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Exogenous putrescine enhances fruit set and yield in tomato under drought through improved water status and antioxidant defense

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Abstract

Tomato (*Solanum lycopersicum* L.) is highly sensitive to limited water availability, especially during the reproductive stage at anthesis, when even short periods of stress can greatly reduce yield. In the present study, we evaluated the effects of exogenously applied putrescine at concentrations ranging from 0 to 10 mM on plant performance, antioxidant activity, and yield under water-deficit conditions. Water stress caused a clear decline in growth and productivity, reducing plant height by 34.83%, fruit set by 61.67%, and yield by 76.35%, while also increasing membrane damage and oxidative stress. However, plants treated with putrescine showed marked improvement, and the response increased with concentration. The 10.0 mM treatment was the most effective, substantially improving plant growth and productivity compared to untreated stressed plants. Fruit set and fruit number also improved considerably under putrescine application. At the physiological level, putrescine helped maintain better plant water status, as indicated by increases in relative water content from 55.10% to 68.36% and in membrane stability from 41.08% to 60.48%. It also reduced electrolyte leakage and lipid peroxidation, indicating lower cellular damage. These improvements were supported by increased activity of key antioxidant enzymes, such as superoxide dismutase, catalase, and ascorbate peroxidase, along with increased expression of their corresponding genes. Overall, the results indicate that putrescine helps plants tolerate water limitation by stabilizing cellular functions and strengthening antioxidant defenses. The findings further show that its effect is dose-dependent and is particularly important at anthesis, where it significantly improves reproductive performance and yield under stress conditions.

Keywords: Putrescine, water stress tolerance, antioxidant activity, membrane integrity, improved fruit set

Introduction

Tomato (*Solanum lycopersicum* L.) remains one of the most widely cultivated vegetables globally, owing to its significant nutritional and economic value. Lycopene, found in tomatoes, possesses antioxidative and anti-cancer properties, making it highly beneficial for health. Additionally, the versatility and vibrant color of tomatoes make them a staple in many cuisines worldwide (Rai et al., 2024). Recent global estimates indicate that tomato is

cultivated on about 4.9-5.0 million hectares, with total production reaching nearly 186 million tonnes(FAO,2024). The demand for tomatoes continues to rise due to their wide use in fresh consumption and in processed products. However, tomato production remains highly vulnerable to abiotic stresses such as cold, heat, water deficit, and salinity.

Among these, drought stress poses a major constraint to the growth, development, and productivity of tomato crops worldwide (Ma et al., 2020; Preeti et al., 2026). It is becoming an increasingly critical global issue, exacerbated by climate change and diminishing water resources (Dos Santos et al., 2022). Severe drought stress significantly affects the entire plant lifecycle (Islam et al., 2022). As global climate change intensifies, the frequency and severity of water shortages are expected to increase, making it essential to develop drought-resistant tomato varieties or implement effective management strategies. Drought stress not only impairs tomato plant health and productivity but also reduces fruit quality (Medyouni et al., 2021; Xu et al., 2020). Lower rainfall and resulting water shortages destabilize plant growth, triggering physiological and biochemical changes, such as the accumulation of osmotic substances, stomatal closure, and reduced net photosynthetic rates (Praveen et al., 2019; Farooq et al., 2020; Seleiman et al., 2021; Jahan et al., 2022).

Furthermore, under water-deficit conditions, plants exhibit increased production of reactive oxygen species due to disruptions in normal metabolic processes (Rai et al., 2021; Hasanuzzaman et al., 2020). The accumulation of these reactive molecules disturbs cellular homeostasis and weakens the plant's defense system. Reactive oxygen species such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals can damage membranes, proteins, and nucleic acids, ultimately impairing cellular function and growth (Gill and Tuteja, 2010; Hasanuzzaman et al., 2020). In addition, they interfere with nutrient transport and hormonal balance, which directly affects reproductive processes such as fruit set and yield (Kawaguchi et al., 2023; Mittler, 2017). Recent studies have further confirmed that drought-induced oxidative stress leads to membrane lipid peroxidation and cellular damage when reactive oxygen species are not efficiently scavenged (Zhang et al., 2024; Singh et al., 2023).

Despite being immobile, plants respond to such stress by activating a coordinated network of physiological and biochemical defense mechanisms (Yang et al., 2021; Rai et al., 2021). A key component of this response is the antioxidant system, which includes enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, and other peroxidases that function together to regulate reactive oxygen species levels across cellular compartments (Apel and Hirt, 2004; Hasanuzzaman et al., 2020). The activity of these enzymes is closely linked to gene expression, and growing evidence suggests that increased transcription of antioxidant-related genes plays a crucial role in improving stress tolerance (Mittler, 2017; Li et al., 2023).

However, under prolonged or severe stress, the intrinsic antioxidant system may be insufficient to maintain redox balance. When the production of reactive oxygen species exceeds the scavenging capacity, oxidative damage accumulates, leading to impaired cellular function and reduced plant performance (Hasanuzzaman et al., 2020; Singh et al., 2023). This

limitation has driven increasing interest in external regulators that can support or enhance the plant's defense system. In this context, polyamines, particularly putrescine, have gained attention for their roles in stabilizing cellular structures, regulating metabolic processes, and interacting with antioxidant pathways, thereby improving plant tolerance under water-deficit conditions (Chen et al., 2019; Jahan et al., 2022; Li et al., 2023).

One emerging cost-effective strategy to enhance drought tolerance in plants is the use of plant growth regulators, particularly polyamines (Mohammadi et al., 2018; Chen et al., 2019; Zhang et al., 2020; Zhao et al., 2021; Jahan et al., 2022; Shao et al., 2022; Tyagi et al., 2022; Li et al., 2023). Polyamines are low-molecular-weight aliphatic compounds, mainly comprising putrescine, spermidine, and spermine, which are widely distributed in plants and play important roles in growth regulation and stress adaptation (Alcázar et al., 2010; Hasanuzzaman et al., 2020). Among these, putrescine acts as the primary precursor in polyamine biosynthesis and is central to maintaining cellular homeostasis under stress conditions. It contributes to membrane stabilization, osmotic adjustment, and regulation of antioxidant systems, thereby enhancing stress tolerance (Gill and Tuteja, 2010; Jahan et al., 2022; Li et al., 2023). Recent studies have further demonstrated that exogenous application of putrescine improves drought tolerance by modulating the balance of reactive oxygen species, enhancing antioxidant enzyme activity, and maintaining cellular integrity under water-deficit conditions (Seifi and Shelp, 2019; Singh et al., 2023; Zhang et al., 2024). Given its central role in polyamine metabolism and its direct involvement in physiological and biochemical regulation, putrescine has emerged as a key regulator in plant responses to water limitation.

However, the role of putrescine in improving drought tolerance in tomatoes remains largely unexplored. Polyamines such as Putrescine, spermidine, and spermine are small molecules that are ubiquitously distributed in plants (Wu et al., 2016; Yuan et al., 2019). In plants, arginine decarboxylase and ornithine decarboxylase convert arginine and ornithine into Put (Fortes et al., 2019). Spermidine and spermine are synthesized from putrescine through the addition of aminopropyl groups by the enzymes spermidine synthase (SPDS) and spermine synthase, with decarboxylated S-adenosylmethionine (dc-SAM) serving as the initial substrate. The enzyme S-adenosylmethionine decarboxylase catalyzes the conversion of S-adenosylmethionine into decarboxylated S-adenosylmethionine, which serves as an essential aminopropyl donor in the biosynthesis of spermidine and spermine. PAs are eventually broken down by diamine oxidase and polyamine oxidase into 4-aminobutanal, 1,3-diaminopropane, and N-(3-aminopropyl)-4-aminobutanal (Li et al., 2016). Research has shown that endogenous polyamine levels increase significantly in crops such as rice, tobacco, and maize under drought stress (Capell et al., 2004; Bae et al., 2007; Alcazar et al., 2010; Li et al., 2018). This accumulation is regulated by PA synthesis genes and the dynamic balance of free, conjugated, and bound PA forms (Shi et al., 2013). Zhou and Yu (2010) found that vetiver grass increased PA levels to cope with drought stress. Exogenous application of PAs has also been shown to modulate drought responses in wheat by enhancing osmolyte accumulation, regulating PA metabolism, and increasing free PA levels (Ebeed et al., 2017). Hamid et al. (2018) reported that Put reduced ROS accumulation during drought stress by

enhancing antioxidant enzyme activity. Similar effects have been observed in maize, where polyamines have been shown to strengthen the antioxidant defense system (Li et al., 2018).

Despite these findings, limited research exists on the specific effects of putrescine, a crucial precursor in PA synthesis, on the antioxidant defense system and polyamine homeostasis in tomatoes under drought conditions. Here, we investigated the effect of exogenous putrescine on drought tolerance in tomato, with a specific focus on the anthesis stage, a critical phase for reproductive success. While the role of polyamines in stress tolerance is known, limited information is available on the stage-specific and dose-dependent effects of putrescine on physiological regulation, antioxidant defense, and polyamine homeostasis during drought conditions.

We hypothesized that exogenous application of putrescine enhances drought tolerance by improving water status, stabilizing cellular membranes, and strengthening antioxidant enzyme activity, thereby reducing oxidative damage during anthesis. We aimed to evaluate the effects of different putrescine concentrations on growth and yield traits, assess changes in physiological parameters related to water status and membrane integrity, and analyze the responses of antioxidant enzymes and polyamine metabolism under water-deficit conditions.

