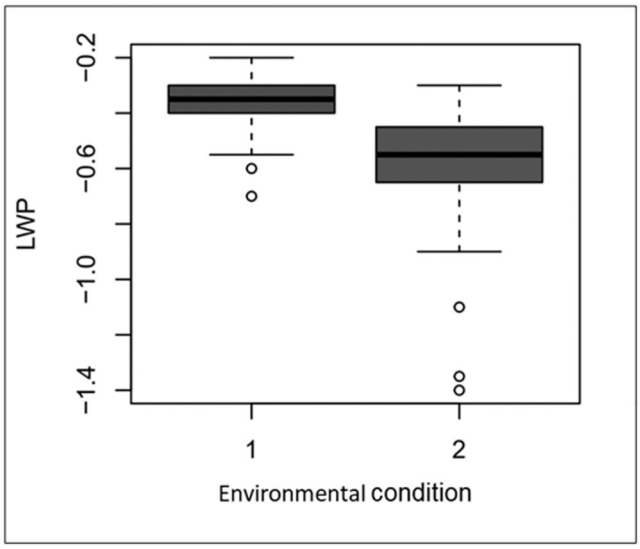


Fig 1. Boxplot of tomato ILs leaf water potential (LWP) in the two environmental conditions. Environmental condition 1 = non-stressed plants, environmental condition 2 = plants subjected to drought stress. Circles indicate outliers in the dataset.

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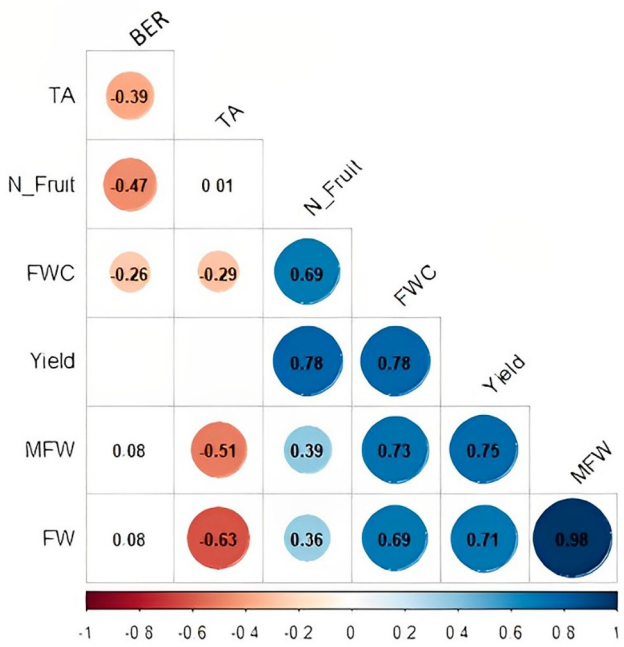


Fig 2. Pearson's correlation matrix of BLUP means for all traits showing significant genotypic effects. Correlations were validated by the t-test at 0.05 significance level, and blank squares mean that the correlation was not significant ($p > 0.05$). BER = blossom-end rot, FW = fruit width, FWC = fruit water content, MFW = mean fruit weight, N_fruits = mean number of fruits per plant, TA = titratable acidity, Yield = total yield. Colors are related to the correlation values. The darker the red the closer to -1 and the daker the blue the closer to 1.

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Overall, the yield attributes were positively correlated. As MFW displayed a strong positive correlation with FW (r = 0.98), we decided to retain only one of them for further analysis and removed FW. The other correlations among the traits were only moderate ($r < 0.7$); hence, all the other traits were retained. Yield showed a moderate positive correlation with N_ fruit (r = 0.78), FWC (r = 0.78), and MFW (r = 0.75).

Single-trait and multi-trait ranking of ILs subjected to drought stress

Fig 3 displays the genotype ranking according to the ideotype for each trait. The ideotype was designed considering maximum values for FWC, N_Fruit, MFW, and yield, minimum values for BER, and mean values for TA. The chromosomal fragments of *S. pennellii* introgressed into cv. M-82 promoted changes in all the ILs for all traits. IL7-1 stood out in terms of yield-related traits. Compared with its parent, cv. M-82, there was an impressive increase in mean fruit weight (68.83%) and total yield (32.24%). This IL also had the highest increase in fruit number. IL 7-4-1 was among the top five genotypes for all traits, and the only trait that was not improved by the *S. pennellii* introgression was BER. When analyzing all the traits together, it is evident that an introgressed region can increase some traits while decreasing others. In addition, a genotype ranked first for one trait could be ranked last for others. For example, the introgression of *S. pennellii* promoted an increase of 49.14% in the MFW of IL 1-4-18; this IL was also ranked second for FW and penultimate for BER.

Fig 4 presents the ranking of the 11 evaluated genotypes according to the multi-trait index FAI-BLUP, and the probability of good drought tolerance in relation to the distance to the ideotype. This method ranked the genotypes according to the proposed ideotype (maximum

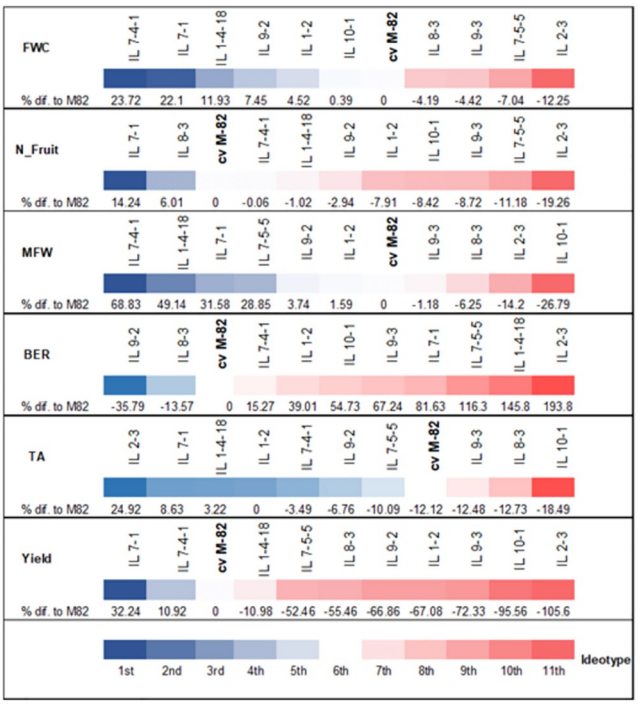


Fig 3. Ranking of genotypes according to the ideotype for each trait and the percentage difference (increase or decrease) in relation to cv. M-82. The genotypes closest to the ideotype are on the left. The colored gradient indicates the intensity of difference in relation to M-82: the deeper the blue and red colors, the more substantial the changes promoted by the S. pennnellii genome away from the ideotype. BER = blossom-end rot, FWC = fruit water content, MFW = mean fruit weight, N_fruits = mean number of fruits per plant, TA = titratable acidity, Yield = total yield.

