JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(4), 369–383 https://doi.org/10.12911/22998993/200337 ISSN 2299–8993, License CC-BY 4.0 Received: 2025.01.07 Accepted: 2025.02.03 Published: 2025.02.17

Mitigating drought stress effects in tomato through seed priming and foliar application of salicylic acid: Impacts on germination and plant growth

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ABSTRACT

Climate change has a detrimental impact on the agricultural sector, resulting in rising temperatures and reduced water availability, which has a direct impact on decreasing crop production and sustainability. This study aimed to evaluate the effect of seed priming and foliar application of SA on seed germination and plant growth under drought-stress conditions. This research was conducted from July to September 2024 at the laboratory and greenhouse in the Faculty of Agriculture, Brawijaya University, Malang, East Java. A Randomized Complete Block Design (RCBD) with two factors was employed in this study. The first factor was drought stress with 0 and 15% polyethylene glycol (PEG), and the second factor was salicylic acid (SA) at concentrations of 0, 50, 75, and 100 μ M. The results showed that PEG 6000 significantly reduced the germination rate and growth of tomato seeds. While SA priming had no significant effect on the seed germination under normal conditions, the seeds priming with SA significantly increased the germination rate, germination stress tolerance index, shoot length stress index, root length stress index, and seed vigor index under drought stress conditions. SA priming also promoted seedling growth by increasing shoot and root lengths. Furthermore, the foliar application of SA significantly improved the vegetative growth of soil-grown tomatoes under drought stress, evidenced by improvements in key growth characteristics, including plant height, number of leaves, plant biomass, RWC, and relative chlorophyll content. Notably, foliar SA application led to a thickened tomato leaves as an early drought stress response. This study demonstrated that both priming and foliar SA application could enhance plant resilience and vigor, helping to alleviate the detrimental effects of drought stress.

Keywords: tomato, PEG, drought, salicylic acid, seed priming.

INTRODUCTION

High temperatures and water scarcity, driven by global climate change, adversely impact the agricultural sector, reducing crop productivity and sustainability. Tomato (*Lycopersicon esculentum*), a member of the Solanaceae family, is an important horticultural commodity known for its high nutritional content, such as carotenoids, lycopene, β -carotene, vitamin C, vitamin B, and

anthocyanins (Amr and Raie, 2022). According to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) (2024), the global population continues to increase yearly, reaching 8.1 billion in 2024 and is projected to increase to 9.8 billion in 2050. Therefore, the demand for tomatoes is expected to rise in parallel. The data from the Central Statistics Agency of Indonesia (2020) has seen a decline in tomato production from 66.758 tons in 2017 to 65,585 tons in 2018, respectively. Although the potential production of tomatoes is estimated at 50-70 t ha⁻¹ (Laginda et al., 2017), the actual productivity was just at 18.4 t ha-1 in 2022 (Central Statistics Agency of Indonesia, 2024). Abiotic stress such as drought, is the major yield-limiting factor in the area of low water availability for production in arid and semi-arid regions. The reduction in agricultural production occurs during the vegetative stage and sometimes during the reproductive stage of plants because of random drought conditions (Selvaraj et al., 2017). Drought stress also causes a reduction in the water potential, relative water content (RWC), and plant transpiration, usually leading to increased leaf temperature (Mahmud et al., 2016).

Additionally, it has been predicted that by 2100, abiotic stress will decrease the production of several agricultural crops worldwide by 20-55% (Ahmad et al., 2022). Moreover, drought causes a variety of physiological and biochemical changes at cellular and organism levels (Madani et al., 2019). These unfavorable conditions lead to a decline in the photosynthetic rate in plants, which results in diminished growth and an increased likelihood of early senescence. In higher plants, drought stress can affect cell elongation (Qiao et al., 2024). The phenomenon is defined by the limitation of water transport from the xylem to the surrounding elongating cells, which eventually lowers growth rates and causes plant death (Nir et al., 2020). In laboratory experiments, polyethylene glycol (PEG-6000), which is highly soluble in water, has been effectively used to mimic drought stress with limited metabolic interferences, which cannot enter the cells due to their molecular weight (Basal et al., 2020). Previous research results showed that PEG-induced plant drought stress significantly reduced seed germination rates and altered root architecture during the early stages of seedling development (Ranjan et al., 2022; Marthandan et al., 2020). Drought stress also increases the accumulation of reactive oxygen species (ROS). Under this

