



RESEARCH ARTICLE

# Physiological and enzyme dynamics of tomato under drought stress

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## ARTICLE HISTORY

Received: 10 May 2024

Accepted: 08 September 2024

Available online

Version 1.0 : 31 October 2024



## Additional information

**Peer review:** Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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## CITE THIS ARTICLE

Tamilselvan I, Velu P, Thangaiah A, Gunasekaran Y, Nedunchezhiyan V, Kathirvel SK, Chelladurai D. Physiological and enzyme dynamics of tomato under drought stress. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.3874>

## Abstract

Climate change leads to an increase in the frequency and severity of droughts, which have a negative impact on agriculture by altering plant growth and lowering water availability, placing food production systems at risk of sustainability. In order to improve drought resistance and ensure food security in the face of growing water shortages, this research was conducted to determine the physiological and enzymatic responses of tomato plants to drought stress. This study was conducted at Tamil Nadu Agricultural University, Coimbatore, involving 3 hybrids, viz., H<sub>1</sub>: EC169966 × LE118, H<sub>2</sub>: EC177824 × LE118 and H<sub>3</sub>: Arka Ashish × LE 27, along with their parents, P<sub>1</sub>: EC177824, P<sub>2</sub>: LE27, P<sub>3</sub>: EC169966, P<sub>4</sub>: LE118 and P<sub>5</sub>: Arka Ashish, under 50 % and 100 % field capacity conditions in factorial completely randomized design (FCRD) with 3 replications. Results indicated a significant change in physiological and enzymatic activities. Here, the parent P<sub>2</sub> and hybrid H<sub>2</sub> showed superior tolerance, with higher relative water content, proline, leaf water potential, membrane and chlorophyll stability index. Added to that, the response of enzyme activities including peroxidase, nitrate reductase, catalase, polyphenol oxidase and superoxide dismutase was found to be increase notably in drought-tolerant hybrids and parents, which correlated strongly with physiological markers of drought resistance. These modifications highlight the capacity of some genotypes to preserve photosynthetic efficiency and cellular integrity in water-limited environments. The results highlight the significance of choosing and developing drought-tolerant cultivars in order to maintain agricultural production in areas vulnerable to drought and address issues related to global food security.

## Keywords

climate change; breeding; enzymes; tomato; water stress

## Introduction

The impact of drought stress on worldwide agricultural production has become more apparent in recent times. As water scarcity is exacerbated by climate change, the consequences for plant growth and productivity are becoming increasingly obvious (1). India's current irrigation programmes have an overall water utilisation efficiency of just 40 %. Drought conditions

impact approximately 68 % of the net sown area, which amounts to 140 million ha. Severe regions, comprising 50 % of this area are characterised by recurrent drought effects (2). Moreover, due to the interdependence of ecosystems, crop production affected by drought may trigger a series of consequences that impact not only social well-being but also economic stability and food security. In developing nations where agriculture constitutes a substantial sector of the economy, diminished agricultural production resulting from drought conditions may precipitate food scarcity, escalating food costs and financial insecurity among producers (3). Recent statistics indicate that drought stress has caused a substantial decline in worldwide agricultural production, with estimates indicating a reduction of 10-25 % in the cultivation of major crops on an international scale. Being sessile organisms, plants react physiologically to drought stress in a variety of ways to minimise water loss and preserve cellular homeostasis. These reactions include modifications to leaf morphology, osmolyte accumulation and stomatal closure (4). Lower soil moisture content can result in reduced water uptake, which can lower the turgor pressure inside plant cells. This restricts the amount of carbon dioxide that can be absorbed by photosynthesis, along with stomatal closure, to lessen transpiration (5). As a result, plant growth is hampered and photosynthetic rates fall along with biomass production. Drought stress also causes the production of reactive oxygen species (ROS), which damages molecules and cellular structures and results in oxidative stress (6).

Among the various crops vulnerable to drought stress, tomatoes hold significant importance due to their widespread cultivation and nutritional significance. The tomato, (*Solanum lycopersicum* L.) originated in Peru, serves as one of the best-known and most significant warm-season vegetable crops farmed for its versatility (7). With an estimated yearly global production of 182.3 million tonnes, tomatoes rank as the second-highest-cultivated vegetable in the world next to potato. In addition to being a substantial supply of vital nutrients like vitamins A and C, tomatoes also play a big role in the global supply of food and financial stability in a number of regions. Tomato plants are vulnerable to drought stress, which puts nutritional security at risk for both quantity and quality of yield (8).

Tomato plants' physiological processes, such as photosynthesis, transpiration and enzyme activity are all greatly impacted by drought stress. Prolonged drought stress can cause altered enzyme kinetics, decreased leaf area and poor carbon assimilation (9). The development of resilient tomato crop varieties that can endure water scarcity without sacrificing quality and yield is critically needed, as tomato crops are susceptible to drought stress (10). So far in India, Arka Vikas and Arka Meghali released from the Indian Institute of Horticultural Research are reported to be rainfed varieties. At present, there is no released hybrids for drought tolerance in tomato. In order to achieve this, it is imperative to develop hybrid cultivars that are resistant to drought by acquiring a thorough comprehension of the physiological and biochemical

processes that govern drought tolerance in tomato plants (11).