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values for FWC, N_Fruit, MFW, and yield; minimum values for BER; and mean values for TA), considering all the parameters simultaneously. The cultivar M-82 was ranked the sixth closest to the drought-tolerant ideotype. Therefore, the five ILs ranked above it were selected as drought-tolerant and investigated for the presence of candidate genes for this trait. Among

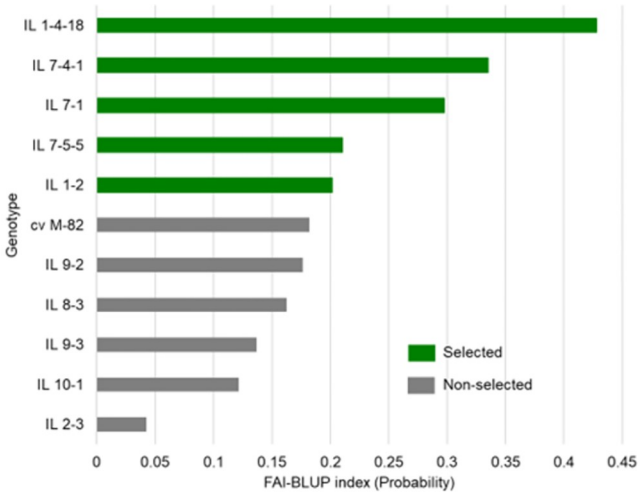


Fig 4. Genotype ranking and selected genotypes using the FAI-BLUP index.

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the five ILs selected as drought-tolerant, IL 1-4-18 and IL-1-2 were previously determined to be drought-tolerant in the germination and seedling stages. In addition, the most drought-tolerant plants at the germination and seedling stages (IL 1-4-18) were also the most drought-tolerant during the vegetative and reproductive stages.

Candidate genes associated with drought tolerance

All the genotypes ranked above M-82 (IL 1-4-18, IL 7-4-1, IL 7–1, IL 7-5-5, and IL 1–2) were further investigated for candidate genes associated with drought tolerance. Gene markers associated with the introgressed segments of the selected ILs were identified using [40]. Their functionality was inferred using information on their respective orthologs in the *A*. *thaliana* genome. Fig 5 provides a summary of all the candidate genes discovered.

In IL 1-4-18, the gene *SCS* was identified. It encodes SnRK2, a protein involved in plant response to water deprivation [42]. In IL 7-4-1, *PRXQ* and *CFS1* were identified. *PRXQ* encodes peroxiredoxin Q, which decomposes peroxides and is therefore involved in the

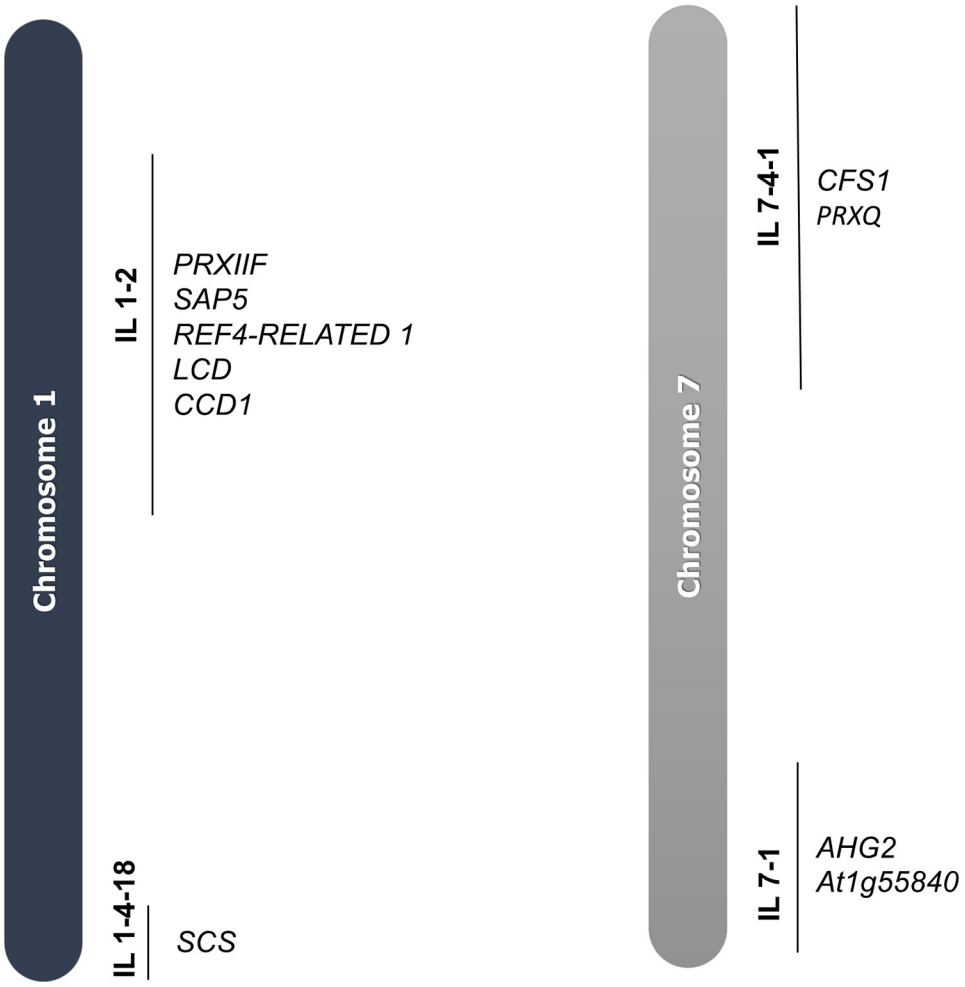


Fig 5. Candidate genes associated with drought tolerance in the selected ILs.