stress, ROS was consistently observed to be associated with decreased plant development during drought stressors. The primary locations for ROS formation in photosynthetic organisms are photosystems I and II (PSI and PSII) in the chloroplast, and oxidative stress is closely related to both photoinhibition and photosynthesis (Alam et al., 2022; Yang et al., 2021). Changes in these physiological functions can result in accumulating toxic metabolites, impair plant development, and compromise defenses. Currently, it has been confirmed that drought stress damages plant cell turgor, membranes, and organelles (Fang and Xiong, 2015). Therefore, given the example of various adverse effects of drought stress, implementing effective technology to increase the resilience of plants would reduce the effects of stress.

Salicylic acid (SA) is a small-molecule phenolic compound commonly found in plants, and moderate application does not harm the plant itself (Hashempour et al., 2014). SA is a group of hormones that help to improve plant resilience under drought stress (Iqbal et al., 2022). Thus, exogenous SA has been widely used to mitigate the detrimental effects of drought stress on plants. SA plays an important role in plant development, for instance the induction of plant flowering, root growth, seed germination, and ion uptake (Jahan et al., 2023; Dawood and Latef, 2022). As an example, seed priming has been considered one of the effective techniques in improving plant growth performance under stress conditions at the early germination stage (Samota et al., 2017). In addition, priming and spraying of 100 ppm SA improved wheat growth performance and yield under drought (Sher et al., 2017). Exogenous SA plays a role as an important signaling agent that regulates the plant antioxidant system as a defense against oxidative stress (Ahmad et al., 2021). Thus, the present study aimed to investigate the effects of seed priming and foliar application of SA on tomato seed germination and growth under PEG-induced plant stress supplementation.

MATERIALS AND METHODS

The research was conducted in the laboratory and greenhouse of the Faculty of Agriculture, Brawijaya University, Malang City, East Java, from July to September 2024. This study used a Randomized Complete Block Design with two factors. The first factor was drought stress with two levels: D0 (control) and D1 (15% PEG 6000). The second factor was the concentration of SA with four levels: S0 (control), S1 (50 µM SA), S2 (75 µM SA), and S3 (100 μ M SA). There were 8 treatment combinations, each repeated 3 times, resulting in 24 experimental units. The tomato variety used in this study was Servo F1, produced by East West Seed Indonesia. During the first stage, tomato seeds were soaked in SA solution at 50 and 70 µM concentrations in sterile water for 12 hours at room temperature with dark conditions. The seeds primed with water were used as control. After soaking, the seeds were removed from the SA solution, rinsed with water, and air-dried until they reached the original moisture content. Petri dishes lined with 6 layers of filter paper were used to germinate seeds. Then, the germination media were supplemented with 5 mL of 15% PEG to induce drought stress and 5 mL of water as a control. Once the media were prepared, 25 seeds are sown in each petri dish and incubated for 48 hours at room temperature of 25 °C. and relative humidity of 35-40%. Additionally, The PEG solution was applied daily once the preceding solution had been fully depleted. Daily observations were performed by counting the number of germinated seeds each day until day 8. Seed germination was determined when the radicle emerged from the seed (2 mm long). After seven days, the germination rate and seedling vigor index were measured according to the International Seed Testing Association (ISTA, 1996) protocol.

Furthermore, tomato seedlings with 3–4 leaves were transferred into the soil pot media (10 cm in diameter) in the greenhouse experiment for foliar application of SA. After a week of transplanting, the plants were further sprayed with SA for a week (same concentration as seed priming), prior to PEG 6000 treatment. These treatments were applied every two days for two weeks. Approximately 100 mL of 15% PEG was poured into the soil medium (until field capacity) to induce drought stress.