In order to ascertain the fundamental mechanisms underlying drought tolerance, the purpose of this study aims to examine the physiological and enzymatic responses of tomato plants subjected to drought stress. It is expected that tomato plants experiencing drought stress will display distinct physiological reactions, such as the regulation of stomata and adjustments in enzymes. These responses are intended to maintain cellular balance and minimise the negative impacts of water scarcity. By elucidating these mechanisms, we aim to contribute to the development of drought-tolerant tomato hybrids, thereby enhancing resilience to water scarcity and ensuring food security in drought-prone regions.

## Materials and methods

### 2.1. Site Description and treatment details

The study was conducted at the college orchard of the Department of Vegetable Science, at Tamil Nadu Agricultural University in Coimbatore. The location is situated at 11° 02' North latitude, 77°03' East longitude, and has an altitude of 426.6 m above mean sea level (Fig. 1). The experiment was designed in a factorial completely randomised design (FCRD) with 3 replications and 2 factors: drought at 50 % field capacity (FC) and control at 100 % field capacity (FC). The hybrids, viz., H<sub>1</sub>: EC169966 × LE118, H<sub>2</sub>: EC177824 × LE118 and H<sub>3</sub>: Arka Ashish × LE27, along with their parents, viz., P<sub>1</sub>: EC177824, P<sub>2</sub>: LE27, P<sub>3</sub>: EC169966, P<sub>4</sub>: LE118 and P<sub>5</sub>: Arka Ashish, were taken for this research. Here, the genotypes were collected from National Bureau of Plant Genetic Resources (EC 177824 and EC169966), Indian Institute of Horticultural Research (Arka Ashish) and Tamil Nadu Agricultural University (LE27 and LE118). The genotypes used LE27 and LE118 were reported to be drought tolerant (12). Seedlings that were 25 days old were transplanted into the pots. Drought was imposed on the plants 15 days after transplanting. Both under control conditions (100 % FC) and drought stress conditions (50 % FC), the following observations were recorded:



Fig. 1. Experimental site.

## 2.2. Relative water content (%)

The relative water content of the leaf was determined by taking 20 leaves from each plant and their fresh weight was taken immediately. They are then placed in the distilled water for 4 h, after which the turgid weight is noted. Then the leaves are dried in an oven at 80 °C till a concordant dry weight was obtained. The experiment was performed by a standard method (13). Relative water content was obtained by the formula

Relative water content (%) = Fresh Weight-Dry weight / Turgid Weight-Dry Weight × 100

## 2.3. Chlorophyll stability index (%)

The procedure used to calculate the chlorophyll stability index (14). A 250 mg leaf sample was collected and homogenised using 80 % acetone. The leaf sample was then centrifuged for 10 min at 3000 rpm. The supernatant was extracted and brought to a volume of 25 mL. The absorbance of the spectrophotometer at 652 nm was recorded.

Chlorophyll stability index (%) = Total chlorophyll content (treated) / Total chlorophyll content (control) × 100

Here, Treated – 50 % field capacity; Control – 100 % field capacity

## 2.4. Membrane stability index (%)

The completely expanded leaves were collected. Fifty discs of leaves were divided into 2 test tubes, to which 10 mL of deionized water was added. The test tubes were then chilled at 10 °C for 18 h. After thoroughly washing the leaves with deionized water, 15 mL of ionised water was introduced. Then, each test tube was maintained in a water bath at 25 °C and 45 °C for 1 h. Following a 1 h boiling period, a subsequent conductivity measurement of the aqueous phase was obtained at 25 °C once the samples had chilled. The stability of the leaf membrane was assessed using the Blum and Ebercon method (15).

MTS (%) =  $[1 - (T1/T2)] \times 100$

MTS (%) =  $[1 - (C1/C2)] \times 100$

Were, T1- Before boiling (at 45 °C), T2 - After boiling, C1- Before boiling (at 25 °C), C2 - After boiling

## 2.5 Leaf water potential (MPa)

The leaf water potential was quantified in MPa utilising an instrument leaf water potential metre (ARIMAD 3000) (16).

## 2.6. Leaf electrolyte leakage (%)

Liu and his co-workers utilised the electrical conductivity metric as a means of evaluating the cell membrane's stability (17). After the leaf samples were washed and cut into little pieces, 10 mL of distilled water were added. The initial electrical conductivity of the leaf samples was evaluated following a 24 h incubation period in complete darkness and at room temperature. After heating the samples in a water bath at 100 °C for 15 min, the final conductivity was determined. The formula utilised to calculate ion leakage is as follows:

Electrolyte leakage (%) = Initial electrical conductivity / Final electrical conductivity × 100

## 2.7. Proline (µg fresh weight)

The leaf sample (250 mg) was taken and homogenised using 10 mL of 2 % sulphosalicylic acid. The 2 mL of extract was taken separately in the test tube along with 2 mL of ninhydrin solution and 2 mL of glacial acetic acid and boiled at 100 °C in a water bath for 1 h. The test tube was then transferred to an ice bath to terminate the reaction. The test tubes were then filled with 4 mL of toluene and transferred to the separating funnel, where the pink-coloured solution was collected from the top layer. The spectrophotometer reading of the pink colour solution at 520 nm was noted. Simultaneously, the blank was also maintained without the leaf extract and the proline content was estimated by a method (18).