MATERIALS AND METHODS

Plant Materials, Growth Environment, and Experimental Treatments

Tomato (*Solanum lycopersicum* L.) cv. 'Kashi Anupam' was obtained from the ICAR-Indian Institute of Vegetable Research in Varanasi, India. The seeds were sterilized for five minutes in a 0.1% sodium hypochlorite (NaOCl) solution and then rinsed several times with deionized water. They were placed in the dark at 28 ± 1°C for 36 hours to facilitate germination. After germination, the seeds were planted in plastic trays filled with a peat-vermiculite mixture (2:1) and then transferred to a greenhouse at the Institute of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India. The greenhouse conditions were maintained at 28 ± 1°C during the day and 18 ± 1°C at night, with relative humidity between 65% and 75%, and a 12-hour light cycle with photosynthetically active radiation (PAR) of 300 µmol $m^{-2}s^{-1}$. Seedlings with well-developed roots were transferred into pots (20 cm × 16 cm) containing a mixture of soil, vermicompost, and sand (4:2:1). All seedlings were grown under optimum controlled conditions of 24 ± 1°C during the day (14-hour light) and 16 ± 1°C at night (10-hour dark), with relative humidity maintained at 55–65%. These conditions were maintained to ensure uniform plant growth and reduce environmental fluctuations during the stress treatment period. A total of 30 uniformly grown tomato plants with fully expanded leaves were selected for drought stress and putrescine (Put) treatment at the anthesis stage. The experiment was conducted in a completely randomized design comprising six treatments with five replications each, including control, drought stress without putrescine application, and drought-stressed plants treated with putrescine at concentrations of 2.5, 5.0, 7.5, and 10.0 mM. Pots were maintained at optimal moisture levels through regular

irrigation until anthesis. Putrescine was applied as a foliar spray according to the respective treatment schedules, with the first application made 1 day before drought imposition and the second 2 days after stress initiation. In contrast, control plants received a spray of double-distilled water. All leaves were sprayed on both sides until the solution dripped off naturally. The day following the Put pretreatment was designated as Day 0, after which irrigation was withheld to induce drought stress. Soil water status was maintained at 35 ± 5% under non-stress conditions and reduced to 8 ± 2% during drought treatment. The gravimetric approach was used to monitor and maintain the required soil moisture levels throughout the experiment. After six days of drought stress, the top three leaves were collected for gene quantification, while the fourth and fifth leaves from each treatment were harvested and frozen at -80°C for subsequent analysis.

Growth and Yield Attributing Indicators

To evaluate the combined effects of putrescine and drought stress on tomato plants at anthesis, various growth- and yield-related indicators were measured. These included plant height, root length, fresh and dry weights of shoots and roots. At the end of the experiment, the number of leaves, fruit set, and number of fruits per plant were recorded. Total plant yield was calculated by summing the harvest yields.

Plant height and root length were measured with a standard ruler, and fresh and dry weights of shoots and roots were determined using an electronic balance after the plants were oven-dried at 65°C for 72 hours. Root length (R) divided by shoot length (S) yielded the R/S ratio. The number of fruits per plant was counted manually at each harvest, and the average fruit weight and yield per plant were also measured using an electronic balance. The fruit setting percentage was calculated by using the following formula:

$$\text{Fruit Setting (\%)} = (\text{Fruit Set Clusters per Plant} / \text{Flower Clusters per Plant}) \times 100$$

Leaf area (cm^2)

Leaf area (LA) was determined on the sixth day after putrescine application from destructively sampled flag leaves using the formula described by Aldesuquy et al. (2014).

$$\text{Leaf area } (cm^2) = \text{Length} \times \text{Width} \times 0.75$$

Photosynthetic pigments

Chlorophyll content was assessed using the method outlined by Lichtenthaler and Buschmann (2001). The following equations were applied to calculate the amounts of chlorophyll a, chlorophyll b, and total chlorophyll in fresh weight (mg g^{-1}) (Wagay et al. 2019), where *V* represents the extraction volume, and *W* is the weight of the sample:

Chlorophyll a (mg g^{-1}) = (12.7 * 663 nm) − (2.69 * 645 nm) * V/W *1000
Chlorophyll b (mg g^{-1}) = (22.91 * 645 nm) − (4.68 * 663 nm) * V/W*1000
Total chlorophyll (mg g^{-1}) = chlorophyll a + chlorophyll b

Carotenoid content was extracted using dimethyl sulfoxide (DMSO) at a ratio of 1.5 ml DMSO per 10 mg of plant tissue. Samples were incubated for 3 hours at 65 °C, and the

absorbance was measured at 480 nm, using β-carotene as a standard. Data were expressed as mg per gram of fresh weight (mg g^{-1} FW). Each treatment was replicated 3 times to ensure accuracy.

Relative water content

After six days, five leaves from each treatment group, along with their replicates, were collected and placed in a polythene bag for immediate transport to the laboratory to assess the relative water content (RWC). In the lab, the fresh weight (FW) of the detached leaves was measured. Subsequently, the leaves were submerged in distilled water and stored in the dark at room temperature for 24 hours to determine their turgid weight (TW). Once the TW was recorded, the leaves were air-dried in a hot-air oven at 70°C until they reached a constant weight, after which the dry weight (DW) was measured with an electronic digital balance. The RWC was calculated using the method outlined by Mullan and Pietragalla (2012).

$$RWC = (FW - DW/TW - DW) \times 100$$

Electrolyte Leakage

The method outlined by Jahan et al. (2019) was used to measure electrolyte leakage from leaves. Initially, a leaf disc measuring approximately 10 mm was positioned in a Petri dish to prevent electrolyte leakage during removal. The disc was washed with distilled water on three occasions. After adding distilled water, the dish was placed in a dark room at room temperature for 24 hours. Following this incubation period, the solution's initial electrical conductivity (EC_1) was measured. Afterward, the specimens were heated in a water bath at 95°C for 20 minutes, then cooled to ambient temperature before measuring electrical conductivity (EC_2) again. The formula below was used to determine the electrolyte leakage:

$$EL\ (\%) = (EC_1/EC_2) \times 100$$

Measurement of Membrane Stability Index

Membrane stability index (MSI) was determined following six days of drought and foliar application of putrescine; fully mature leaves were harvested from each treated plant. Two samples of fresh flag leaves, each weighing 0.2 g, were cut into small strips and placed in separate glass test tubes containing 10 ml of distilled water. Two sets of test tubes were prepared for the analysis. The first set was incubated in a water bath at 40°C for approximately 30 minutes, after which the electrical conductivity (EC) of each sample was measured using an EC meter (C_1). The second set of test tubes was subjected to a water bath at 100°C for about 15 minutes, and the EC values were recorded as C_2. The MSI was calculated using the formula provided by Sairam et al. (1997).

$$MSI = [1 - (C_1/C_2)] \times 100$$

Assessment of Lipid Peroxidation

Lipid peroxidation levels were evaluated by measuring malondialdehyde (MDA) equivalents, following a modified method based on Rai et al. (2024). Initially, 0.5 g of leaf tissue was homogenized in 80% ethanol using a mortar and pestle. The homogenate was then centrifuged at 3000× g for 10 minutes at 48°C. The resulting pellet was extracted twice

more with the same solvent. The supernatants were combined, and 1 mL of this solution was mixed with an equal volume of a reagent solution containing 20% trichloroacetic acid, 0.01% butylated hydroxytoluene, and 0.65% thiobarbituric acid in a test tube. This mixture was heated to 95°C for 25 minutes and then cooled to room temperature. Absorbance was measured at wavelengths of 440 nm, 532 nm, and 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and reported as MDA μmol g^{-1} of fresh weight (FW).

H_2O_2 Measurement

The concentration of H_2O_2 in leaves was determined using the procedure outlined by Zhao et al. (2021). In short, 1g of plant leaf samples was crushed in 2 mL of cold acetone and then centrifuged at 10,000× g for 15 minutes at 4°C to obtain the supernatant. This supernatant was then combined with 0.05 mL of $TiCl_4$ (20%) solution and 0.1 mL of concentrated ammonia water, followed by centrifugation at 4000× g for 10 minutes. The remaining residue was washed three times with cold acetone, and then 3 mL of 2 M H_2SO_4 was added to dissolve the precipitate. The absorbance values were measured at 415 nm using a spectrophotometer. The H_2O_2 content was determined using a standard curve and expressed as μmol g^{-1} FW.

Proline content (μmol g^{-1} FW)

Proline was quantified using the method described by Pantha et al. (2024). 0.5 g of tomato leaf tissue was homogenized in 10 mL of 3% aqueous sulfosalicylic acid. Following this, 2 mL of the extract was combined with 2 mL of the ninhydrin reagent and incubated at 100 °C for one hour. After the reaction, proline was extracted with 4 mL of toluene, vigorously mixed, and left at room temperature for 30 min until the two phases separated. chromophore-containing toluene was cooled at room temperature, and absorbance was measured at 520 nm using a spectrophotometer. Proline content was determined using a standard curve for L-proline and expressed as μmol g^{-1} fresh weight (FW).

Soluble Sugars (mg g^{-1} FW)

Total soluble sugars were measured in an ethanolic extract of fresh tomato flag leaves following the method described by Kaur and Asthir (2017). To prepare the sample, 0.1 mL of the ethanol extract was mixed with 3 mL of fresh anthrone solution (150 mg anthrone dissolved in 100 mL 72% H_2SO_4) and heated in a boiling water bath for 10 minutes. After cooling the mixture, absorbance was recorded at 625 nm using a spectrophotometer. The total soluble sugars (TSS) were determined using a glucose standard curve.

Antioxidant Enzyme Assay

Three hundred milligrams of fresh tomato leaf were ground in liquid nitrogen, then mixed with 100 mM sodium phosphate buffer (pH 7.4) containing 1% polyvinyl pyrrolidone (PVP) and 0.5% Triton X-100, and vortexed. The resulting homogenate was centrifuged at 20,000 rpm for 20 minutes at 4 °C. The supernatant was used for antioxidant activity assays and stored at −20 °C for protein estimation using the Bradford method (1976) with a UV-Visible spectrophotometer (Rai et al., 2024).

Superoxide Dismutase

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to the method of Sarkar S and Oba S (2018). The reaction mixture consisted of 1.5 mL of 50 mM sodium phosphate buffer (pH 7.8), 0.3 mL of 130 µM methionine, 0.3 mL of 750 µM nitro blue tetrazolium (NBT), 0.3 mL of 100 µM EDTA-Na_2, 0.3 mL of 20 µM riboflavin, 100 µL distilled water, and 0.1 mL enzyme extract. The reaction mixture was illuminated at a light intensity of 4000 lux for 20 min, and absorbance was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT photo reduction. Each treatment was analyzed in six replicates.