Morphological analysis of seed germination and seedling growth

Germination rate

The germination rate was observed based on the following formula:

$$= \frac{Germination rate (G \%)}{Total seeds germinated} \times 100$$
(1)

Promptness index

The promptness index (*PI*) was calculated using the formula employed by Ashraf et al., (1990) as follows:

$$PI = nd2 (1.00) + nd4 (0.75) ++ nd6 (0.5) + nd8 (0.25)$$
(2)

where: *PI*-promptness index, *nd*-number of seeds that germinated on the observation day.

Germination stress tolerance index

The germination stress tolerance index (GSTI) was calculated using the formula by Ashraf et al., (2008) as follows:

$$GSTI(\%) = \frac{PI \text{ of seeds under drought stress}}{PI \text{ of seeds without stress (control)}} \times 100 (3)$$

Shoot and root length stress index

The shoot length stress index (SLSI) and root length stress index (RLSI) were calculated using the formula by Shah et al., (2020) as follows:

$$SLSI(\%) = \frac{Seedling length under drought stress}{Seedling length without drought stress} \times 100(4)$$

$$RLSI(\%) = \frac{Root \ length \ under \ drought \ stress}{Root \ length \ without \ drought \ stress} \times 100$$
(5)

Vigor index

The vigor index (VI) was calculated based on the method used by Abdul-Baki and Anderson, (1973) as follows:

$$Vigor Index (VI) = Germination rate (\%) \times \times [root length + shoot length (cm)]$$
(6)

Plant growth analysis

Relative chlorophyll content

The three youngest completely expanded tomato leaves (3-week-old) were used to quantify relative chlorophyll content (Chl). The chlorophyll content was determined using the measurement index obtained by SPAD-value; SPAD502, KONI-CAMINOLTA. Inc., Tokyo. and quantified by the method used by Cerovic et al., (2012) as follows:

$$Chl = (99 SPAD)/(144-SPAD)$$
(7)

Relative water content

Relative water content (RWC) was measured according to Barrs and Weatherly (1962). Leaf tissues from randomly selected plants were weighed as fresh weight (FW), then immediately immersed in sterilized water and placed in the dark for 8 hours. Afterwards, turgid weight (TW) was weighed, and samples were placed in an oven at 80 °C for 48 hours to determine dry weight (DW). RWC was subsequently calculated using the following formula:

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100 \tag{8}$$

Leaf thickness

The thickness of the leaf tissue was measured using the paraffin method (Nakamura, 1995). Plant tissue thickness was observed on tomato leaves by making cross-sections using semi-permanent preparations. Leaf samples were taken from 3-week-old tomatoes. The leaves were cut into 1×1 cm pieces using a razor blade. The slices were then placed on a microscope slide, treated with glycerin, and covered with a coverslip. Subsequently, they were observed using an Olympus BX 43 computer microscope at 40x magnification.

Data analysis

The statistical analysis used the Analysis of Variance (ANOVA) to assess the significant differences among treatments. Tukey's Honestly Significant Difference (HSD) test at the 5% level was used to compare means, with significance established at p < 0.05. RStudio version 2024.01.1-394 was also employed to generate PCA and Pearson's analysis.

RESULTS AND DISCUSSION

Effect of seed priming on seed germination and seedling morphology

The result of this study indicated that several characteristics related to seed germination and seedling growth were significantly affected by PEG 15% treatment. Although drought stress affected all treated seeds primed with different concentrations of SA, there was a notable variation in their responses to the stress. As shown in Figure 1(A-E), SA priming was observed to improve seed germination rate, germination stress tolerance index (GSTI), shoot length stress index (SSLI), and root length stress index (RLSI) under drought stress conditions. Higher concentration of SA resulted in a higher germination rate. Notably, SA priming greatly improved seed germination rate

under drought stress compared to untreated plants (Figure 1A; Figure 2). The highest germination rate was observed at 75 and 100 µM SA, with an increase of 40% and 45%, respectively, compared to unprimed seeds. These findings suggest that SA could improve plant tolerance against drought stress. Adhikari et al. (2020) and Zulfiqar (2021) reported that under drought stress, SA reduces ABA accumulation and mitigates the decline in cytokinin and indole-3-acetic acid (IAA) levels typically induced by water deficit. These effects promote the activation of seed metabolic processes, such as enzymatic activity and mobilization of nutrient stores to promote seed germination and radicle emergence. In addition, treating SA seed priming stimulates germination rates (Ali et al., 2019; Samota et al., 2017).