Proline content =  $36.23 \times \text{Optical density} \times \text{Volume of aliquot taken} / \text{Fresh weight of the sample}$

## 2.8. Peroxidase (changes in OD/min/g leaves)

A volume of 5 mL of 100 mM phosphate buffer was utilised to homogenise the leaf sample (0.5 g). The mixture went through a 15 min centrifugation at 13000 rpm and 4 °C. A volume of 5 mL is obtained by diluting the surface containing the peroxidase enzyme in a solution composed of 6.25 µL of 100 mM phosphate buffer, 2.5 µL of 50 µM H<sub>2</sub>SO<sub>4</sub>, 50 µM pyrogallol and distilled water in a 1:20 ratio. After 1 min of incubation at 25 °C, 0.5 mL of 5 % H<sub>2</sub>SO<sub>4</sub> was introduced to the reaction mixture in order to terminate it. The 445 nm spectrophotometer reading was recorded. The absorbance of 1 unit of peroxidase is 0.1 (19).

## 2.9. Nitrate reductase (µg NO<sub>2</sub>/g/h)

As per the method suggested by Nicholas, the nitrate reductase activity was determined in fully expanded functional leaves and expressed in µg NO<sub>2</sub>/g/h (20). The leaf sample (7 g) was grinded by using 20 mM potassium phosphate buffer, at pH 7.5 for 90 sec. This solution is then added to the charcoal, stirred and centrifuged at 5000 rpm. The supernatant was used for the assay. Incubate 0.1 mL of sodium nitrate, 0.2 mL of phosphate buffer, 0.1 mL of methyl viologen, a desirable volume of enzyme and 0.8 mL of water at 30 °C. The reaction was started by adding 0.2 mL of the dithionite reagent and incubating it for 10 min. The reaction was stopped by shaking the mixture vigorously until the dye colour disappeared and dithionite was completely oxidized. Run the blank simultaneously, without the enzyme. Follow the nitrate reductase assay to measure nitrate in an adequately diluted reaction mixture aliquot.

## 2.10. Catalase (µg of H<sub>2</sub>O<sub>2</sub>/min)

The leaf sample (0.1 g) was homogenized with 0.1 M phosphate buffer. The sample was then centrifuged at 15000 rpm at 4 °C for 30 min. 1 mL of the supernatant was used as the enzyme source; along with it 2 mL of hydrogen peroxide and 3 mL of phosphate buffer were taken and incubated for 1 min at 20 °C. After 1 min the reaction was terminated by adding 10 mL of 0.7 N H<sub>2</sub>SO<sub>4</sub>. Then the reaction mixture was titrated against 0.01 N KMnO<sub>4</sub> until the light purple colour persisted for at least 15 sec to find the residual hydrogen peroxide. The blank is also prepared by adding the enzyme extract to an acidified solution of

the reaction mixture at zero time. This method was done according to the procedure (21).

### 2.11. Polyphenol oxidase (change in OD of H<sub>2</sub>O<sub>2</sub>/min/g)

The activity of polyphenol oxidase was measured in accordance with the procedure (22). The standard reaction mixture comprised 0.5 mL of 0.01 M catechol, 1.5 mL of 0.1 M phosphate buffer (pH 6.5) and 0.5 mL of enzyme preparation. At 495 nanometers, the absorbance was set to zero to initiate the enzyme reaction. The variations in absorbance were monitored for 3 min at 30 sec intervals and the polyphenol oxidase activity was calculated as a change in OD minute<sup>-1</sup> g<sup>-1</sup> of fresh weight.

### 2.12. Superoxide Dismutase (SOD)

Ascertaining SOD activity required by a standard method. 100 mL of hydroxylamine hydrochloride, 500 mL of nitro blue tetrazolium, 1.3 mL of buffer and 70 mL of enzyme extract make up the reaction mixture. At 540 nm, the absorbance was measured (23).

### 2.13. Statistical Analysis

The physiological and enzyme values for 2 factors (50 % and 100 % field capacity) were analysed and then Duncan's multiple range test was done using STAR software. Differences having a *p*-value of <0.01 was considered statistically significant. The Pearson correlation coefficient between the physiological and enzyme parameters was determined. The figures were generated with Origin and R software.

## Results and Discussion

### 3.1. Changes in physiological parameters

#### 3.1.1. Relative water content (%)

The capacity of plant tissues to retain water under various circumstances is indicated by the relative water content (RWC), which is particularly pertinent in situations where drought stress is present (24). Upon analyzing the data, it becomes apparent that the drought stress situation has a considerable influence on the relative water content (RWC) of both the parents and hybrids (Fig. 2a). Reductions in RWC are detected consistently across all the samples. Significantly, among the parents, P<sub>2</sub> demonstrates the maximum RWC under both conditions, suggesting that it possesses a resilient resistance to drought stress. Similarly, H<sub>2</sub> has superior tolerance in hybrids, as evidenced by its highest RWC values. Several adaptation processes may account for the tolerant parent/hybrid greater RWC. Tolerant parent/hybrid exhibit higher RWC due to their capacity to sustain optimal cellular hydration levels even in conditions of limited water availability (25). This indicates the presence of effective water retention techniques and adaptive responses when subjected to drought stress, which are critical for ensuring plants' survival and productivity in water-scarce environments (24).