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cellular response to oxidative stress [43]. *CFS1* encodes a RING-type zinc-finger family protein involved in the response to ABA [44].

In IL 7–1, the genes *At1g55840* and *AHG2* were identified. *At1g55840* encodes a Sec14p-like phosphatidylinositol transfer family protein that is involved in the defense response to abiotic stress [45]. *AHG2* encodes a poly(A)-specific ribonuclease AtPARN. The expression of AtPARN is upregulated by ABA or stress treatment, acting upstream of or within the response to ABA and osmotic stress [46]. No genes related to drought tolerance were found in the selected IL 7-5-5.

In IL 1–2, the genes *PRXIIF*, *SAP5*, *REF4-RELATED 1*, *LCD*, and *CCD1* were identified as candidates. *PRXIIF* encodes a mitochondrial matrix localized peroxiredoxin involved in redox homeostasis, which is involved in oxidative stress response [47]. *SAP5* encodes a protein with E3 ligase activity that positively regulates stress responses in Arabidopsis and responds to water deprivation [48]. *REF4-RELATED 1*, encodes a protein shown to physically associate with the conserved transcriptional coregulatory complex, mediator, and is involved in the regulation of phenylpropanoid homeostasis. It regulates the phenylpropanoid metabolic process related to plant response to abiotic stress [49]. *LCD* encodes an enzyme that decomposes L-cysteine into pyruvate, H2S, and NH3, which is involved in plant response to drought [50]. Finally, *CCD1* encodes 9-cis-epoxycarotenoid dioxygenase, which responds to water deprivation [51].

Discussion

An ideal drought-tolerant genotype must display drought tolerance at all developmental stages of plant growth [12]. Therefore, specific stages throughout the ontogeny of the plant must be evaluated separately [13]. Here, we selected genotypes previously screened and ranked for drought tolerance during seed germination and seedling growth and evaluated them during the vegetative and reproductive stages under two water regimes (100% ASW and drought exposure imposed by water withholding for up to 20 d).

The significant difference among the environments observed for LWP ($p < 0,05$) highlights that the adopted methodology was efficient in promoting drought stress in the plants from which water was withheld. Even the outliers displayed a much lower LWP when submitted to drought stress. Despite the wide range of methodologies proposed to induce drought stress in tomatoes [13, 27, 52, 53], multiple studies, from the pioneering work of Wudiri [25] to the recent experiments of Cui [26], suggest that the flowering and fruit-setting stage is when tomato plants are the most sensitive to drought. Therefore, promoting severe water deprivation (soil almost reaches wilting point) during this stage, as we did in our study, proved to be an accurate method of screening and selecting plants exhibiting drought tolerance.

As expected, the plants from environmental condition 2, which were exposed to drought, showed lower LWP than those from environmental condition 1. Predawn LWP usually corresponds to a daily maximum or base water potential, which is presumed to correspond to the equilibrium between the soil and plant water potentials [54]. It is believed that predawn plant water potential is in equilibrium with the "wettest" soil accessed by the roots [55, 56]. Therefore, the predawn LWP was used as a surrogate for the soil water potential. After irrigation suspension in environmental condition 2, evapotranspiration consumed the water available in the soil over the entire period of drought stress. By the end of the period, the soil reached a much lower water potential than that in the pots that were watered daily.

We did not identify significant genotype × environment relationships for any of the evaluated traits. Like most herbaceous plants, 90% of the total fresh weight of tomato plants is water [57]. For tomato fruits, this percentage is even higher; water accounts for 93–95% of tomato

composition [15]. All chemical and biochemical cellular processes, including photosynthesis, take place within an aqueous media. Water deficit immediately stops cell division and enlargement and stem and leaf elongation, processes that require a minimum turgor pressure. A lack of water also inhibits photosynthesis, closes stomata, and decreases respiration rates and other enzyme-mediated processes [58]. Thus, it was expected that the genotypes under drought stress would show different responses for the evaluated traits than those under watered conditions. The LWP values confirmed that the plants in environmental condition 2 were under drought stress conditions. However, the drought period was short, considering the extent of the growth cycle, which was approximately five months. A hypothesis for the non-interaction between genotype and environment is that after drought stress suspension, the plants subjected to water deficit adjusted their physiological and biochemical machinery to adapt to these conditions. This suggests that they might be a valuable set of genetic resources for the further investigation of drought tolerance.

Genotypic differences ($p < 0,05$) were identified for only seven of the 18 evaluated traits. This can be explained by the fact that the 11 genotypes used as plant material were highly genetically related (10 ILs from the collection developed by [20] and their genitor, the commercial cultivar M-82). Therefore, it is likely that the genomic portions shared among the genotypes, rather than the introgressed fragments, are associated with the traits for which significant genotypic differences were not observed. In this study, the differences promoted by the introgressed fragments are the ones of interest; therefore, only the traits displaying genotypic differences were further analyzed.

The chromosomal fragments of *S. pennellii* introgressed into cv. M-82 promoted changes in fruit quality- and yield-related traits (FWC, MFW, FL, FW, yield, BER, and TA) of ILs. M-82 was not the first-ranking genotype for any of these, indicating that the genomic introgressions were favorable for some of the ILs. While *S. pennellii* produces small green fruits unlike cultivated varieties, certain wild alleles may exhibit counterintuitive effects compared to what one would expect when examining the phenotype of a crop's wild relative [12]. While examining the function of wild tomato alleles in a background of tomato cultivars through QTL analysis, were identified two ILs with larger fruit sizes than the cultivated parent, and QTL mapping in the IL population revealed that the allele leading to larger fruit size was, surprisingly, contributed by *S. pennellii* [20]. Moreover, pyramiding different genetic loci derived from *S. pennellii* into *S. lycopersicum* lines in a heterozygote state increased fruit yield by 30–50% under field conditions compared to an elite variety [59].

From the single-trait ranking, it is evident that an introgressed region can increase some traits while decreasing others. In addition, a genotype ranked first for one trait could be ranked last for others. Therefore, single-trait ranking is not helpful in selecting the genotypes best suited to drought conditions during the vegetative and reproductive stages, justifying the use of a multi-trait approach.