Additionally, SA priming also improved the germination stress tolerance index (GSTI) (Figure 1B and Figure 2). The GSTI was significantly increased by 250% and 275% with 75 and 100 µM of SA priming, respectively, compared to the untreated plants. These findings highlight the importance role of SA in plant stress tolerance. Under stress conditions, plants produce high levels of the hormone abscisic acid (ABA) that induce seed dormancy, resulting in delaying seed germination (Vishal and Kumar, 2018). However, salicylic acid (SA) can counteract this effect by reducing the ABA levels in plants, thereby facilitating seed germination and resilience (Safari et al., 2018). Similarly, Bagautdinova et al., (2022) also demonstrated that SA mitigates the inhibitory effect by ABA and improves plant adaptation under unfavorable environmental conditions. Furthermore, SA priming also improved the shoot length stress index (SSLI) and root length stress index (RLSI) (1C, 1D, and Figure 3). Increasing SA resulted in improved SSLI and RLSI of tomato seeds. The highest SLSI values (65% and 70%) were obtained when the seed was primed with 75 and 100 µM, respectively. For RLSI, 75 and 100 µM of SA treatments also showed significant increase compared to the non-SA-primed variants with improvements of 5.9% and 10.6%, respectively. These results suggest that SA plays a vital role in promoting shoot and root elongation during germination, enhancing plant resilience under drought stress.

According to Azad et al. (2021), SA increases the tolerance of plants to unfavorable conditions, such as drought and salinity, helping to maintain normal growth and development. In addition, it



Figure 1. Comparison of germination rate (a), GSTI (b), SLSI (c), RLSI (d), and vigor index (e) of tomato seedlings at various levels of salicylic acid application under drought stress. Different letters on the top bars indicate significant differences among treatments by Tukey's test: p < 0.05. GST: germination stress index, SLSI: shoot length stress index, RLSI: root length stress index



Figure 2. Germination quality of tomato seedlings 6 days after showing (DAS) with the addition of various SA concentrations under normal and drought stress conditions

can also induce root formation, improving water and nutrient absorption. Galviz-Fajardo et al., (2020) showed that SA priming increases the performance of tomato seedlings under drought stress; effective in increasing shoot length and general vigor. Exogenous SA has also enhanced root architecture at an earlier stage of seedling development. In this study, SA priming does not only lead to a significant increased the germination rate but also the vigor index, under both



Figure 3. Morphological appearance of tomato seedlings the addition of various concentrations of SA under normal and drought stress conditions. The figure represents 10 days old seedlings

normal or drought conditions. Moreover, under drought stress conditions, the seeds treated with SA showed higher viability than the control (Figure 1E and Figure 3). Meanwhile, Pranata and Zakariyya (2021) also reported that applying SA significantly enhanced seed viability and vigor index, especially under drought-stress conditions. The study also has shown that SA priming can modulate the levels of growth hormones such as gibberellins and abscisic acid, which are critical for germination and seedling establishment. This hormonal regulation promotes germination and enhances the resilience of seedlings to subsequent drought stress, improving their survival and growth rates (Abdelaal et al., 2020).