Ascorbate Peroxidase

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed following the method of Murshed et al. (2008). Fresh leaf tissue (1g) was homogenized in liquid nitrogen with sodium phosphate buffer (pH 7.2) containing polyvinylpolypyrrolidone using a pre-chilled mortar and pestle. The homogenate was centrifuged at 6,700 rpm for 20 min, and the resulting supernatant was used as the crude enzyme extract. The reaction mixture comprised 1500µL of phosphate buffer, 20 µL of EDTA, 1000µL of sodium ascorbate, and 20 µL of enzyme extract. The reaction was initiated by adding 480µL of hydrogen peroxide (H_2O_2), and the decline in absorbance was monitored at 290 nm every 2 min against a blank. Each treatment consisted of six replicates.

Catalase

Catalase (CAT; EC 1.11.1.6) activity was determined following the method of Chance and Maehly (1955) with slight modifications. The reaction mixture consisted of 1.5mL of 50 mM sodium phosphate buffer (pH 7.8), 300µL of 0.1 M hydrogen peroxide (H_2O_2), 1.0mL distilled water, and 200µL enzyme extract. Catalase activity was estimated by monitoring the decomposition of H_2O_2 through the decrease in absorbance at 240nm per min using a spectrophotometer. Each treatment was analyzed in six replicates.

Total RNA Isolation and Gene Expression Analysis

Leaf samples from tomato plants were collected in TRIzol, and total RNA was extracted according to the manufacturer's guidelines using the GeneJET Plant RNA Purification Kit (Thermo Fisher Scientific, USA; sourced from Mumbai, India). Following this, cDNA synthesis was carried out using the Maxima H minus first-strand cDNA synthesis kit with dsDNase (Thermo Fisher Scientific, USA). Sequences for superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and Actin, which served as a housekeeping gene, were obtained from the Sol genome browser and NCBI GeneBank. Alignment of these sequences was performed using MAFFT alignment software (version 6.240). Conserved sequences were identified, and primers were designed using Primer3 software (version 4.1.0), as detailed in Table 1. Gene expression analysis was executed with four biological and four technical replicates. Quantitative PCR (qPCR) was conducted in 96-well plates (CFX 96 Touch™ Real-Time PCR, Bio-Rad, Hercules, CA, USA). Each reaction comprised a final volume of 20µL, including 10µL of 2X SYBR Green Master Mix (Thermo Scientific, Waltham, MA, USA), 6µL of cDNA samples, and 200nM primers. The cycling conditions were established as

follows: an initial denaturation at 95 °C for 3 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 1 minute. The housekeeping gene Actin was used as an internal reference to normalize expression levels (Li et al., 2014). The specificity of the amplification was verified through melting curve analysis. Relative expression levels were calculated using the 2^−ΔΔCT method (Pfaffl, 2001).

Table 1. List of antioxidant gene primers for gene expression profiling.

Primer name	Sequence	Annealing Temp. (°C)
β-actin (F)	TTGACTGAGGCACCACTTAACCCT	68.7
β-actin (R)	GCTTTCAGGTGGTGCAACGACTTT	71.0
SOD (F)	CACGTCTTCAAAGCAAGTGG	63.5
SOD (R)	CTAAGAAGAAGGGCATTCTTTGGCAT	68.7
CAT (F)	GATGAGCACACTTTGGAGCA	64.1
CAT (R)	TGCC CTTCTATTGTGGTTCC	63.8
APX (F)	GAAACTCAGAGGACTCATTGCTGAGAAGAATTG	72.9
APX (R)	GAAACTGCTCCCTAATGGGCTCCAAGAG	73.9

SOD: Superoxide dismutase; CAT: Catalase; APX: Ascorbate peroxidase; F: Forward; R: Reverse.

Statistical analysis

Statistical analysis of the data was performed using R Studio software (R Studio Team, 2021). Normality and homoscedasticity were evaluated using the Shapiro-Wilk test and Bartlett's test, respectively, through the default functions in R Studio. For data that followed a normal distribution, a one-way ANOVA model was applied, considering five sources of variation: irrigation, drought stress and putrescine concentration. Statistical significance was determined using the D'Agostino test of skewness on the residuals, followed by post-hoc Tukey's Honest Significant Difference test.

Results

Effect of putrescine on growth and yield-related parameters of tomato

Drought stress severely affects plant growth and productivity, resulting in reduced tomato growth and yield. In the present study, different concentrations of putrescine were evaluated for their effects on growth and yield parameters of tomato plants under water-deficit conditions. Growth and yield attributes were significantly influenced by drought stress and putrescine application (Figure 1a and 1b).

Under drought stress, untreated plants with putrescine (Put) showed a significant 34.83% reduction in plant height compared to the control (79.7 cm), clearly highlighting the adverse effects of drought. However, Put treatment mitigated this impact in a concentration-

dependent manner, with higher concentrations (7.5 or 10.0 mM) showing more pronounced effects. Application of 7.5 mM Put improved plant height by 35.0%, while 10.0 mM Put resulted in a 39.08% improvement. Compared to unstressed control plants, the reductions in plant height deviations were 12.03% and 9.32%, respectively, demonstrating the effectiveness of higher Put concentrations in alleviating drought stress. The application of Put also significantly improved leaf number under drought stress, with a consistent increase as Put concentrations rose, particularly at 5.0 mM or higher. In the control group, plants had 114.92 leaves. With 5.0 mM Put, leaf number increased by 28.70%, reducing the gap to control by 16.39%. Notably, higher concentrations (7.5 mM and 10.0 mM) showed even greater benefits, with leaf counts improving by 43.64% and 48.73%, respectively, compared to untreated drought-stressed plants, narrowing the difference from the control to just 6.64% and 3.32%, respectively. This indicates that higher Put concentrations are particularly effective in mitigating drought-induced leaf loss and restoring leaf numbers to control levels.

Drought stress reduced leaf area by 18.83% relative to the control, underscoring its negative impact on leaf development. Adding 2.5 mM Put resulted in only a slight improvement (1.23%), though still 17.81% lower than the control. At 5.0 mM, leaf area increased by 16.57%, reducing the gap to the control to 5.36%. The leaf area further improved with increasing Put concentrations, particularly at 7.5 mM and 10.0 mM, where the respective increases were 21.36% and 21.89%, and were only 1.48% and 1.03% below the control. These results suggest that higher Put concentrations effectively mitigate drought-induced reductions in leaf area, nearly restoring it to control levels.

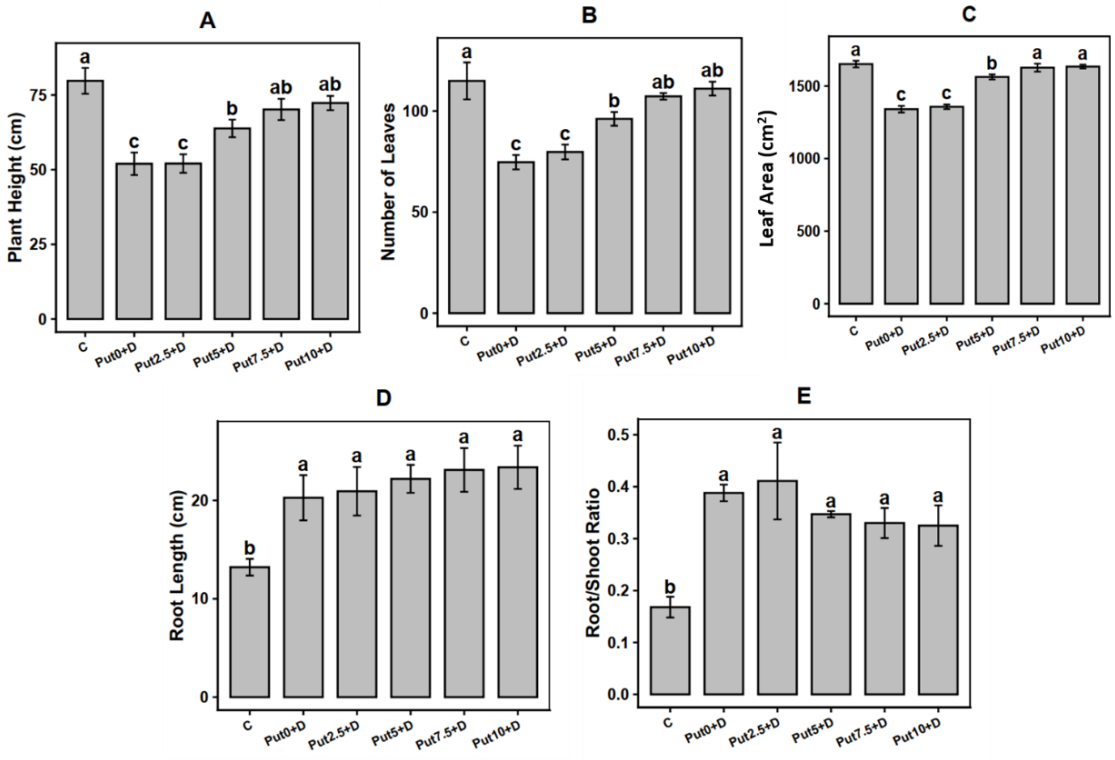


Figure 1a. Effect of putrescine application on growth parameters of tomato under drought stress:(A) Plant height, (B) number of leaves, (C) leaf area, (D) root length, and (E) root-to-shoot ratio. Values represent mean ± SE. Different lowercase letters indicate significant differences among treatments at $P \leq 0.05$ (HSD test). C = well-watered

control; Put0+D = drought stress without putrescine; Put2.5+D, Put5+D, Put7.5+D, and Put10+D indicate foliar application of putrescine at 2.5, 5.0, 7.5, and 10.0 mM under drought stress.

Under drought conditions, control plants had the shortest root length, averaging 13.21 cm. Drought stress alone increased root length by 53.46% compared to the control, reflecting the plant's natural response to seek deeper water. Adding 2.5 mM Put further increased root length by 58.42%, and 5.0 mM Put increased it by 68.02% over the control. With 7.5 mM Put, root length increased by 74.87%, and at the highest concentration of 10.0 mM, the greatest root length was recorded, with a 76.87% increase over the control and a 15.29% improvement over untreated drought plants. These results indicate that root length consistently increased with higher Put concentrations, particularly at 5.0 mM and above, suggesting that drought stress induces root growth as an adaptive response to water scarcity.