The FAI-BLUP index ranked five ILs above M-82, confirming that the *S. pennellii* genomic fragments in these ILs increased their drought tolerance. *S. pennellii* possesses several adaptive mechanisms that ensure its survival in arid environments, including morphophysiological and anatomical modifications in the aerial part of the plant, such as a cuticular composition associated with increased resistance to water flux, a smaller leaf surface area, and greater leaf thickness. Therefore, it was expected that the introgressed *S. pennellii* fragments in the ILs would carry genes that enhance drought tolerance. Moreover, IL 1-4-18 and IL-1-2, which were ranked as drought-tolerant in this study, were also ranked as drought-tolerant in their germination and seedling stages. Drought tolerance is a stage-specific phenomenon that is controlled by many genes with different effects [14, 15, 32]. Therefore, most probably, the genes controlling the drought tolerance of IL 1-4-18 and IL-1-2 during germination and seedling growth

were not the same as those controlling this trait in the stages evaluated in our study. Hence, the fragments of *S. pennellii* inherited by these ILs are a rich source of drought tolerance-related genes, with genes acting during seed germination and seedling growth, and genes expressed during the vegetative and reproductive stages. Our results suggest that IL 1-4-18 and IL-1-2 are promising genotypes for inclusion as genitors in breeding programs aimed at developing drought-tolerant tomato cultivars.

IL 1-4-18 was ranked the most drought-tolerant genotype and the gene *SCS*, which encodes SnRK2, was identified in this IL. SnRK2 was described as a crucial regulator of plant responses to abiotic stresses [60]. This protein acts as a positive central regulator of ABA signaling during water stress at the vegetative and reproductive stages [42]. Water deficit stress, such as drought, triggers various biochemical and physiological responses in plants, including alterations in gene expression and the accumulation of the phytohormone ABA [61]. ABA regulates diverse plant processes, including the adaptation of plants to water stress [62, 63]. Numerous drought stress-responsive genes have been reported, many of which are induced by ABA [64]. SnRK2s pathways regulate the plant response to ABA by the direct phosphorylation of various downstream targets, such as SLAC1, KAT1, AtRbohF, and transcription factors required to express numerous stress response genes [60]. Therefore, the gene discovered in our study might have played a central role in the response of IL 1-4-18 to water deprivation, resulting in drought tolerance.

In IL 7-4-1, the genes *PRXQ* and *CFS1* were identified. *CFS1* encodes a RING/FYVE/PHD-type protein. This group of proteins is nuclear ubiquitin E3 ligases. Although they do not seem to be directly involved in transcriptional regulation, they can interact with some transcription factors to regulate their transcriptional activities, resulting in improved abiotic and biotic stress tolerance in several species [44]. *PRXQ* encodes peroxiredoxin Q, which plays a role in the cellular response to oxidative stress caused by peroxide decomposition. Peroxiredoxins are part of the enzymatic ROS-scavenging system developed by plants to scavenge ROS generated during biotic stress events such as drought [65]. Peroxiredoxin Q, one of the four plant subtypes, is a chloroplast gene associated with responses to several abiotic stresses [43]. By scavenging peroxides generated under stress, peroxiredoxin Q protects the photosynthetic machinery, enabling photosynthesis to continue in non-optimal conditions. IL 7-4-1 was ranked as the second-most drought-tolerant genotype and showed outstanding behavior for all evaluated traits under drought conditions. *PRXQ* and *CFS1* may be key genes in the drought-tolerance strategy of this genotype.

In IL 7–1, the genes *At1g55840* and *AHG2* were identified. *At1g55840* encodes a Sec14p-like phosphatidylinositol transfer family protein. In soybeans, this protein is a component of a stress response pathway that serves to protect adult plants under osmotic stress [66]. Proteins with a Sec14p-like domain play a role in the lipid regulation of the Rho-mediated signaling pathway, which is involved in water stress tolerance in wild barley [67]. *AHG2* encodes AtPARN. Although the specific roles of this protein are not well-described, they were found to be expressed when plants were subjected to drought stress [46], and according to Covarrubias [68] they are involved in the post-transcriptional gene regulation of salinity and drought responses. These two genes are good candidates for explaining the drought tolerance of IL 7–1.

Although IL 7-5-5 was determined to be the fourth closest genotype to the drought tolerance ideotype (Fig 3), we were unable to identify any candidate genes that could account for this finding. The adopted methodology excluded genes that lacked an *A. thaliana* ortholog or had unknown functions from the list of potential candidate genes. Hence, it is possible that one or several of these excluded genes are related to the drought tolerance displayed by IL 7-5-5 in this study.

In IL 1–2, the genes *PRXIIF*, *SAP5*, *REF4-RELATED 1*, *At3g61140*, *LCD*, *At3g62010*, *CCD1*, *At4g15530*, and *At5g49480* were identified as potential candidates. *PRXIIF* encodes a mitochondrial matrix localized peroxiredoxin involved in redox homeostasis, which is involved in oxidative stress response. Under less than optimal conditions, such as drought and salinity, this mitochondrial thioredoxin is necessary for the effective operation of key metabolic pathways, including antioxidant metabolism and stomatal function [47]. Upon experiencing drought episodes, Arabidopsis plants become more drought-resistant as a result of metabolite adaptations in both primary and secondary metabolism triggered by the deactivation of the mitochondrial TRX system [69]. As this is a mitochondrial trait, IL 1–2 should be considered a maternal donor for use in breeding programs. *SAP5* encodes a protein with E3 ligase activity that acts as a positive regulator of stress responses. In wheat (*Triticum aestivum*) and *Arabidopsis thaliana*, STRESS-SAP5 is involved in drought tolerance and acts as an E3 ubiquitin ligase to target DRIP and MBP-1 for degradation [70]. *REF4-RELATED 1* is involved in the regulation of the phenylpropanoid metabolic process [71]. The phenylpropanoid biosynthetic pathway is activated under abiotic stress conditions such as drought, resulting in the accumulation of various phenolic compounds, which, among other roles, scavenge harmful ROS [49]. It was found that an elevated phenylpropanoid content enhanced wheat tolerance to drought stress by maintaining an improved water status, leading to higher photosynthesis rates and lower membrane damage [72]. The gene *LCD* encodes L-cysteine desulfhydrase. L-cysteine desulfhydrase has been identified as the primary catalyst for the breakdown of cysteine into H2S, which is critical in the regulation of stomatal closure in plant responses to drought stress [73]. H2S may serve as a vital intermediary in the ABA-mediated regulation of ion channels involved in stomatal control. H2S influences the expression of ABA receptor candidates, and ABA, in turn, affects H2S production. As a result, H2S interacts with ABA to regulate the stomatal activity, thereby contributing to drought stress tolerance in Arabidopsis [50]. In addition, rice plants exhibit enhanced drought tolerance when LCD is overexpressed, leading to increased H2S production and the persulfidation of total soluble protein [6]. *CCD1* encodes 9-cis-epoxycarotenoid dioxygenase, which is involved in the response to water deprivation. This enzyme is thought to be a key enzyme in ABA biosynthesis [74] involved in stress responses, and is quickly accumulated by many plant species when exposed to drought stress [63]. 9-cis-epoxycarotenoid dioxygenase and the response to water deprivation was linked by demonstrating that drought induces the expression of the gene encoding this enzyme, which in turn controls the level of endogenous ABA under drought-stressed conditions [51].