Effect of SA and PEG 6000 on soil-grown tomatoes

In this study, SA priming was also applied to tomato plants to assess its effect on the growth of soil-grown tomatoes. Drought stress is known to adversely affect plant growth, leading to morphological changes such as necrotic and curled leaves, which are indicative of stress responses (Figure 4). According to Ziogas et al., (2021), drought stress causes detrimental effects on plant morphology and induces leaf necrosis and abscission that might related to programmed cell death. The obtained results also demonstrated that foliar application of SA affects several plant growth parameters in tomatoes (Figure 5A-E), including a significantly higher plant height under normal and drought stress conditions compared to their respective controls (Figure 5A). Under normal conditions, increasing the SA concentration led to an increase in plant height, although no significant difference was observed between the 75 and 100 µM concentrations. Conversely, under drought conditions, there was a decrease in plant height at SA concentrations of 100 µM. The plant height recorded at SA concentrations of 0, 50, 75, and 100 µM were 25 cm, 35 cm, 38 cm, and 30 cm, respectively. This suggests that while moderate



Figure 4. Morphological appearance of tomato leaves with various concentrations of SA under normal and drought stress conditions



Figure 5. Comparison of plant height (a), number of leaves (b), biomass (c), RWC (d), and chlorophyll (e) of tomatoes at various levels of SA under normal and stress conditions. Different letters on the top bars indicate significant differences among treatments by Tukey's test: p < 0.05. RWC: relative water content

concentrations of SA can enhance growth, higher concentrations may inhibit it, particularly under stress conditions. Tang et al. (2023), reported that SA is essential for plant growth and stress adaptation, with optimal SA promoting positive impact on plant growth. Conversely, excessive SA concentrations can inhibit plant growth. These observations are consistent with the findings by Sofy et al. (2020) and Hara et al. (2012), who observed that low SA concentrations enhanced stress tolerance without affecting plant growth, while higher concentrations had negative impact and even causing cell death. In addition, the study exhibited that SA plays an important role in regulating cell division and cell elongation, which influences increased vegetative growth (Fujikura et al., 2020).

Furthermore, under both normal and drought stress conditions, foliar SA application significantly increased the number of leaves (Figure 5B). Under well-watered conditions, the exogenous SA significantly increased the number of leaves compared to untreated plants with 13.8%, 17.2%, and 17.2% at 50, 75, and 100 μ M concentrations, respectively. Meanwhile, under drought

conditions, no significant differences were found at a concentration of 100 µM compared to the control plants. However, at the concentrations of 50 μ M and 75 μ M, the number of leaves were significantly increased. In addition, exogenous SA was observed to have increased plant biomass under both normal and drought stress conditions (Figure 5C). Under normal conditions, the SAtreated plants showed higher biomass than the untreated plants, with the 75 µM SA treatment resulting the highest increase of 44.4% compared to the control. In comparison, the 50 and 100 µM treatments showed increases of 22.2% and 27.8%, respectively. Notably, the increase in plant biomass under drought further support the ameliorative effect of foliar SA in response to such stress. In fact, the plant biomass was marginally higher in the plant treated with 75 µM SA + drought compared to control plants under well-watered conditions. Overall, the increase in biomass subjected to drought with foliar SA at 50, 75, and 100 µM was 18.75%, 37.5%, and 15% higher than the control plants, respectively.

Better morphology and biomass in the plants under drought can also be attributed to the application of SA. Previous studies have shown that SA plays an important role in plant growth and interacts with the hormone auxin. SA helps increase the synthesis of auxin, which is important for the growth and development of plant organs, including leaves (Pasternak et al., 2019). Furthermore, Emamverdian et al. (2020) reported that SA can interact with other hormones, such as gibberellin and auxin, which are involved in promoting vegetative growth and biomass production. Additionally, the growth-promoting effects of SA are often attributed to its ability to regulate photosynthesis and transpiration, the processes that are crucial for biomass accumulation (Sari et al., 2023). Moreover, foliar application of exogenous SA on tomato seedlings under drought stress conditions significantly increased RWC compared to the untreated plants (Figure 4D). Specifically, under stress conditions, RWC increased by 5.5%, 36.4%, and 18.2% in 50, 75, and 100 µM SA concentrations, respectively. Nevertheless, no significant differences in RWC were observed under normal conditions. Interestingly, all treated seedlings with foliar SA subjected to drought have shown no significant difference in relative to all treatments under well-watered condition. These findings highlight a significant role of SA in maintaining water potential under drought stress conditions. In addition, this study found that exogenous SA increased the relative chlorophyll content under both normal and drought conditions (Figure 4E). The increase in the concentration of SA was found to increase the relative chlorophyll content. Under normal conditions, 50, 75, and 100 μ M of foliar SA increased relative chlorophyll content by 6.7%, 13.3%, and 16.7%, respectively. Meanwhile, chlorophyll content was increased by 7.7%, 30.8%, and 26.9% under drought conditions. Interestingly, foliar application of 75 and 100 µM SA under drought stress resulted in higher relative chlorophyll content compared to untreated plants under both water regimes. These findings suggest that foliar SA treatment not only improves the chlorophyll content in drought-stressed plants but also enhances the chlorophyll content in the plants grown under normal water conditions.