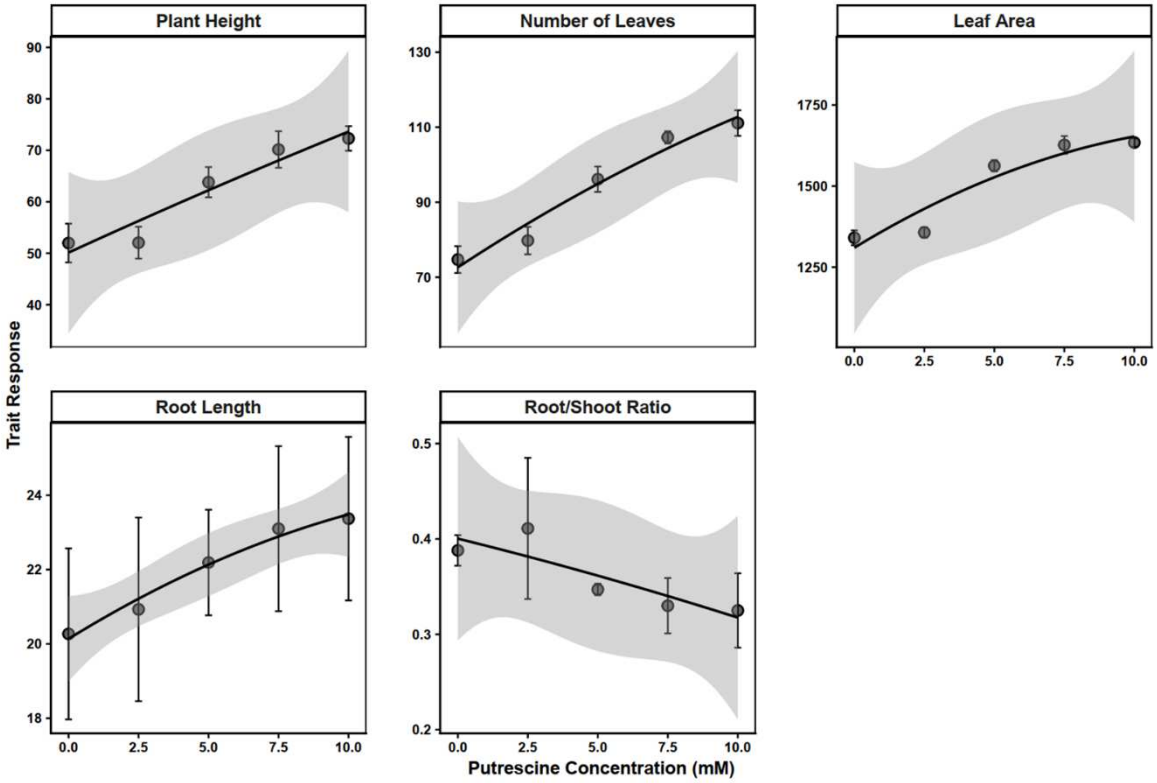


Figure 1b. Dose response relationship of putrescine application on growth traits of tomato under drought stress: Dose-dependent changes in plant height, number of leaves, leaf area, root length, and root-to-shoot ratio in response to foliar application of putrescine under drought stress conditions. Points represent mean values ± SE, while solid lines indicate fitted polynomial regression curves with 95% confidence intervals. Putrescine concentrations of 0, 2.5, 5.0, 7.5, and 10.0 mM were evaluated under drought stress conditions.

Under drought stress, control plants showed the lowest root-to-shoot ratio, 0.168. In the absence of putrescine, the ratio increased significantly to 0.388, a 130.95% rise, indicating a shift towards greater root growth ($p < 0.05$). With 2.5 mM putrescine, the ratio further improved to 0.411, representing a 144.64% increase over the control and a 5.93% increase over untreated drought plants. At 5.0 mM, the ratio was 0.347, a 106.55% increase over the control, but a 15.59% decrease from the 2.5 mM treatment. At 7.5 mM, the ratio dropped slightly to 0.330 (a 96.43% increase over the control), and at the highest concentration, 10.0 mM, it was 0.325 (a 93.45% increase over the control). Despite slight

declines at higher concentrations, all putrescine treatments resulted in significant improvements over the control.

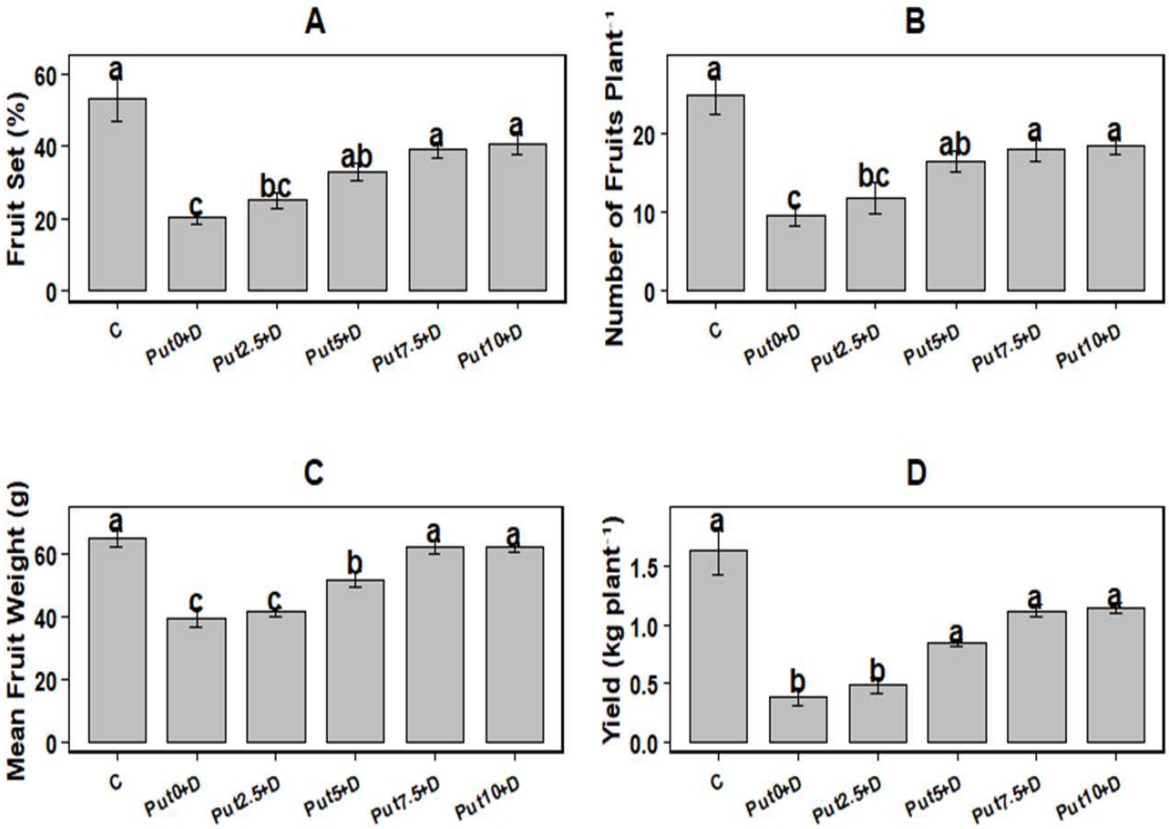


Figure 2a. Effect of putrescine application on fruit set, yield, and yield-attributing traits of tomato under drought stress: (A) Fruit set, **(B)** number of fruits per plant, **(C)** mean fruit weight, and **(D)** yield per plant of tomato plants subjected to different concentrations of putrescine under drought stress. Values represent mean ± SE. Different lowercase letters indicate significant differences among treatments at $P \leq 0.05$ according to the HSD test. C = well-watered control; Put0+D = drought stress without putrescine; Put2.5+D, Put5+D, Put7.5+D, and Put10+D represent foliar application of putrescine at 2.5, 5.0, 7.5, and 10.0 mM, respectively, under drought stress conditions.

Fruit set was significantly influenced by drought stress and putrescine application (Figure 2a and 2b). The well-watered control recorded the highest fruit set (52.95%), whereas drought-stressed plants without putrescine treatment exhibited a sharp decline to 20.27%, representing a 61.67% reduction relative to the control. Foliar application of putrescine progressively improved fruit set as concentration increased. Plants treated with 2.5 mM putrescine recorded a fruit set of 25.03%, corresponding to a 23.53% increase over untreated drought-stressed plants. Similarly, fruit set increased to 33.06% at 5.0 mM putrescine, indicating a 63.10% improvement over drought-stressed plants without putrescine application. The maximum response was observed at 7.5 and 10.0 mM putrescine, with fruit set reaching 38.99% and 40.83%, respectively, representing improvements of 92.35% and 101.43% over untreated drought-stressed plants. Despite these improvements, fruit set across all drought treatments remained lower than that of the well-watered control. Similarly, fruit production per plant dropped from 24.89 fruits in

control plants to 9.55 fruits without putrescine, a 61.64% decrease. With 2.5 mM putrescine, production increased to 11.90 fruits, a 24.59% rise, while at 5.0 mM, it rose to 16.49 fruits, a 72.91% increase. At 7.5 mM, production increased to 18.08 fruits, an 89.56% improvement; at 10.0 mM, it increased to 18.47 fruits, a 93.22% increase. For fruit weight, control plants averaged 64.99 g, which dropped to 39.51 g in the absence of putrescine, a 39.22% reduction. With 2.5 mM putrescine, the weight rose to 41.37 g, a 4.73% increase, while at 5.0 mM, it increased to 51.74 g, a 31.07% improvement. At 7.5 mM, fruit weight increased to 62.18 g, a 57.46% rise, and at 10.0 mM, it yielded 61.78 g, a 56.32% increase. Finally, the yield per plant dropped from 1.625 kg in controls to 0.384 kg without putrescine, a 76.35% decrease. With 2.5 mM putrescine, the yield increased to 0.487 kg, a 26.81% improvement, and at 5.0 mM, it rose to 0.846 kg, a 120.16% increase. At 7.5 mM, yield increased to 1.117 kg, a 190.33% rise, and at 10.0 mM, yield reached 1.137 kg, a 196.66% improvement. Overall, putrescine application significantly improved fruit set, production, weight, and yield under drought stress, with the most substantial effects observed at 5.0 mM and higher concentrations