Uncovering genes in IL populations is a powerful method for mining genetic variation present in wild crop relatives for traits with the potential to improve desired characteristics in elite varieties. Several traits identified in ILs and advanced background populations have been used in tomato breeding [21]. In this study, we identified several candidate genes related to drought tolerance in ILs. We provide useful information for classical breeding approaches by indicating promising genotypes to be used as parents, and for molecular breeding approaches by identifying candidate genes that can be introgressed into elite varieties through marker-assisted selection. Our results, therefore, represent a significant step toward developing drought-tolerant tomato cultivars.

Conclusion

The FAI-BLUP index ranked IL 1-4-18, IL 7-4-1, IL 7–1, IL 7-5-5, and IL 1–2 as closest to the drought-tolerant ideotype. The introgressed lines IL 1-4-18 and IL1-2 were also previously identified as drought-tolerant during the germination and seedling stages. The following candidate genes associated with drought tolerance were identified in selected ILs: *AHG2*,

At1g55840, *PRXIIF*, *SAP5*, *REF4-RELATED 1*, *PRXQ*, *CFS1*, *LCD*, *CCD1*, and *SCS*. Because they are already associated with genetic markers, they can be transferred to elite tomato cultivars through marker-assisted technology after validation.

Supporting information

S1 Fig. Diagram illustrating the trial set. Environmental condition 1 = plants were kept in non-stressed conditions throughout the growing season, with the soil water content maintained at field capacity. Environmental condition 2 = plants were challenged with drought by withholding water for up to 20 d after flowering. 1 to 11 indicates the genotypes, 1 = IL 1-4-18, 2 = IL 2–3, 3 = IL 1–2, 4 = IL 9–2, 5 = IL 10–1, 6 = IL 8–3, 7 = IL 7-4-1, 8 = IL 7-5-5, 9 = IL 9–3, 10 = IL 7–1, 11 = cv. M-82.
(TIF)

S1 File. Data set underlying data used to reach the conclusions drawn in the manuscript.
(XLSX)

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References

1. FAO. Fruit and vegetables—your dietary essentials. The International Year of Fruits and Vegetables, 2021, background paper. 1st ed. Rome, Italy: FAO; 2020
2. Foolad MR, Subbiah P, Zhang L. Common QTL affect the rate of tomato seed germination under different stress and nonstress conditions. International Journal of Plant Genomics. 2008; 2007(97386): 1–10. https://doi.org/10.1155/2007/97386 PMID: 18317505
3. Ayankojo IT, Morgan KT, Ozores-Hampton M, Migliaccio KW. Effects of real-time location-specific drip irrigation scheduling on water use, plant growth, nutrient accumulation, and yield of Florida fresh-market tomato. HortScience. 2018. 53(9): 1372–1378. https://doi.org/10.21273/HORTSCI13183-18