#### 3.1.2. Chlorophyll stability index (per cent)

The chlorophyll stability index (CSI) is a significant measure of plant well-being and capacity to cope with stress, especially during periods of drought. It indicates the extent

to which chlorophyll content is maintained in leaves (26). Upon analyzing the data, it is clear that drought stress at 50 % field capacity (FC) leads to a significant decrease in CSI when compared to the control at 100 % FC for all parents/hybrids (Fig. 2b). This demonstrates the harmful impact of water shortage on the stability of chlorophyll. Out of all the parental lines, P<sub>2</sub> stands out as the most tolerant genotype, showing the greatest CSI under both normal and drought stress conditions. Similarly, H<sub>2</sub> has the greatest CSI values in hybrids and shows higher tolerance. There are a number of adaptive processes that might be responsible for the greater CSI in tolerant hybrid/parent. These may include effective antioxidant defense mechanisms, such as the buildup of enzymatic and non-enzymatic antioxidants, which assist in reducing oxidative stress caused by drought (27). In addition, genotypes with high tolerance may have improved abilities to retain water, which might decrease the extent of damage caused to chlorophyll molecules under conditions of water deficiency. Furthermore, the resistance that has been seen may be attributed to the overexpression of genes related to the production and maintenance of chlorophyll, which guarantees the preservation of photosynthetic activity even in situations when water is scarce (28).

#### 3.1.3. Membrane stability index (per cent)

The membrane stability index (MSI) is a vital measure of cell membrane integrity, especially in the presence of drought stress. It indicates the plant's capacity to sustain cellular stability in the face of limited water availability (29). Among the parents in the provided data, P<sub>2</sub> has the greatest resistance to drought stress, as seen by continuously higher MSI levels compared to the other parents in both conditions. Similarly, H<sub>2</sub> is the most drought-tolerant among the hybrids, exhibiting higher MSI values in both normal and drought stress conditions (Fig. 2c). The observed pattern highlights the importance of MSI in selecting hybrid/parent that are resilient to drought. Tolerant hybrid/parent have higher MSI values, indicating their improved capacity to preserve membrane integrity and functioning in settings of low water availability. This probably incorporates processes that, taken together, contribute to prolonged cellular stability and enhanced drought resistance, such as effective osmotic adjustment, antioxidant defense systems and decreased membrane lipid peroxidation (30).

#### 3.1.4. Leaf water potential (MPa)

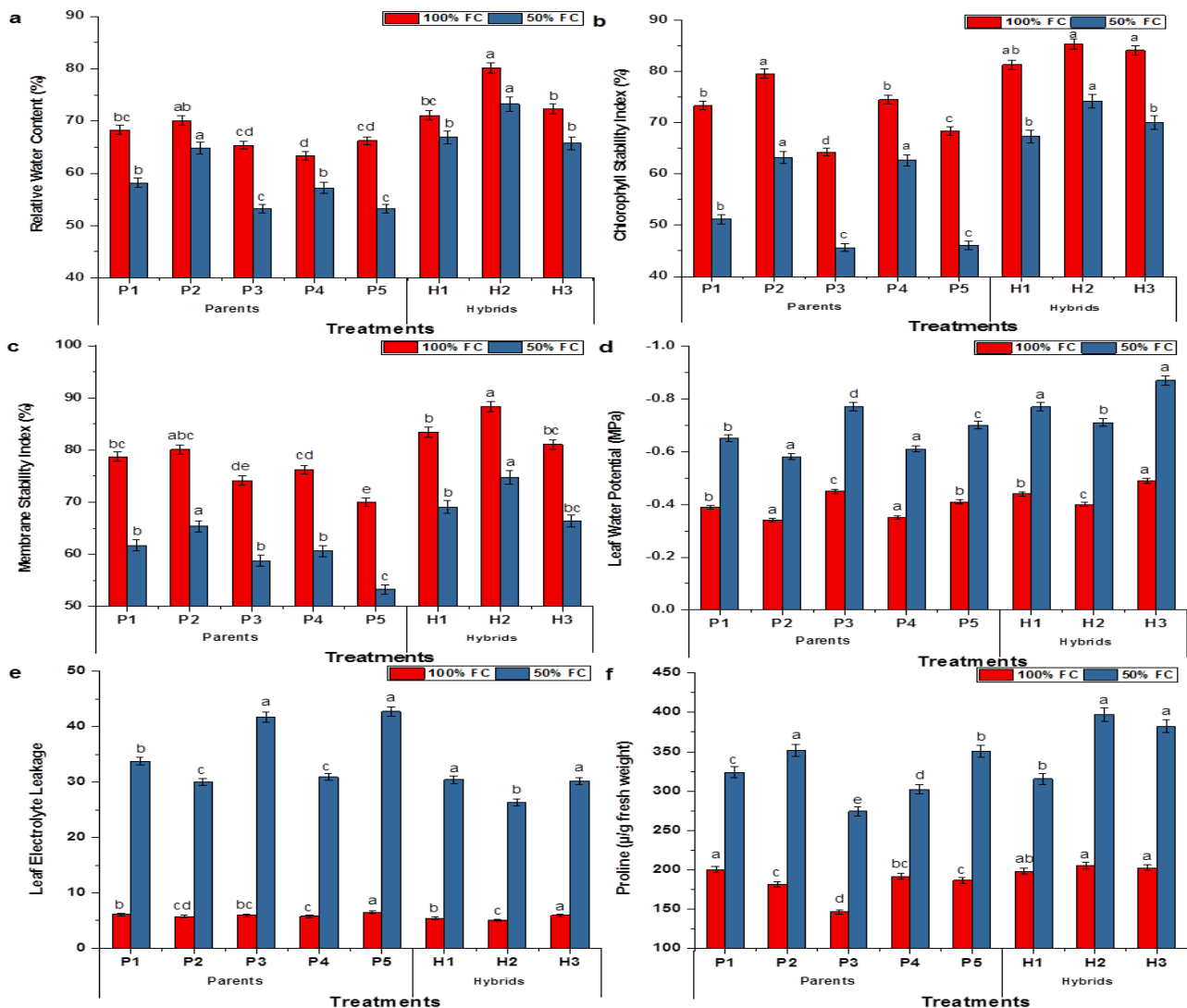
Leaf water potential (LWP) is an important measure of plant water status, especially during periods of drought stress. In general, LWP values decrease in response to water stress, reflecting the water deficit response of plants (31). Out of all the parents, P<sub>2</sub> has the greatest ability to withstand drought stress, as it consistently has higher negative leaf water potential (LWP) values in both normal and stressful situations. Similarly, H<sub>2</sub> has the greatest resilience to drought-induced stress among the hybrids (Fig. 2d). The greater LWP found in tolerant parent/hybrid is due to many physiological changes, including effective water absorption, decreased rates of water loss via transpiration, osmotic adjustment and improved water retention techniques including deeper root systems and alterations to the cuticle (32).