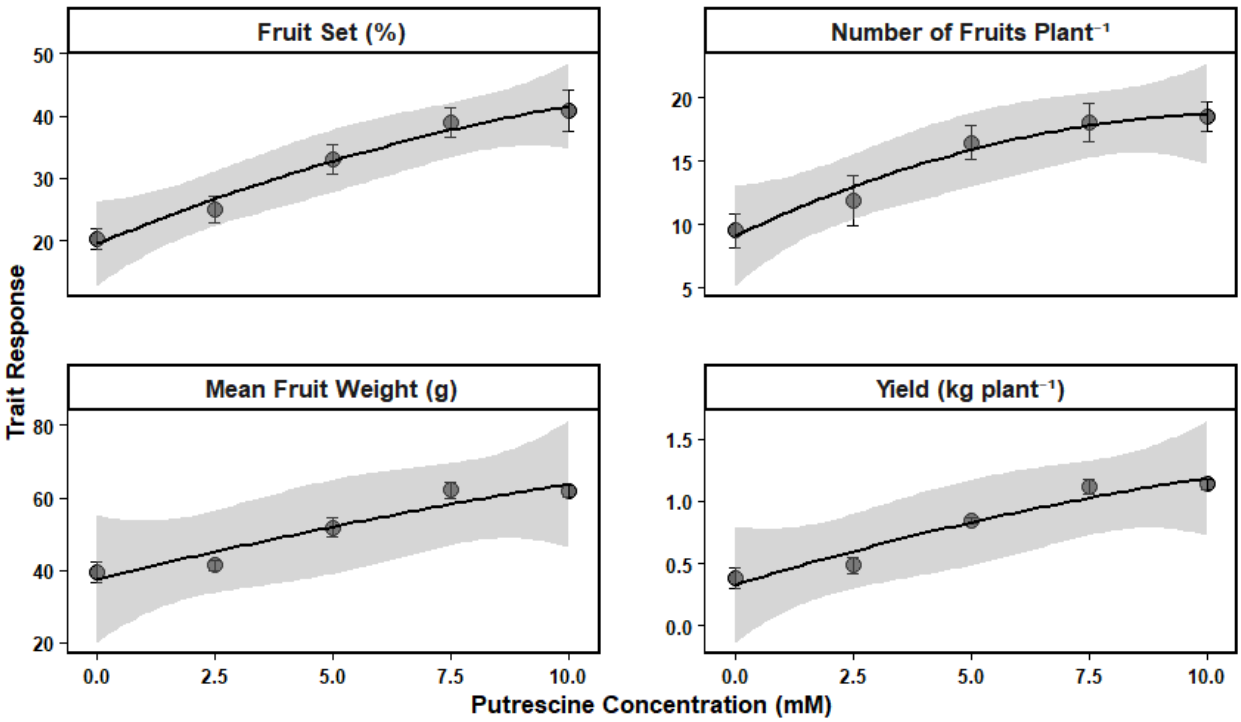


Figure 2b. Dose-dependent modulation of reproductive performance and yield traits in drought-stressed tomato plants following putrescine application: The figure depicts the effects of increasing putrescine concentrations on fruit set, number of fruits per plant, mean fruit weight, and yield per plant under drought stress. Symbols represent mean values ± SE, and solid lines denote fitted polynomial regression models with 95% confidence intervals. Increasing putrescine concentrations progressively enhanced reproductive efficiency and yield performance, with maximum responses observed at 7.5-10.0 mM under drought stress.

Putrescine Application and dynamics of chlorophyll and carotenoid content in drought stressed tomato plants

Drought stress severely impacts plant physiology, especially chlorophyll content, which is vital for photosynthesis and overall plant health. The data in Figure 1 highlight the

effect of putrescine on drought-induced chlorophyll degradation, showing varying degrees of effectiveness as putrescine concentration increases. Under drought stress, chlorophyll a (Chl a) content was significantly higher in control plants (1.833 ± 0.153 units) compared to drought-stressed plants without putrescine (1.297 ± 0.047 units). Putrescine treatments mitigated this decline in a concentration-dependent manner, with 2.5 mM (1.453 ± 0.124 units), 5.0 mM (1.570 ± 0.053 units), 7.5 mM (1.553 ± 0.056 units), and 10.0 mM (1.620 ± 0.087 units) all showing significantly higher chlorophyll a content than untreated drought plants. The 10.0 mM concentration was the most effective, yielding the highest chlorophyll a content among drought-stressed plants. Similarly, chlorophyll b (Chl b) content in drought-stressed plants decreased significantly from the control (0.807 ± 0.051 units) to 0.447 ± 0.056 units. Putrescine treatments restored Chl b levels in a dose-dependent manner, with the 10.0 mM concentration achieving the highest Chl b content (0.683 ± 0.042 units), representing a 52.8% increase over untreated drought-stressed plants. Total chlorophyll content followed a similar pattern, with control plants showing 2.640 ± 0.204 units, and drought stress reducing it to 1.743 ± 0.103 units. All putrescine treatments significantly improved total chlorophyll content, with 10.0 mM (2.283 ± 0.129 units) providing the highest levels, which were not significantly different from those of the 5.0 mM and 7.5 mM treatments. The chlorophyll a/b ratio, which measures the balance between the two chlorophyll types, was highest in untreated drought plants (2.94 ± 0.23) but decreased with putrescine application, with 7.5mM yielding the highest ratio (2.65 ± 0.36) and 10.0mM the lowest (2.45 ± 0.14). Although all putrescine treatments resulted in a lower ratio compared to untreated drought-stressed plants, the differences among them were not statistically significant.

Carotenoid content increased significantly under drought stress, from 0.853 ± 0.065 units in the control to 1.220 ± 0.057 units in untreated drought-stressed plants. Putrescine treatments led to a dose-dependent reduction in carotenoid content, with 10.0 mM resulting in 1.057 ± 0.068 units, slightly lower than in untreated drought stress. Although lower than in untreated drought plants, higher putrescine concentrations (7.5 mM and 10.0 mM) brought carotenoid levels closer to those of control plants. The carotenoid/chlorophyll ratio, which increased dramatically under drought stress (0.699 ± 0.013), was moderated with putrescine application. The ratio for untreated drought-stressed plants was the highest. In contrast, putrescine-treated plants showed significantly lower ratios: 2.5mM (0.449 ± 0.037), 5.0mM (0.438 ± 0.015), 7.5mM (0.479 ± 0.055), and 10.0mM (0.464 ± 0.024), all of which were higher than the control (0.324 ± 0.024) but significantly lower than the untreated drought plants.

These findings suggest that putrescine application effectively mitigates the negative effects of drought stress on tomato plants, enhancing both chlorophyll and carotenoid content, improving the chlorophyll a/b ratio, and moderating the carotenoid/chlorophyll ratio. Higher concentrations of putrescine, particularly 10.0 mM, were most effective at alleviating the impact of drought stress on these physiological parameters.

Putrescine Application and behavior of tomato plants for various physiological parameters under drought stress

Drought stress negatively affects various physiological aspects of tomato plants, as shown in Figures 3, 4, and 5. Electrolyte leakage, a marker of membrane damage, was lowest in control plants (49.84%) and highest in drought-stressed plants without putrescine (72.54%). Putrescine treatments reduced leakage in a concentration-dependent manner, with 2.5 mM reducing leakage to 67.89%, 5.0 mM to 62.10%, 7.5 mM to 55.46%, and 10.0 mM to 56.96%. Membrane stability, measured as the Membrane Stability Index (MSI), was highest in control plants (75.26%) and lowest in untreated drought plants (41.08%). Putrescine application improved MSI: 2.5 mM and 5.0 mM increased it to 43.63% and 52.52%, respectively, and 7.5 mM and 10.0 mM reached 59.59% and 60.48%, respectively, indicating better membrane integrity under drought conditions. Lipid peroxidation, an indicator of oxidative stress, was lowest in controls (0.67 nmol g^{-1} FW) and highest in drought-stressed plants (2.36 nmol g^{-1} FW). Putrescine application reduced lipid peroxidation, with 7.5 mM and 10.0 mM showing the greatest reductions to 0.98 and 1.02 nmol g^{-1} FW, respectively. Hydrogen peroxide (H_2O_2) levels, a key oxidative stress marker, were lowest in control plants (40.33 µmol g^{-1} FW) and highest in untreated drought plants (80.82 µmol g^{-1} FW). Putrescine treatments lowered H_2O_2 levels, with 7.5mM and 10.0mM being the most effective, reducing H_2O_2 to 61.48 µmol g^{-1} FW and 66.51 µmol g^{-1} FW, respectively.