4. Sharma P, Kothari M, Dalavi P. Water Requirement of Drip-Irrigated Tomato Inside a Shade Net House. In: Goyal MR, Aladakatti BK, editors. Engineering Interventions in Sustainable Trickle Irrigation water requirements, uniformity, fertigation, and crop performance. Waretown: Apple Academic Press; 2018. pp. 35–42. https://doi.org/10.1201/9781315184241-2
5. George S, Jatoi SA, Siddiqui SU. Genotypic differences against PEG simulated drought stress in tomato. Pakistan Journal of Botany. 2013; 45(5): 1551–1556.
6. Zhou H, Zhou Y, Zhang F, Guan W, Su Y, Yuan X, et al. Persulfidation of Nitrate Reductase 2 Is Involved in l-Cysteine Desulfhydrase-Regulated Rice Drought Tolerance. International Journal of Molecular Sciences. 2021; 22(22): 12119. https://doi.org/10.3390/ijms222212119 PMID: 34829996
7. Yang H, Du T, Qiu R, Chen J, Wang F, Li Y, et al. Improved water use efficiency and fruit quality of greenhouse crops under regulated deficit irrigation in northwest China. Agricultural Water Management. 2017; 179: 193–204. https://doi.org/10.1016/J.AGWAT.2016.05.029
8. Dariva FD, Pessoa HP, Copati MGF, Almeida GQ, Castro Filho MN, Picoli EAT, et al. Yield and fruit quality attributes of selected tomato introgression lines subjected to long-term deficit irrigation. Scientia Horticulturae. 2021; 289: 110426. https://doi.org/10.1016/J.SCIENTA.2021.110426
9. Mangat PK, Shim J, Gannaban RB, Singleton JJ, Angeles-Shim RB. Alien introgression and morpho-agronomic characterization of diploid progenies of Solanum lycopersicoides monosomic alien addition lines (MAALs) toward pre-breeding applications in tomato (S. lycopersicum). Theoretical and Applied Genetics. 2021; 134(4): 1133–1146. https://doi.org/10.1007/s00122-020-03758-y PMID: 33386862
10. Zhao T, Wu T, Pei T, Wang Z, Yang H, Jiang J, et al. Overexpression of SlGATA17 Promotes Drought Tolerance in Transgenic Tomato Plants by Enhancing Activation of the Phenylpropanoid Biosynthetic Pathway. Frontiers in Plant Science. 2021; 12: 634888. https://doi.org/10.3389/fpls.2021.634888 PMID: 33796125
11. Anjum SA, Ashraf U, Zohaib A, Tanveer M, Naeem M, Ali I., et al. Growth and developmental responses of crop plants under drought stress: a review. Zemdirbyste-Agriculture. 2017; 104 (3): 267–276.
12. Ebert AW, Schafleitner R. Utilization of Wild Relatives in the Breeding of Tomato and Other Major Vegetables. In: Redden R, Yadav SS, Maxted N, Dulloo E, Guarino L, Smith P, editors. Crop Wild Relatives and Climate Change. New Jersey: Wiley Blackwell; 2015 pp. 141–172. https://doi.org/http%3A//dx.doi.org/10.1002/9781118854396.ch9
13. Foolad MR. Genome mapping and molecular breeding of tomato. International Journal of Plant Genomics. 2007; 2007: 064358. https://doi.org/10.1155/2007/64358 PMID: 18364989
14. Bacallao MF, Fundora LB. Tolerancia a estrés por déficit hídrico en tomate (Solanum lycopersicum L.). Cultivos Tropicales. 2014; 35(3): 70–88.
15. Foolad MR, Foolad MR. Recent advances in genetics of salt tolerance in Tomato. Plant Cell, Tissue and Organ Culture, 2004; 76: 101–119. https://doi.org/10.1023/B:TICU.0000007308.47608.88
16. Mohammadi R. Efficiency of yield-based drought tolerance indices to identify tolerant genotypes in durum wheat. Euphytica. 2016; 211(1): 71–89.
17. Bolger A, Scossa F, Bolger ME, Lanz C, Maumus F, Tohge T, et al. The genome of the stress-tolerant wild tomato species Solanum pennellii. Nature Genetics. 2014; 46(9): 1034–1038. https://doi.org/10.1038/ng.3046 PMID: 25064008
18. Moyle LC, Muir CD. Reciprocal insights into adaptation from agricultural and evolutionary studies in tomato. Evolutionary Applications. 2010; 3(5–6): 409–421. https://doi.org/10.1111/j.1752-4571.2010.00143.x PMID: 25567935
19. Kumar PP, Acharya B, Dutta S, Saha S, Hazra P. Variation among the genotypes of tomato for cross-ability to its wild relatives. International Journal of Chemical Studies. 2019; 7(3): 3423–3426.
20. Eshed Y, Zamir D. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield- associated QTL. Genetics. 1995; 141(3), 1147–1162. https://doi.org/10.1093/genetics/141.3.1147 PMID: 8582620
21. Lippman ZB, Semel Y, Zamir D. An integrated view of quantitative trait variation using tomato interspecific introgression lines. Current Opinion in Genetics and Development. 2007; 17(6): 545–552. https://doi.org/10.1016/j.gde.2007.07.007 PMID: 17723293
22. Schauer N, Semel Y, Roessner U, Gur A, Balbo I, Carrari F, et al. Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nature Biotechnology. 2006; 24(4): 447–454. https://doi.org/10.1038/nbt1192 PMID: 16531992
23. Pessoa H P. Linhagens de introgressão derivadas de Solanum pennellii: tolerância ao déficit hídrico durante a germinação e no estádio de plântula. M.Sc dissertation, Universidade Federal de Viçosa. 2018. https://locus.ufv.br//handle/123456789/29053

24. Ahammed GJ, Li X, Yang Y, Liu C, Zhou G, Wan H, et al. Tomato WRKY81 acts as a negative regulator for drought tolerance by modulating guard cell H2O2–mediated stomatal closure. Environmental and Experimental Botany. 2020; 171: 103960. https://doi.org/10.1016/J.ENVEXPBOT.2019.103960

25. Wudiri BB, Henderson DW. Effects of water stress on flowering and fruit set in processing-tomatoes. Scientia Horticulturae. 1985; 27: 189–198. https://doi.org/10.1016/0304-4238(85)90022-6

26. Cui J, Shao G, Lu J, Keabetswe L, Hoogenboom G. Yield, quality and drought sensitivity of tomato to water deficit during different growth stages. Scientia Agricola. 2020; 77(2): e20180390. https://doi.org/10.1590/1678-992X-2018-0390

27. Dariva FD, Copati MGF, Pessoa HP, Alves FM, Dias FO, Picoli EAT, et al. Evaluation of anatomical and physiological traits of Solanum pennellii Cor. associated with plant yield in tomato plants under water-limited conditions. Scientific Reports. 2020; 10(1): 1–13. https://doi.org/10.1038/s41598-020-73004-4 PMID: 32994541

28. van Genuchten MTh. A Closed-form Equation for Predicting the Hydraulic Conductivity of Unsaturated Soils. Soil Science Society of America Journal. 1980; 44(5): 892–898. https://doi.org/10.2136/SSSAJ1980.03615995004400050002X

29. Seki K. SWRC fit—a nonlinear fitting program with a water retention curve for soils having unimodal and bimodal pore structure. Hydrol. Earth Syst. Sci. [Preprint] 2007; 4, 407–437. https://doi.org/10.5194/hessd-4-407-2007

30. Bernardo S, Soares AA, Mantovani EC. Manual de irrigação. 8th ed. Viçosa: Editora UFV; 2019.

31. Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT. Sap Pressure in Vascular Plants: negative hydrostatic pressure can be measured in plants. Science. 1965; 148(3668): 339–346. https://doi.org/10.1126/science.148.3668.339 PMID: 17832103

32. Egea I, Albaladejo I, Meco V, Morales B, Sevilla A, Bolarin MC, et al. The drought-tolerant Solanum pennellii regulates leaf water loss and induces genes involved in amino acid and ethylene/jasmonate metabolism under dehydration. Sci Rep. 2018; 8: 2791. https://doi.org/10.1038/s41598-018-21187-2 PMID: 29434236

33. Kader AA, Stevens MA, Albright M, Morris LL. Amino Acid Composition and Flavor of Fresh Market Tomatoes as Influenced by Fruit Ripeness When Harvested. Journal of the American Society for Horticultural Science. 1978; 103(4): 541–544.