Salicylic acid is believed to regulate stomatal closure through its interaction with abscisic acid (ABA), hereby reducing water loss during drought and maintaining cellular relative water content (Park et al., 2021; Mimouni et al., 2016). SA also plays a pivotal role in controlling transpiration and enhancing plant resilience to drought stress (Ma et al., 2017; Majeed et al., 2016). Furthermore, SA stimulates the accumulation of osmolytes, such as proline and soluble sugars, which facilitate osmotic adjustment and protect cellular structures under drought conditions (Abdelaal et al., 2020). Exogenous SA promotes chlorophyll synthesis and enhancing the activity of key enzymes involved in photosynthesis, such as ribulose-1,5-bisphosphate carboxylase/ oxygenase (RubisCO) (Yousefvand et al., 2022). The synergistic effect of SA with other nutrients, such as zinc, has also been reported to further enhance chlorophyll content and antioxidant activity, thereby improving photosynthetic efficiency (Choudhary et al., 2016). Moreover, enhancing chlorophyll content is associated with improved relative water content and reduced electrolyte leakage, indicating better cell membrane stability and function (Amiri et al., 2023).

SA increased leaf thickness under drought stress

The obtained results exhibited that either SA or PEG affects the leaf thickness of tomatoes. The application of PEG 6000, which simulates drought stress, resulted in a significant reduction in leaf thickness by up to 31%, compared to normal conditions. Conversely, the foliar application of SA appears to mitigate some of the adverse effects of drought stress. Under normal conditions (without PEG), increasing SA concentrations of 50, 75, and 100 µM significantly decreased leaf thickness by 18%, 25%, and 27%, respectively (Table 1). Moreover, the tomato leaves treated with SA tend to be thinner than the control plants (Figure 6A-D). However, under drought stress conditions, foliar application of 50 µM SA significantly increased leaf thickness compared to the 0 µM SA (Figure 6E-H). Interestingly, leaf thickness at 50 µM SA was 21% higher than the control plants. Those results suggest that SA affects leaf thickness differently under normal and stress conditions, primarily through its role in physiological and biochemical adaptations that improve water retention and photosynthetic efficiency. Plants may decrease or increase the thickness of these leaves to adapt to drought stress. Previous studies have shown that drought stress reduces the water potential in plant cells, causing osmotic imbalance and inhibiting

Trootmonto	Leaf thickness (µm)					
Treatments	Control	PEG 15 %				
0.044.64	457.37 a	316.00 b				
0 μινι 5Α	А	С				
EQ UM SA	377.30 b	399.70 a				
50 µM SA	В	А				
75 µM SA	343.80 a	355.73 a				
	С	В				
100	332.94 a	343.51 a				
	С	В				
HSD 5%	14.905					

Table 1. Interaction between PEG 6000 induceddrought stress and SA concentration on leaf thicknessof tomato

Note: numbers accompanied by the same lowercase letter in the same row or the same capital letter in the same column are not significantly different based on the HSD test at the 5% level.