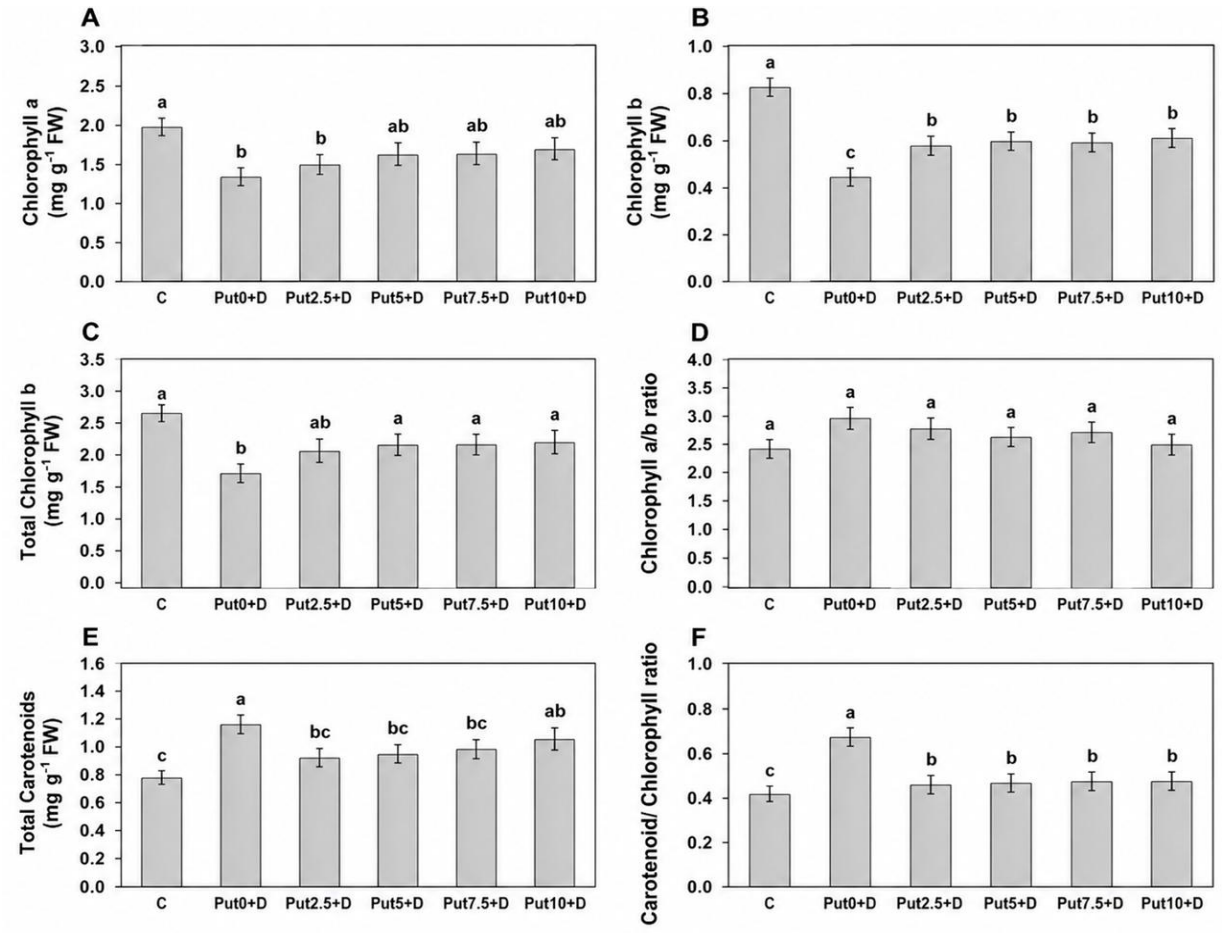


Figure 3. Putrescine-mediated modulation of photosynthetic pigments and carotenoid balance in tomato under drought stress.

(A) Chlorophyll a, (B) chlorophyll b, (C) total chlorophyll, (D) chlorophyll a/b ratio, (E) total carotenoids, and (F) carotenoid/chlorophyll ratio in tomato plants subjected to different concentrations of putrescine under water deficit conditions. Values represent mean ± SE. Different lowercase letters indicate significant differences among treatments at $P < 0.05$. C = well-watered control; Put0+D = drought stress without putrescine; Put2.5+D, Put5+D, Put7.5+D, and Put10+D represent foliar application of putrescine at 2.5, 5.0, 7.5, and 10.0 mM, respectively, under drought stress. FW = fresh weight.

Relative water content (RWC) in drought-stressed plants was lowest at 55.10% without putrescine and highest in control plants at 75.24%. Putrescine treatments improved RWC, with 2.5 mM increasing it to 61.43%, 5.0 mM to 65.59%, and 7.5 mM and 10.0 mM reaching 68.85% and 68.36%, respectively, indicating better water retention.

Proline content, which increases under stress, was lowest in control plants (14.32 μmol g^{-1} FW) and highest in drought-stressed plants (19.12 μmol g^{-1} FW). Putrescine increased proline content in a dose-dependent manner, with 7.5mM and 10.0mM treatments resulting in the highest levels (23.01 μmol g^{-1} FW and 23.18 μmol g^{-1} FW, respectively). Soluble sugar content was lowest in control plants (0.76 mg g^{-1} FW) and highest in drought-stressed plants (1.64 mg g^{-1} FW). Putrescine treatment increased soluble sugars, with the highest levels recorded at 10.0mM (1.86 mg g^{-1} FW), suggesting that putrescine enhances sugar accumulation under drought stress. These results demonstrate that putrescine plays a protective role in tomato plants under drought stress by improving membrane stability, reducing oxidative damage, enhancing water retention, and modulating osmotic regulators such as proline and soluble sugars, with higher putrescine concentrations conferring the most significant benefits.

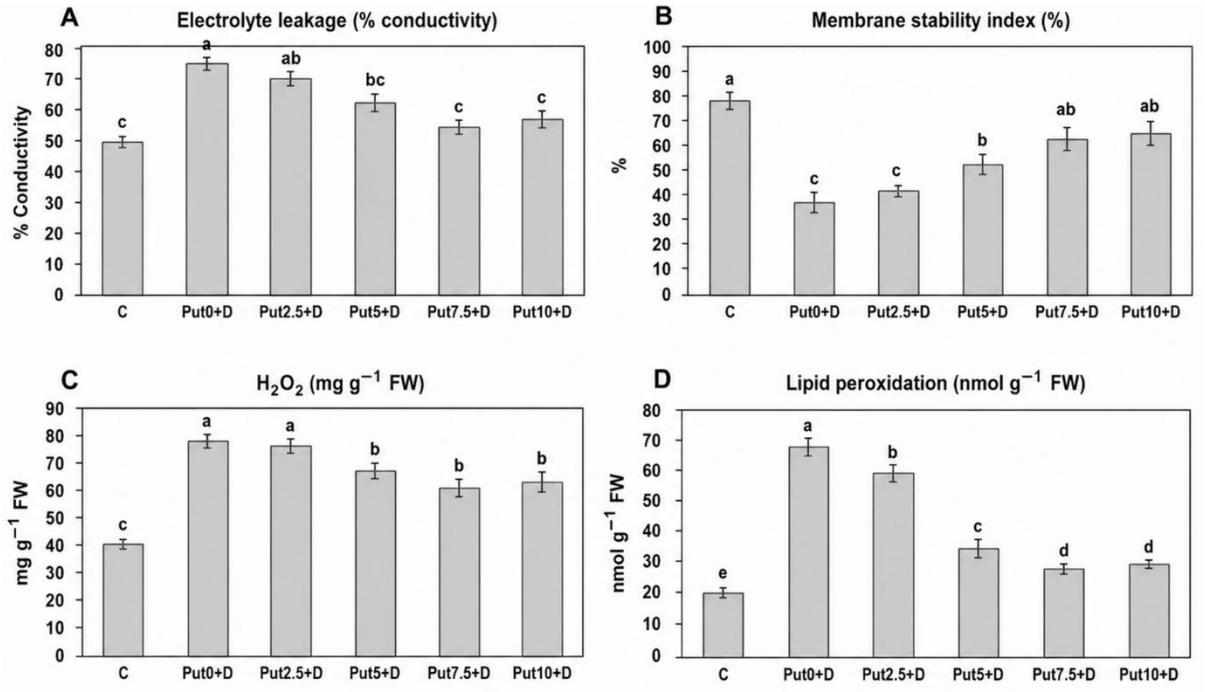


Figure 4. Putrescine-mediated regulation of membrane integrity and oxidative stress markers in tomato under water deficit conditions.

(A) Electrolyte leakage, (B) membrane stability index, (C) H_2O_2 content, and (D) lipid peroxidation in tomato plants subjected to different concentrations of putrescine under water deficit conditions. Values represent mean ± SE. Different lowercase letters indicate significant differences among treatments at $P < 0.05$. C = well-watered control; Put0+D = drought stress without putrescine; Put2.5+D, Put5+D, Put7.5+D, and Put10+D represent foliar application of putrescine at 2.5, 5.0, 7.5, and 10.0 mM, respectively, under drought stress. FW = fresh weight.