34. Patterson HD, Thompson R. Recovery of Inter-Block Information when Block Sizes are Unequal. Biometrika. 1971; 58(3): 545. https://doi.org/10.2307/2334389

35. Henderson CR. Best Linear Unbiased Estimation and Prediction under a Selection Model. Biometrics. 1975; 31(2): 423–447. https://doi.org/10.2307/2529430 PMID: 1174616

36. Rocha JRASC, Machado JC, Carneiro PCS. Multitrait index based on factor analysis and ideotype-design: proposal and application on elephant grass breeding for bioenergy. GCB Bioenergy. 2018; 10 (1): 52–60. https://doi.org/10.1111/gcbb.12443

37. Kaiser HF. The varimax criterion for analytic rotation in factor analysis. Psychometrika. 1958; 23: 187–200. https://doi.org/10.1007/BF02289233

38. Bartlett MS. The statistical conception of mental factors. British Journal of Psychology. 1937; 28: 97–104. https://doi.org/10.1111/j.2044-8295.1937.tb00863.x

39. Toubiana D, Semel Y, Tohge T, Beleggia R, Cattivelli L, Rosental L, et al. Metabolic profiling of a mapping population exposes new insights in the regulation of seed metabolism and seed, fruit, and plant relations. PLoS Genetics. 2012; 8(3): e1002612. https://doi.org/10.1371/journal.pgen.1002612 PMID: 22479206

40. Fernandez-Pozo N, Menda N, Edwards JD, Saha S, Tecle IY, Strickler SR, et al. The Sol Genomics Network (SGN)—from genotype to phenotype to breeding. Nucleic Acids Research. 2015; 43(D1): D1036–D1041. https://doi.org/10.1093/nar/gku1195 PMID: 25428362

41. TAIR. The Arabidopsis Information Resource. 2022. https://www.arabidopsis.org/.

42. Fujita Y, Nakashima K, Yoshida T, Katagiri T, Kidokoro S, Kanamori N, et al. Three SnRK2 Protein Kinases are the Main Positive Regulators of Abscisic Acid Signaling in Response to Water Stress in Arabidopsis. Plant and Cell Physiology. 2009; 50(12): 2123–2132. https://doi.org/10.1093/pcp/pcp147 PMID: 19880399

43. Jing LW, Chen SH, Guo XL, Zhang H, Zhao YX. Overexpression of a Chloroplast-located Peroxiredoxin Q Gene, SsPrxQ, Increases the Salt and Low-temperature Tolerance of Arabidopsis. Journal of Integrative Plant Biology. 2006; 48(10): 1244–1249. https://doi.org/10.1111/J.1744-7909.2006.00357.X

44. Zang D, Wang C, Ji X, Wang Y. Tamarix hispida zinc finger protein ThZFP1 participates in salt and osmotic stress tolerance by increasing proline content and SOD and POD activities. Plant Science. 2015; 235: 111–121. https://doi.org/10.1016/j.plantsci.2015.02.016 PMID: 25900571

45. Huang J, Ghosh R, Bankaitis VA. Sec14-like phosphatidylinositol transfer proteins and the biological landscape of phosphoinositide signaling in plants. Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids. 2016; 1861(9): 1352–1364. https://doi.org/10.1016/j.bbalip.2016.03.027 PMID: 27038688

46. Zhang JF, Yuan LJ, Shao Y, Du W, Yan DW, Lu YT. The disturbance of small RNA pathways enhanced abscisic acid response and multiple stress responses in Arabidopsis. Plant, Cell & Environment. 2008; 31(4): 562–574. https://doi.org/10.1111/j.1365-3040.2008.01786.x PMID: 18208512

47. Geigenberger P, Thormählen I, Daloso DM, Fernie AR. The Unprecedented Versatility of the Plant Thioredoxin System. Trends in Plant Science. 2017 22(3): 249–262. https://doi.org/10.1016/J.TPLANTS.2016.12.008 PMID: 28139457

48. Kang M, Fokar M, Abdelmageed H, Allen RD. Arabidopsis SAP5 functions as a positive regulator of stress responses and exhibits E3 ubiquitin ligase activity. Plant Molecular Biology. 2011; 75(4–5): 451–466. https://doi.org/10.1007/s11103-011-9748-2 PMID: 21293909

49. Sharma A, Shahzad B, Rehman A, Bhardwaj R, Landi M, Zheng B. Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants under Abiotic Stress. Molecules. 2019; 24(13): 2452. https://doi.org/10.3390/molecules24132452 PMID: 31277395

50. Jin Z, Xue S, Luo Y, Tian B, Fang H, Li H, et al. Hydrogen sulfide interacting with abscisic acid in stomatal regulation responses to drought stress in Arabidopsis. Plant Physiology and Biochemistry. 2013; 62: 41–46. https://doi.org/10.1016/j.plaphy.2012.10.017 PMID: 23178483

51. Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, et al. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. The Plant Journal. 2001; 27(4): 325–333. https://doi.org/10.1046/j.1365-313x.2001.01096.x PMID: 11532178

52. Aydöner Çoban G, Altunlu H, Gül A. Effectiveness of In Vitro and In Vivo Tests for Screening of Tomato Genotypes against Drought Stress. Ege Üniv. Ziraat Fak. Derg. 2020; Özel Sayı: 143–150. https://doi.org/10.20289/zfdergi.835798

53. Vanisree P, Sankari A, Pugalendhi L, Djanaguiraman M. Effect of deficit irrigation on physiological and biochemical traits of tomato (Solanum lycopersicum) genotypes. The Pharma Innovation Journal. 2021; 10(10): 2134–2140. https://www.thepharmajournal.com/archives/?year=2021&vol=10&issue=10&ArticleId=8495

54. Sellin A. Base water potential of Picea abies as a characteristic of the soil water status. Plant and Soil. 1996; 184(2): 273–280. https://doi.org/10.1007/BF00010456

55. Richter H. Water relations of plants in the field: some comments on the measurement of selected parameters. Journal of Experimental Botany. 1997; 48(1): 1–7. https://doi.org/10.1093/JXB/48.1.1

56. Ritchie GA, Hinckley TM. The Pressure Chamber as an Instrument for Ecological Research. Advances in Ecological Research. 1975; 9: 165–254. https://doi.org/10.1016/S0065-2504(08)60290-1