plant growth, leading to morphological changes, including changes in leaf area and thickness (Avivi, 2023; Kumar et al., 2017). The application of PEG 6000, which simulates drought stresses by lowering water potential, may induce physiological stress in plants, resulting in various modifications in leaf anatomy (Din et al., 2020). Under stress conditions, particularly drought stress, leaves normally become thicker as an ecophysiological adaptation to enhance survival (Aneja et al., 2024; Lamalakshmi et al., 2017). In the current study, the observed increase in leaf thickness in the plants subjected to drought may be attributed to an elevation in chlorophyll content, which could directly enhance photosynthetic activity and carbohydrate production. Additionally, Conesa et al. (2020) reported that increased leaf thickness is associated with the thickening of the upper epidermis, which serves to reduce water loss from leaves under dry conditions. Thicker leaf anatomy facilitates higher absorption of light, thereby optimizing the process of photosynthesis under stress conditions (Chang et al., 2016). Moreover, leaf thickness allows the leaf to have greater gas exchange capacity and can retain water when water availability is limited (Hoque et al., 2018). The thickened leaves may commonly be an anatomical adaptation in plants as a response to drought stress to reduce transpiration rate, thereby increasing water use efficiency in their environment (Tang et al., 2023).

Principal components analysis (PCA) and Pearson's correlation

PCA analysis was performed to determine the relationship between different SA concentrations and their impact on tomato plants' seed germination and growth variables under normal and stress conditions. The result showed that Dim1 and Dim2 explained 68.2% and 25.7% of the total data variation, reflecting the major impact of SA on plant growth variables. Under normal conditions, untreated plants showed lesser variability in most growth parameters. In contrast, the addition of 50 µM of SA showed very strong positive relations with the variables of growth-related attributes, which include promptness, germination rate, vigor index, and leaf number, which also indicated that this dose is an optimal concentration that enhances such growth attributes (Rhaman et al., 2021). In addition, higher SA concentrations (75 and 100 μ M) had the tendency to be positively



Figure 6. Salicylic acid on leaf thickness of tomato under normal and drought stress conditions. A) 0 μ M SA, 0% PEG treatment; B) 50 μ M, 0% PEG; C) 75 μ M SA, 0% PEG; D) 100 μ M, 0% PEG; E) 0 μ M, 15% PEG; F) 50 μ M, 15% PEG; G) 75 μ M, 15% PEG; H) 100 μ M, 15% PEG



Figure 7. PCA analysis of seed germination characteristics and the growth of tomatoes under normal and drought stress conditions. Blue indicates variables, and red indicates treatments. RLSI: root length stress index, SLSI: shoot length stress index, GST: germination stress index, RWC: relative water content

correlated with other growth variables like RWC and plant height. Exogenous application of SA can enhance physiological responses in plants, especially under stress conditions (Isa, 2021). Under drought stress, the 75 and 100 µM SA significantly affected variables such as GSTI, RLSI, SLSI, chlorophyll content, and biomass production. These results align with previous studies that have exhibited the role of SA in mitigating stress effects and improving plant resilience (Ximénez-Embún et al., 2018). In contrast, untreated plants and the plants treated with 50 μ M SA showed less relationship with those parameters under drought conditions, indicating that the SA concentration may exert a threshold effect on plant responses against stress. This is in accordance with another study which reported that low concentrations of SA are generally not sufficient to activate the whole stress response pathway. In contrast, higher concentrations can enhance stress tolerance by regulating physiological and biochemical processes (Vijayakumar et al., 2021).

Furthermore, Pearson's correlation coefficients demonstrated significant relations between several variables, as illustrated in the correlation matrix in Figure 8. The germination rate and promptness were highly positively correlated (0.98), which means that a high germination rate is strongly associated with high promptness. Significant positive correlations between germination rates and various growth parameters indicate that prompt germination is critical for optimal plant development (Shibeshi and Kassa, 2021; Thapa et al., 2023). The vigor index and germination also showed a strong positive association of 0.97, indicating that the more vigorous the seeds result in better germination. A previous study reported that the seed vigor is an important determinant of germination speed and overall plant performance (Singh and Shweta, 2020). Furthermore, RLSI, SLSI, and GSTI showed a very strong correlation with each other (about 0.99); such consistency among stress indices is crucial index to be considered reliable in the evaluation of plant robustness under different conditions (Pugh et al., 2018; Arnoult et al., 2015).