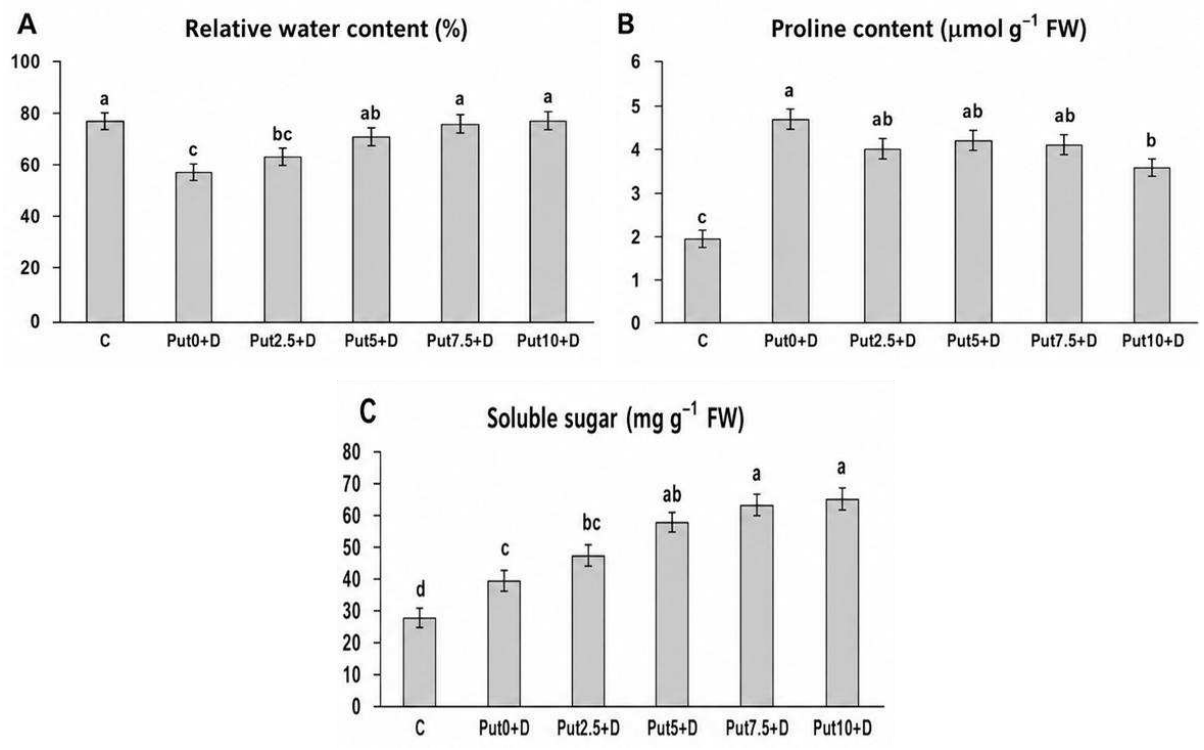


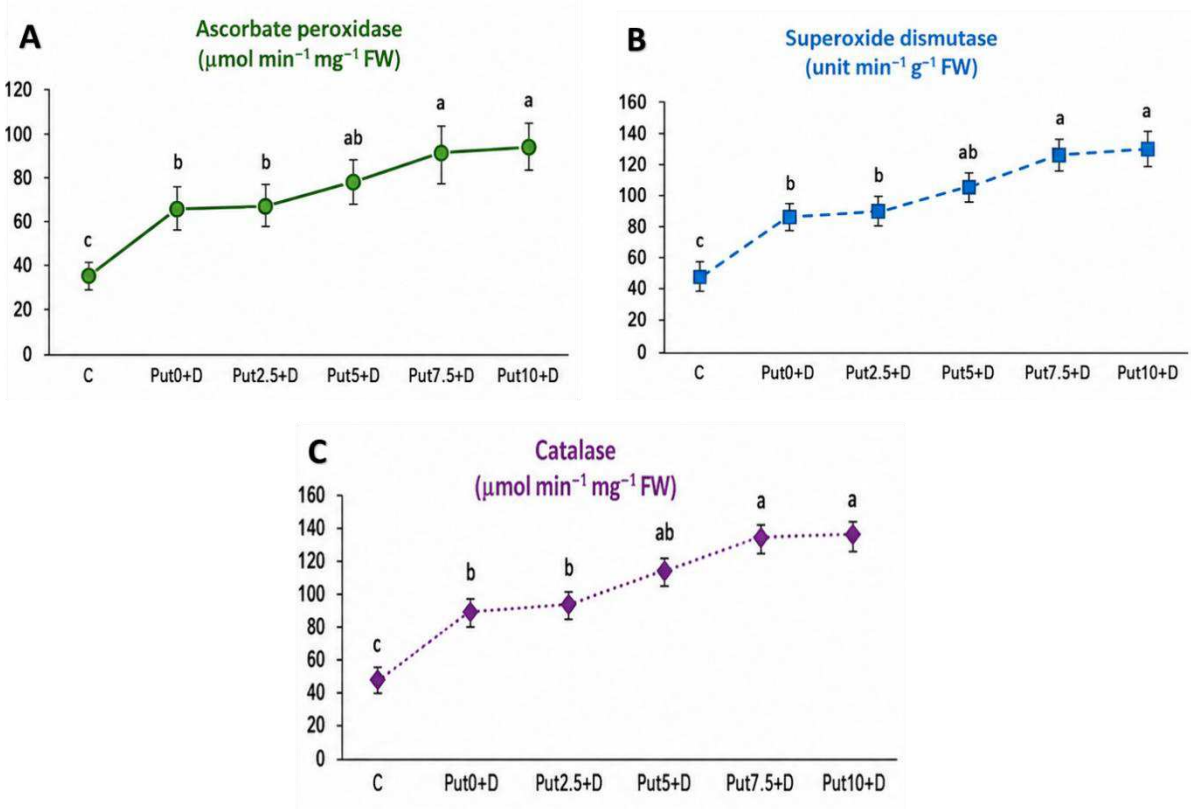
Figure 5. Putrescine-mediated regulation of osmotic adjustment and water relations in tomato under water-deficient conditions:(A) Relative water content, (B) proline content, and (C) soluble sugar content in tomato plants subjected to different concentrations of putrescine under water deficit conditions. Values represent mean ± SE. Different lowercase letters indicate significant differences among treatments at $P < 0.05$. C = well-watered control; Put0+D = drought stress without putrescine; Put2.5+D, Put5+D, Put7.5+D, and Put10+D represent foliar application of putrescine at 2.5, 5.0, 7.5, and 10.0 mM, respectively, under drought stress. FW = fresh weight.

Putrescine-induced modulation of antioxidant enzyme activity and associated gene expression dynamics in tomato plants under drought stress

Drought stress in plants triggers a cascade of physiological changes primarily due to oxidative stress, leading to the accumulation of reactive oxygen species (ROS). In response, plants activate a complex antioxidant defense system, including key enzymes like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), to combat oxidative damage. The data presented in Figures 6 and 7 highlight the impact of putrescine, applied at various concentrations, on the activity of these antioxidant enzymes, as well as on the expression of the associated genes, in drought-stressed tomato plants. APX activity in control plants was low (100.27 μmol min^{-1} mg^{-1} FW), but under drought stress, it increased significantly to 155.61 μmol min^{-1} mg^{-1} FW. Application of putrescine further enhanced APX activity, with the highest levels observed at 7.5 mM and 10.0 mM, reaching 175.71 μmol min^{-1} mg^{-1} FW and 178.06 μmol min^{-1} mg^{-1} FW, respectively. Similarly, SOD activity, initially low in control plants (22.22 units min^{-1} g^{-1} FW), increased to 32.47 units min^{-1} g^{-1} FW in drought-stressed plants, and further enhanced with putrescine treatments, reaching a peak of 38.68 units min^{-1} g^{-1} FW at 10.0 mM. Catalase activity followed a similar trend, with control plants showing the lowest activity (28.35 μmol min^{-1} mg^{-1} FW), which increased under drought stress to 58.58 μmol min^{-1} mg^{-1} FW. Putrescine treatments further increased

catalase activity, with the highest level (71.05 µmol min^{-1} mg^{-1} FW) at 7.5 mM. However, a higher concentration (10.0 mM) resulted in a slight decrease to 70.04 µmol min^{-1} mg^{-1} FW. These findings demonstrate that putrescine enhances antioxidant enzyme activity in a dose-dependent manner, with higher concentrations providing the most effective protection against drought-induced oxidative stress, particularly through the activation of APX, SOD, and CAT. The results suggest that putrescine helps strengthen the plant's antioxidant defense system, improving its ability to tolerate drought stress.

Figure 6. Putrescine-induced enhancement of antioxidant enzyme activities in tomato under water deficit conditions: (A) Ascorbate peroxidase (APX), **(B)** superoxide dismutase (SOD), and **(C)** catalase (CAT) activities in tomato plants subjected to different concentrations of putrescine under water deficit conditions. Values represent mean ± SE. Different lowercase letters indicate significant differences among treatments at P



< 0.05. C = well-watered control; Put0+D = drought stress without putrescine; Put2.5+D, Put5+D, Put7.5+D, and Put10+D represent foliar application of putrescine at 2.5, 5.0, 7.5, and 10.0 mM, respectively, under drought stress. FW = fresh weight.

Drought stress induces significant changes in the gene expression of antioxidant enzymes, and the application of putrescine at varying concentrations affects this response in tomato plants. Ascorbate peroxidase (APX) gene expression was lowest in control plants at

1.70-fold but increased to 3.90-fold in drought-stressed plants without treatment. Putrescine enhanced APX gene expression in a dose-dependent manner, with 2.5 mM, 5.0 mM, 7.5 mM, and 10.0 mM treatments showing expression levels of 3.95-, 5.10-, 6.05-, and 5.87-fold, respectively. The highest expression occurred at 7.5 mM, with 10.0 mM showing a slight decrease, indicating that 7.5 mM is the optimal concentration for maximizing APX gene expression. Similarly, superoxide dismutase (SOD) gene expression was lowest in the control group at 1.91-fold but increased significantly with putrescine treatment. Expression at 2.5 mM reached 5.16-fold, with higher concentrations (5.0 mM, 7.5 mM, and 10.0 mM) further increasing SOD activity, peaking at 6.60-fold in plants treated with 10.0 mM. Finally, catalase (CAT) gene expression also increased with putrescine treatment, starting at 1.68-fold in control plants. Treatment with 2.5 mM and 5.0 mM putrescine increased CAT expression to 4.72-fold and 4.81-fold, respectively, while treatments with 7.5 mM and 10.0 mM putrescine increased CAT expression to 5.84-fold and 6.63-fold, respectively. The statistical analysis showed significant increases in APX, SOD, and CAT expression at higher putrescine concentrations, with the 7.5 mM and 10.0 mM treatments providing the most effective boost to antioxidant defenses against drought-induced oxidative stress. These results suggest that putrescine enhances antioxidant gene expression in a concentration-dependent manner, with 7.5 mM being the most effective concentration in improving the plant's response to drought-induced oxidative stress.