57. Mitchell JP, Shennan C, Grattan SR. Developmental changes in tomato fruit composition in response to water deficit and salinity. Physiologia Plantarum. 1991; 83(1): 177–185. https://doi.org/10.1111/J.1399-3054.1991.TB01299.X

58. Zsögön A, Vicente MH, Reartes DS, Peres LEP. Understanding and improving water-use efficiency and drought resistance in tomato. In: Mattoo A, Handa A, editors. Achieving sustainable cultivation of tomatoes. Cambridge: Burleigh Dodds Science Publishing; 2017. pp. 117–142. https://doi.org/10.19103/AS.2016.0007.01

59. Gur A, Semel Y, Osorio S, Friedmann M, Seekh S, Ghareeb B, et al. Yield quantitative trait loci from wild tomato are predominately expressed by the shoot. Theoretical and Applied Genetics. 2011; 122 (2): 405–420. https://doi.org/10.1007/s00122-010-1456-9 PMID: 20872209

60. Kulik A, Wawer I, Krzywińska E, Bucholc M, Dobrowolska G. SnRK2 protein Kinases—Key regulators of plant response to abiotic stresses. OMICS A Journal of Integrative Biology. 2011; 15(12): 859–872. https://doi.org/10.1089/omi.2011.0091 PMID: 22136638

61. Pashkovskiy PP, Vankova R, Zlobin IE, Dobrev P, Ivanov YV, Kartashov AV, et al. Comparative analysis of abscisic acid levels and expression of abscisic acid-related genes in Scots pine and Norway spruce seedlings under water deficit. Plant Physiology and Biochemistry. 2019; 140: 105–112. https://doi.org/10.1016/j.plaphy.2019.04.037 PMID: 31091491

62. Daszkowska-Golec A. The Role of Abscisic Acid in Drought Stress: How ABA Helps Plants to Cope with Drought Stress. In: Hossain M, Wani S, Bhattacharjee S, Burritt D, Tran LS, editors. Drought Stress Tolerance in Plants. Springer; 2016. Vol 2, pp. 123–151. https://doi.org/10.1007/978-3-319-32423-4_5

63. Lim CW, Baek W, Jung J, Kim JH, Lee SC. Function of ABA in Stomatal Defense against Biotic and Drought Stresses. International Journal of Molecular Sciences. 2015; 16(7): 15251–15270. https://doi.org/10.3390/ijms160715251 PMID: 26154766

64. López-Galiano MJ, García-Robles I, González-Hernández AI, Camañes G, Vicedo B, Real MD, et al. Expression of miR159 Is Altered in Tomato Plants Undergoing Drought Stress. Plants. 2019; 8(7): 201. https://doi.org/10.3390/plants8070201 PMID: 31269704

65. Liebthal M, Maynard D, Dietz KJ. Proxiredoxins and Redox Signaling in Plants. Antioxidants and Redox Signaling. 2018; 28(7): 609–624. https://doi.org/10.1089/ARS.2017.7164/ASSET/IMAGES/LARGE/FIGURE7.JPEG

66. Monks DE, Aghoram K, Courtney PD, Wald DB, Dewey RE. Hyperosmotic Stress Induces the Rapid Phosphorylation of a Soybean Phosphatidylinositol Transfer Protein Homolog through Activation of the Protein Kinases SPK1 and SPK2. The Plant Cell. 2001; 13(5): 1205–1219. https://doi.org/10.1105/tpc.13.5.1205 PMID: 11340192

67. Suprunova T, Krugman T, Distelfeld A, Fahima T, Nevo E, Korol A. Identification of a novel gene (Hsdr4) involved in water-stress tolerance in wild barley. Plant Molecular Biology. 2007; 64(1–2): 17–34. https://doi.org/10.1007/s11103-006-9131-x PMID: 17238046

68. Covarrubias AA, Reyes JL. Post-transcriptional gene regulation of salinity and drought responses by plant microRNAs. Plant, Cell & Environment. 2010; 33(4): 481–489. https://doi.org/10.1111/j.1365-3040.2009.02048.x PMID: 19781008

69. Fonseca-Pereira P, Daloso DM, Gago J, Nunes-Nesi A, Araújo WL. On the role of the plant mitochondrial thioredoxin system during abiotic stress. Plant Signaling & Behavior. 2019; 14(6): 1592536. https://doi.org/10.1080/15592324.2019.1592536 PMID: 30885041

70. Zhang N, Xu J, Liu X, Liang W, Xin M, Du J, et al. Identification of HSP90C as a substrate of E3 ligase TaSAP5 through ubiquitylome profiling. Plant Science. 2019; 287: 110170. https://doi.org/10.1016/j.plantsci.2019.110170 PMID: 31481192

71. Bonawitz ND, Soltau WL, Blatchley MR, Powers BL, Hurlock AK, Seals LA, et al. REF4 and RFR1, subunits of the transcriptional coregulatory complex mediator, are required for phenylpropanoid homeostasis in Arabidopsis. Journal of Biological Chemistry. 2012; 287(8): 5434–5445. https://doi.org/10.1074/jbc.M111.312298 PMID: 22167189

72. Li X, Zhang X, Liu G, Tang Y, Zhou C, Zhang L, et al. The spike plays important roles in the drought tolerance as compared to the flag leaf through the phenylpropanoid pathway in wheat. Plant Physiology and Biochemistry. 2020; 152: 100–111. https://doi.org/10.1016/j.plaphy.2020.05.002 PMID: 32408177

73. Jin Z, Shen J, Qiao Z, Yang G, Wang R, Pei Y. Hydrogen sulfide improves drought resistance in Arabidopsis thaliana. Biochemical and Biophysical Research Communications. 2011; 414(3): 481–486. https://doi.org/10.1016/j.bbrc.2011.09.090 PMID: 21986537

74. Iuchi S, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K. A Stress-Inducible Gene for 9-cis-Epoxycarotenoid Dioxygenase Involved in Abscisic Acid Biosynthesis under Water Stress in Drought-Tolerant Cowpea. Plant Physiology. 2000; 123(2): 553–562. https://doi.org/10.1104/pp.123.2.553 PMID: 10859185