A positive correlation of 0.77 was also obtained between the chlorophyll content and RWC, thus indicating that chlorophyll levels are related to water availability in plant tissues. This correlation is important, because it underlines the role of chlorophyll in photosynthesis and its dependence on an optimal water supply to support plant growth (Li et al., 2018). This gave a very high value of the correlation coefficient, up to 0.91, showing the dependency of biomass accumulation on plant height. This is confirmed by previous

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Germination	1.00	0.98	0.97	0.25	0.25	0.25	0.46	0.82	0.28	0.91	0.91		
Promptness	0.98	1.00	0.96	0.11	0.11	0.11	0.29	0.71	0.17	0.86	0.86		- 0.8
Vigor index	0.97	0.96	1.00	0.38	0.37	0.38	0.52	0.84	0.37	0.93	0.93		- 0.6
RLSI	0.25	0.11	0.38	1.00	0.97	0.99	0.90	×	0.70	0.55	0.55		- 0.4
SISI	0.25	0.11	0.37	0.97	1.00	0.99	0.91	×	0.83	0.52	0.52		- 0.2
GSTI	0.25	0.11	0.38	0.99	0.99	1.00	0.92	×	0.77	0.53	0.53		- 0
Chlorophyll	0.46	0.29	0.52	0.90	0.91	0.92	1.00	>>>	0.81	0.66	0.66		0.2
RWC	0.82	0.71	0.84	▶	X	>>>	₩	1.00		0.86	0.86		0.4
Biomass	0.28	0.17	0.37	0.70	0.83	0.77	0.81	\mathbf{M}	1.00	0.41	0.41		0.6
Height	0.91	0.86	0.93	0.55	0.52	0.53	0.66	0.86	0.41	1.00	1.00		0.8
Leaf number	0.91	0.86	0.93	0.55	0.52	0.53	0.66	0.86	0.41	1.00	1.00		-0.0

Figure 8. Correlation matrix visualization of the correlation among variables under normal and drought stress conditions. The cross in the image indicates no correlation. Positive correlations (closer to 1, blue) indicate a strong positive relationship. Negative correlations (closer to -1, red) suggest an inverse relationship. RLSI: root length stress index, SLSI: shoot length stress index, GST: germination stress index, RWC: relative water content

studies that emphasized plant height as a critical factor for predicting biomass production (Wang et al., 2022). The leaf number and the height also showed a highly positive correlation of 0.93, indicating taller plants tend to have a higher number of leaves. Leaf area is a key factor for light interception and photosynthetic capacity (Harkel et al., 2019), correlated with increasing plant biomass. In contrast, SLSI and chlorophyll, or RLSI and RWC, were weaker correlates, closer to 0.4 or lower. These results indicate that there is almost no dependence between these variables. This shows that the problem of plant physiological responses caused by environmental stress varies, as shown in the correlation between certain indices.

CONCLUSIONS

The findings of this study concluded that drought stress affected the germination performance of tomato seeds. PEG 6000 as a drought stress mimics decreased germination parameters, including germination rate, Germination stress index, shoot length stress index, root length stress index, and vigor index. However, SA priming increased seed germination and vigor under stress conditions. The obtained results also highlighted that the foliar application of SA effectively enhanced the growth of soil-grown tomatoes. SA increased plant height, leaf number, biomass, relative water content, and relative chlorophyll content under normal and water-limited conditions. Seed priming and foliar application treatments at 75 ppm SA showed the most positive results on all observed germination or growth parameters among the SA concentrations tested. The findings of this study highlighted the potential of SA not only in reducing the negative impacts of drought stress but also in increasing overall plant resistance and vigor. However, future studies are recommended to test different levels of PEG or SA to optimize the accurate concentration and tolerance in plants.

Acknowledgments

The authors would like to thank the Faculty of Agriculture, Universitas Brawijaya, for providing

funding to finish all experimental processes with grant number 00810.22/UN10.F0401/B/KS/2024.

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