### 3.1.5. Leaf electrolyte leakage (%)

Leaf electrolyte leakage (LEL) is a reliable indication of cellular membrane damage, especially under drought stress. When there is a water shortage and increased membrane instability, EL values often rise in response to drought stress (33). P<sub>2</sub> demonstrates the greatest resistance to drought stress among the parents, as evidenced by its consistently low LEL values under both control and stress conditions. In a similar way, H<sub>2</sub> shows the greatest resistance to drought stress among the hybrids (Fig. 2e). The lower reported LEL in tolerant hybrid/parent may be ascribed to many biochemical and physiological changes. Tolerant hybrid/parent often use strategies such as producing osmoprotectants and antioxidants, regulating cellular ion balance and strengthening cell membranes by accumulating membrane-stabilizing substances such as proline and glycine betaine. Tolerant hybrid/parent are able to retain cellular integrity and function under extreme drought stress conditions by collectively mitigating cellular membrane breakdown and electrolyte leakage via adaptations (34, 35).

### 3.1.6. Proline ( $\mu\text{g}$ fresh weight)

Proline, a frequently occurring amino acid, plays a vital role as an important osmolyte in plants, especially during periods of drought stress. Its buildup assists in adjusting osmotic balance and enhancing stress tolerance (36). P<sub>2</sub> had the greatest resistance to drought stress among the parents, whereas H<sub>2</sub> showed the highest level of tolerance among the hybrids (Fig. 2f). The increased proline accumulation in these resistant hybrid/parent under drought stress might be attributed to many mechanisms. Firstly, proline functions as a compatible solute, which helps to maintain cell turgor and osmotic equilibrium. This, in turn, prevents water loss and protects cells from injury. Secondly, it has been associated with the stability of subcellular structures and the removal of reactive oxygen species, hence decreasing oxidative stress (37). Furthermore, its function in controlling stomatal closure aids in reducing water loss via transpiration. Moreover, the inherent genetic inclination of tolerant hybrid/parent to augment proline production in the presence of stressful circumstances adds to their exceptional ability to withstand drought. Overall, the substantial buildup of proline in the identified tolerant hybrid/parent highlights its significance as a biochemical indicator for drought tolerance in tomato plants (38).



**Fig. 2.** Changes in a) Relative water content (%); b) Chlorophyll stability index (%); c) Membrane stability index (%); d) Leaf water potential (MPa); e) Leaf electrolyte leakage (%) and f) Proline ( $\mu\text{g}$  fresh weight) at 100 % field capacity and 50 % field capacity of tomato parents and hybrids (P<sub>1</sub>: EC177824, P<sub>2</sub>: LE27, P<sub>3</sub>: EC169966, P<sub>4</sub>: LE118 and P<sub>5</sub>: Arka Ashish, H<sub>1</sub>: EC169966 × LE118, H<sub>2</sub>: EC177824 × L118 and H<sub>3</sub>: Arka Ashish × LE 27).