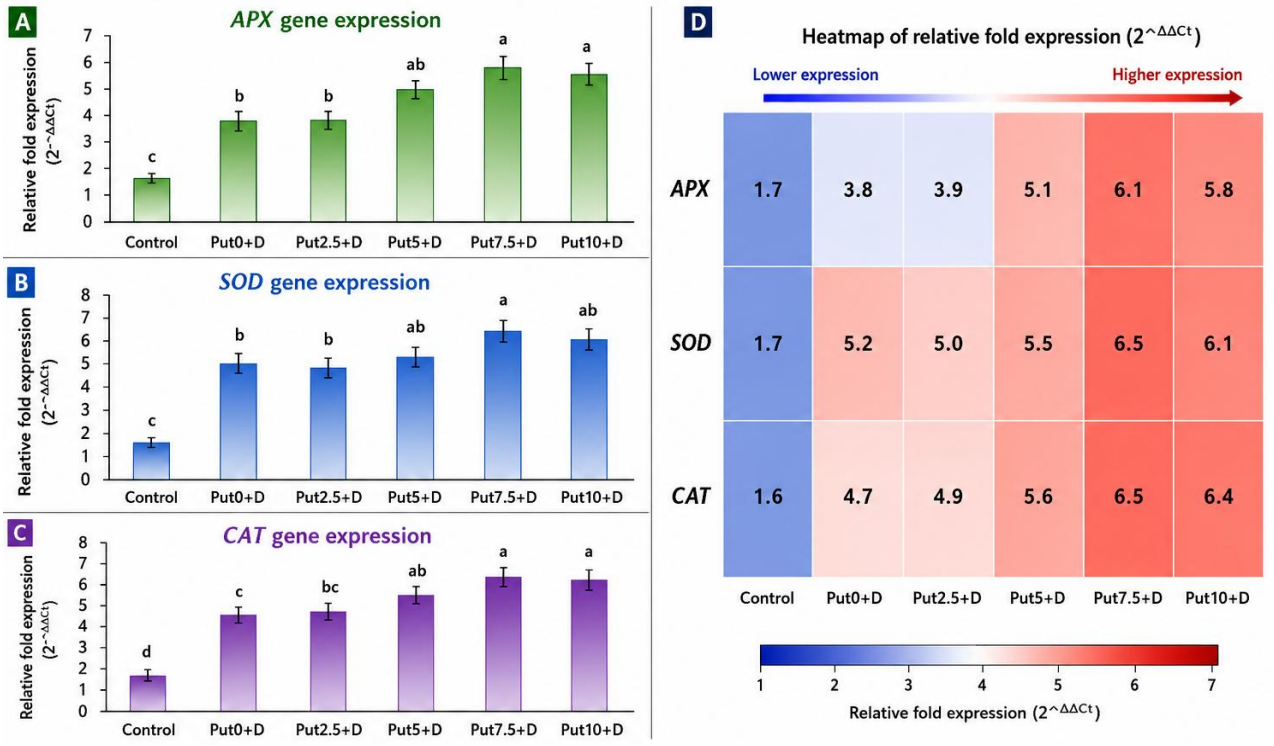


Figure 7. Putrescine-mediated transcriptional regulation of antioxidant defense genes in tomato under water deficit conditions: Relative fold expression profiles of antioxidant genes in tomato plants subjected to different concentrations of putrescine under drought stress conditions. Panels represent **(A)** APX, **(B)** SOD, and **(C)** CAT gene expression, while **(D)** presents a heatmap illustrating overall expression patterns across treatments. Values in bar graphs represent mean ± SE, and different lowercase letters indicate significant differences among treatments at $P < 0.05$. The heatmap color gradient represents relative expression intensity from lower (blue) to higher (red) transcript accumulation. C = well-watered control; Put0+D = drought stress without putrescine; Put2.5+D, Put5+D, Put7.5+D, and Put10+D represent foliar application of putrescine at 2.5, 5.0, 7.5, and 10.0 mM, respectively, under drought stress conditions.

Discussion

Drought stress exerts severe constraints on plant morphological, physiological, and biochemical processes, leading to reduced photosynthetic efficiency, impaired growth, and ultimately lower crop productivity (Oguz et al., 2022; Savchenko & Tikhonov, 2021; Preeti et al., 2026). Recent studies have further highlighted that water-deficit conditions adversely affect nutrient uptake, metabolic activity, and overall physiological efficiency in vegetable crops, thereby reducing crop performance and quality under stressful conditions (Gruda et al., 2024; Gruda et al., 2025a). The reduction in cellular turgor under water deficit conditions limits cell division and elongation, thereby restricting cell expansion, assimilate translocation, and biomass accumulation (Bhandari et al., 2023). In this context, polyamines, particularly putrescine, play a crucial role in enhancing plant resilience to abiotic stress (Shao et al., 2022). Owing to its polycationic nature, putrescine contributes to the regulation of ion homeostasis and stabilization of cellular structures (Hassan et al., 2020). Consistent with earlier findings, drought stress in tomato resulted in significant declines in growth attributes, including plant height, leaf area, and root development (Khan et al., 2023). However, foliar application of putrescine at the anthesis stage effectively mitigated these adverse effects, leading to notable improvements in growth, fruit set, and yield potential, indicating its role in facilitating metabolic and physiological adjustments under water-limited conditions. Similar observations have been reported in vegetable production systems where stress-alleviating approaches improved physiological efficiency and productivity under adverse environmental conditions (Gruda et al., 2025b). Moreover, exogenous putrescine enhances reproductive success by regulating ethylene-mediated hormonal balance, improving pollen performance, and strengthening antioxidant and osmotic defense systems, thereby reducing flower abscission and promoting fruit retention (Alcázar et al., 2010; Gill & Tuteja, 2010; Farooq et al., 2009; Chen et al., 2019).

Drought stress also disrupts photosynthetic pigments, reducing chlorophyll and carotenoid content, which limits plant productivity (Seleiman et al., 2021). Recent reviews have also emphasized that environmental stress conditions negatively influence chlorophyll stability, photosynthetic activity, and nutritional quality of vegetable crops grown under protected and open-field conditions (Gruda et al., 2025a). In this study, chlorophyll content decreased significantly under drought stress, while foliar application of putrescine enhanced chlorophyll and carotenoid levels. This improvement is likely due to putrescine's anti-

senescence properties and its ability to maintain thylakoid membrane integrity, which protects the photosynthetic apparatus under stress (Islam et al., 2022; Hussein et al., 2023). Higher carotenoid levels, which are involved in ROS scavenging, further enhance plant tolerance to oxidative damage under drought conditions (Ni et al., 2022).

Relative water content (RWC) is a key indicator of plant water status, and drought-induced reductions in RWC are often associated with increased membrane permeability and water loss (Lu et al., 2022). In our study, putrescine-treated plants maintained a higher RWC than untreated plants, suggesting improved osmoregulation and membrane stability. This is likely due to increased accumulation of proline and soluble sugars, which help regulate cell membrane permeability and detoxify ROS (Mansour et al., 2020; Sharma et al., 2023). Similar findings indicate that putrescine promotes the accumulation of these osmolytes, thereby enhancing drought tolerance by stabilizing cellular structures and improving antioxidant defense (Jahan et al., 2022). Improved osmotic adjustment and maintenance of cellular hydration have also been recognized as important adaptive mechanisms for sustaining crop productivity and quality under drought stress conditions (Gruda et al., 2024).

Drought also leads to increased accumulation of ROS, resulting in oxidative stress that damages cellular membranes, proteins, and lipids (Sachdev et al., 2021). In this study, putrescine application reduced ROS levels, membrane damage, and lipid peroxidation, particularly at higher concentrations (7.5 and 10.0 mM). This suggests that putrescine maintains cellular homeostasis under drought stress by boosting antioxidant enzyme activity and promoting the accumulation of protective osmolytes such as proline and soluble sugars. These findings are consistent with El-Beltagi et al. (2023), who reported that enhancing antioxidant defense via putrescine treatment mitigates oxidative damage and improves plant growth under water-deficit conditions. Recent studies have likewise emphasized that maintenance of antioxidant balance and cellular redox homeostasis is essential for minimizing drought-induced oxidative injury and sustaining plant productivity under stressful environments (Gruda et al., 2025b).

Antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) are critical for mitigating oxidative damage under drought stress. In the present study, foliar application of putrescine significantly enhanced the activity of these enzymes, particularly at higher concentrations (7.5 and 10.0 mM). This boost in antioxidant activity was reflected in the upregulation of the corresponding genes, APX, SOD, and CAT, which were activated under drought stress. Interestingly, SOD transcript abundance reached its highest at 7.5 mM putrescine but slightly declined at 10.0 mM, whereas APX activity remained consistently high. This indicates that changes in gene expression may not be immediately reflected at the enzymatic level. The observed variation between transcript abundance and enzyme activity could be associated with regulatory processes occurring after transcription, including differences in mRNA stability, translation efficiency, protein accumulation, and enzyme activation. Furthermore, the persistence of APX activity at higher putrescine concentrations may be due to the presence of previously synthesized APX

proteins and their continued functional role in maintaining antioxidant defense under stress conditions. The increased transcript levels of these genes play a key role in strengthening the plant's antioxidant defense system, enhancing its ability to tolerate oxidative stress (Doneva et al., 2021; Zhao et al., 2021). Similar upregulation of antioxidant genes has been observed in other crops, such as wheat (Kumar et al., 2014; Hassan et al., 2020), soybean (Zhao et al., 2021), and rice (Islam et al., 2022), were enhanced gene expression improved drought tolerance. Collectively, these findings support the hypothesis that putrescine treatment bolsters antioxidant defenses through gene activation, a vital strategy for improving plant resilience to drought-induced oxidative stress.

Conclusion

Exogenous application of putrescine substantially reduced the adverse effects of drought stress in tomato plants, particularly at concentrations of 7.5 and 10.0 mM. Putrescine-treated plants maintained better water status, higher chlorophyll retention, and improved membrane stability under water-deficit conditions. The treatment also promoted the accumulation of proline and soluble sugars, contributing to osmotic adjustment and protection of cellular structures. In addition, lower electrolyte leakage and lipid peroxidation in treated plants indicated reduced oxidative damage under drought stress. These responses were accompanied by increased activity and expression of antioxidant enzymes, including APX, SOD, and CAT, suggesting that putrescine strengthens antioxidant defense and helps maintain cellular redox balance under stress conditions. The positive physiological and biochemical responses were reflected in improved reproductive performance and yield. Under drought stress, fruit yield increased from 0.384 to 1.137 kg plant^{-1}, while fruit set improved from 20.27% to 40.83% following application of 10.0 mM putrescine. Overall, the findings demonstrate that foliar application of putrescine during anthesis can effectively improve drought tolerance in tomato by enhancing osmotic regulation, membrane stability, and antioxidant protection under limited water availability.

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Author contributions statement

G.K.R. and P.K.R. conceived and designed the study. G.K.R. and G.J. conducted the experiments and collected the data. G.K.R., R.R.K., and D.M.K. assisted in physiological and biochemical analyses and data interpretation. G.K.R., G.J. and R.R.K. contributed to laboratory analyses and data validation. M.S. and P.K. supported statistical analysis and visualization. G.K.R. and P.K.R. Prepare the figure and table. P.K. and N.S.G. provided critical input and methodological guidance and revised the manuscript. G.K.R. supervised the research, interpreted results, and finalized the manuscript. N.S.G. edits the manuscript. All authors read and approved the final manuscript.

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