## 3.2. Changes in enzyme Activities

### 3.2.1. Peroxidase (changes in OD/min/g leaves)

Peroxidase, an essential enzyme in plant stress responses, has a vital function in reducing the effects of drought stress by eliminating reactive oxygen species (39). Within the group of parents, P<sub>2</sub> showed the greatest degree of tolerance to drought stress, and among the hybrids, H<sub>2</sub> showed the highest level of tolerance (Fig. 3a). The increase in peroxidase activity found in drought-tolerant hybrid/parent may be related to their function in detoxifying reactive oxygen species, thereby reducing cellular damage and preserving cellular homeostasis. Tolerant hybrid/parent may exhibit elevated levels of peroxidase as part of their adaptive response to drought-induced oxidative stress, thereby enhancing their resilience to water scarcity conditions (40). This emphasizes the significance of peroxidase in bestowing drought tolerance in tomato plants and its potential as a focal point for breeding initiatives aiming at creating drought-resistant cultivars.

### 3.2.2. Nitrate reductase ( $\mu\text{g NO}_2/\text{g/h}$ )

Nitrate reductase, a vital enzyme in nitrogen metabolism, plays a critical role in plant adaptation to drought stress by regulating nitrogen uptake and stress responses (41). P<sub>2</sub> demonstrated the greatest resistance to drought stress among the parents, whereas H<sub>2</sub> exhibited the highest tolerance among the hybrids (Fig. 3b). The elevated activity of nitrate reductase in drought-tolerant hybrid/parent can be attributed to its function in facilitating osmotic adjustment and optimizing nitrogen utilization efficiency, both of which are critical mechanisms for adapting to water deficit conditions (42). Hybrid/parent that are tolerant exhibit an elevated level of nitrate reductase activity as a component of their adaptive approach to uphold nitrogen homeostasis and support essential metabolic pathways necessary for drought tolerance.

### 3.2.3. Catalase ( $\mu\text{g of H}_2\text{O}_2/\text{g/min}$ )

Catalase is a crucial enzyme that plays a vital role in the conversion of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a potentially toxic byproduct of cellular metabolism, into water and oxygen via a process known as detoxification. Catalase is of paramount importance in the context of drought stress as it effectively reduces oxidative harm resulting from the buildup of reactive oxygen species (ROS) under conditions of water scarcity (43). The data provided clearly indicates that catalase activity differs between parental and hybrid plants under conditions of control and drought stress. Out of the parents, P<sub>2</sub> shows the greatest resistance to drought stress, whereas among the hybrids, H<sub>2</sub> has the maximum tolerance (Fig. 3c). The observed enhancement in catalase activity in tolerant hybrid/parent under drought stress might be due to many mechanisms. Catalase primarily aids in the decomposition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which in turn helps to minimise the harm caused by oxidative stress to various cellular constituents (44). The tolerant hybrid/parent may exhibit effective regulatory mechanisms that increase the production of catalase in

response to drought stress, allowing them to maintain cellular balance in adverse situations. Moreover, the presence of increased catalase activity in drought-tolerant hybrid/parent may suggest a heightened capacity to remove reactive oxygen species (ROS), thereby maintaining the structural integrity of cells and promoting the general well-being of plants during water-deficient times (45).

### 3.2.4. Polyphenol oxidase (change in OD of H<sub>2</sub>O<sub>2</sub>/min/g)

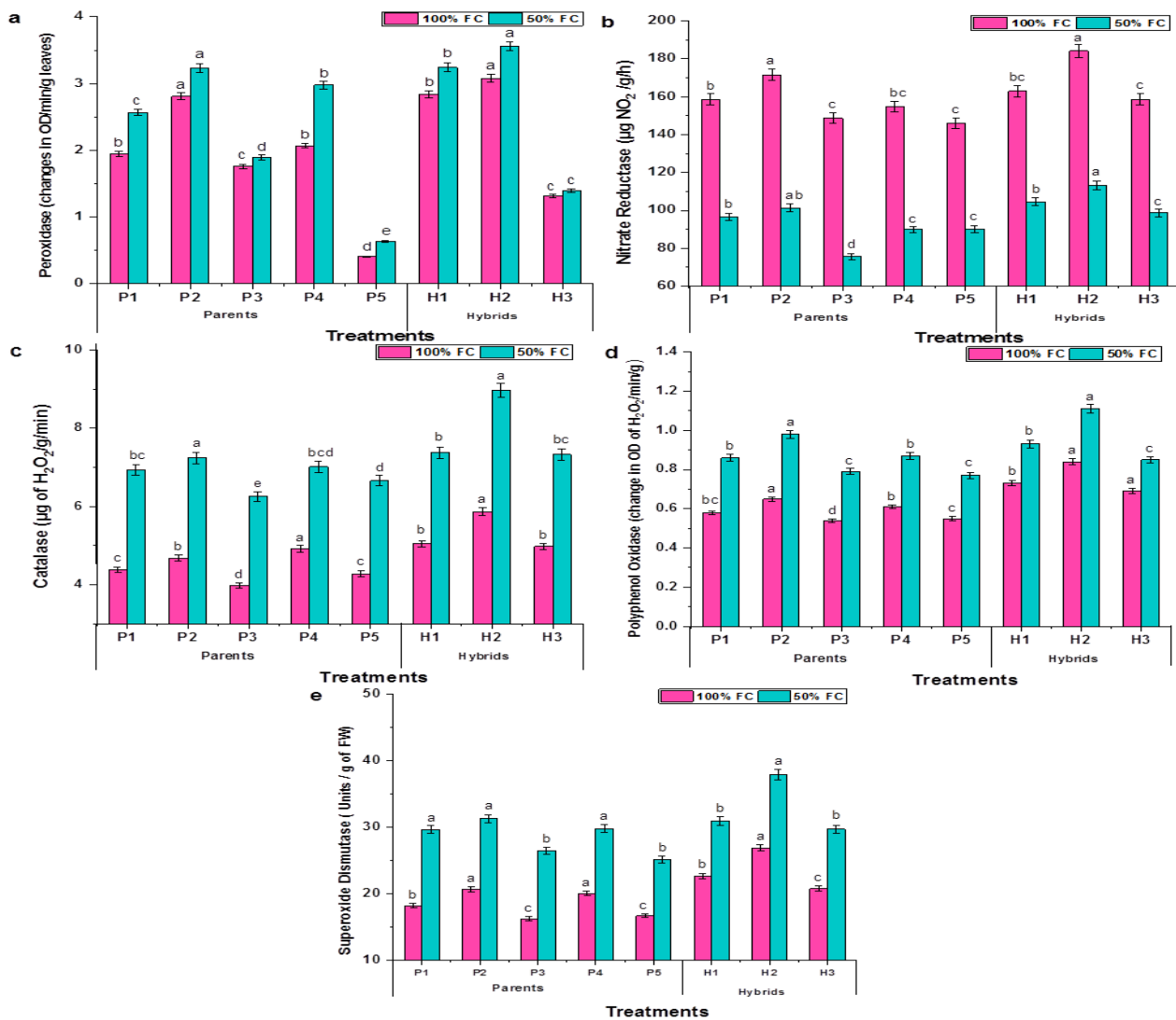
Polyphenol oxidase (PPO) is an enzyme that catalyses the oxidation of phenolic substances, serving as a defensive mechanism against stress conditions. The data provided shows that there are differences in PPO activity across tomato parents and hybrids under both normal and drought stress circumstances. P<sub>2</sub> has the greatest resistance to drought stress among the parents, but H<sub>2</sub> displays the highest level of tolerance among the hybrids (Fig. 3d). The observed elevation in PPO activity in drought-tolerant genotypes may be attributed to many sources. Firstly, PPO has a role in the production of secondary metabolites like flavonoids and lignin. These compounds strengthen cell walls and improve a plant's ability to withstand water scarcity. Secondly, increased PPO activity may promote the browning processes in injured tissues, effectively closing off areas of harm and decreasing water loss via transpiration (46). In addition, the oxidation of phenolic compounds by PPO produces quinones, which possess antibacterial characteristics and potentially assist in protecting against opportunistic infections during periods of drought-induced stress (47).

### 3.2.5. Superoxide Dismutase (SOD)

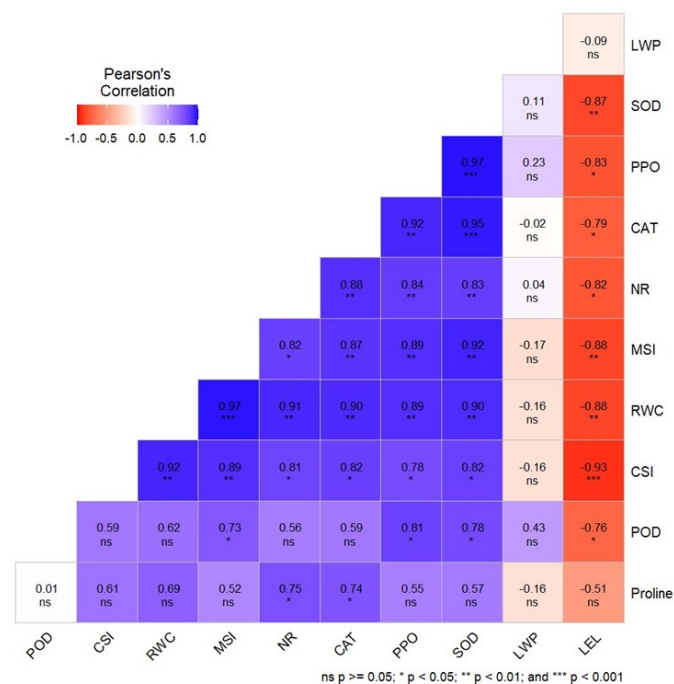
Superoxide dismutase (SOD) is an essential enzyme that plays a critical role in antioxidant defence systems, especially in situations of drought stress when reactive oxygen species (ROS) build up. Notably, P<sub>2</sub> had the greatest resilience to drought stress among the parents, and H<sub>2</sub> showed the highest level of tolerance among the hybrids (Fig. 3e). The increased superoxide dismutase (SOD) activity found in drought-tolerant genotypes may be attributable to many reasons. Firstly, these genotypes may have effective regulatory mechanisms for the expression of the SOD gene, providing a quick response to stress. Furthermore, the heightened SOD activity may augment the process of detoxifying reactive oxygen species (ROS), consequently diminishing oxidative harm to cellular constituents. In addition, hybrid/parent that are tolerant may possess a greater capacity to remove superoxide radicals, preserving cellular homeostasis even when exposed to oxidative stress caused by dehydration (48, 49).

## 3.3. Correlation of enzymes with physiological parameters under drought stress condition

The correlation matrix displays many highly significant correlations, denoted by 3 asterisks, which indicate strong associations under drought conditions in tomatoes (Fig. 4). Notably, SOD displays extremely high positive correlation with CAT and PPO, indicating that these enzymes synergistically enhance their activity to counteract oxidative stress generated by drought stress. The



**Fig. 3.** Changes in a) Peroxidase (changes in OD/min/g leaves) (%); b) Nitrate reductase ( $\mu\text{g NO}_2/\text{g/h}$ ); c) Catalase ( $\mu\text{g of H}_2\text{O}_2/\text{g/min}$ ); d) Polyphenol oxidase (change in OD of  $\text{H}_2\text{O}_2/\text{min/g}$ ) (MPa) and e) Superoxide dismutase (Units/g of FW) at 100 % field capacity and 50 % field capacity of tomato parents and hybrids (P<sub>1</sub>: EC177824, P<sub>2</sub>: LE27, P<sub>3</sub>: EC169966, P<sub>4</sub>: LE118 and P<sub>5</sub>: Arka Ashish, H<sub>1</sub>: EC169966  $\times$  LE118, H<sub>2</sub>: EC177824  $\times$  LE118 and H<sub>3</sub>: Arka Ashish  $\times$  LE27).



**Fig. 4.** Correlation between enzymes and physiological parameters of tomato under drought stress; \*, \*\* and \*\*\* indicate a significant difference at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively. (RWC - Relative Water Content (%); CSI - Chlorophyll Stability Index (%); MSI - Membrane Stability Index (%); LWP Leaf Water Potential (MPa); LEL - Leaf Electrolyte Leakage; Proline ( $\mu\text{g}$  fresh weight); POD- Peroxidase (changes in OD/min/g leaves) (%); NR - Nitrate reductase ( $\mu\text{g NO}_2/\text{g/h}$ ); CAT - Catalase ( $\mu\text{g of H}_2\text{O}_2/\text{g/min}$ ); PPO - Polyphenol oxidase (change in OD of  $\text{H}_2\text{O}_2/\text{min/g}$ ) (MPa) and SOD - Superoxide dismutase (Units/g of FW)).

coordinated activity strengthens the plant's capacity to counteract reactive oxygen species, protecting cell membranes and maintaining metabolic processes (50). The most significant negative correlation is observed between LEL and CSI. The observed significant negative correlation indicates that when the loss of electrolytes from the leaf rises, there is a simultaneous decrease in the stability of chlorophyll. LEL is a metric for membrane permeability and integrity; higher LEL levels indicate more damage to the membranes of cells. The damage to the cellular membranes is likely to result in the depletion of essential cellular components, such as chlorophyll, which is required for the process of photosynthesis. The depletion of chlorophyll, therefore, undermines the plant's capacity to capture sunlight and carry out photosynthesis with efficiency, resulting in a reduction in CSI. These interactions emphasise the important balance between water conservation systems and oxidative stress control in drought-stricken plants, emphasising the significance of these physiological and enzymatic features in tomato survival and production under water-limited conditions (51, 52).

## Conclusion

In conclusion, our research demonstrates the complicated physiological and enzymatic dynamics of tomato plants under conditions of drought, exposing the basic reactions that drive their response to water limitation. Hybrid H<sub>1</sub> and parent P<sub>2</sub> exhibited remarkable resilience to drought indicated by remarkable variations in relative water content, chlorophyll stability index, membrane stability index and leaf electrolyte leakage under stress conditions. These findings suggested that drought tolerance can be improved by selective breeding that focuses on these traits. The study also highlighted the importance of enzyme activity such as peroxidase, nitrate reductase, catalase, polyphenol oxidase and superoxide dismutase in conferring drought resistance. The potential for these enzymes to detoxify reactive oxygen species and support cellular homeostasis under situations of water deficit is suggested by the reported increase in their activity in drought-tolerant hybrid/parent. The correlations observed between these enzyme activity and physiological indicators highlight the interdependence of these responses and provide an improved comprehension of the intricate processes plants employ for surviving off drought stress. Finally, this study helps to the larger goal of assuring food security by increasing the drought tolerance of tomato, paving the door for more sustainable farming techniques in drought-prone areas.

## Acknowledgements

The authors are grateful to Department of Vegetable Science, Tamil Nadu Agricultural University for their support.

## Authors' contributions

IT carried out the experiment, data collection and analysis. PV and AT contributed to conceptualised and supervised the research design and experimental planning. YG and VN contributed for the writing and reviewing the manuscript. SKK and DC helped in statistical analysis.

## Compliance with ethical standards

**Conflict of interest:** The authors have no conflict of interest.

**Ethical issues:** None.

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