



Exogenous Salicylic Acid Induced Drought Stress Tolerance in Immature Tea (*Camellia sinensis* L.) Plants

M.M.N. Damayanthi^{1,3*}, T.L. Wijeratne¹ and J.W. Damunupola^{2,3}

¹Plant Physiology Division, Tea Research Institute of Sri Lanka, Talawakele

²Department of Botany, Faculty of Science, University of Peradeniya, Sri Lanka

³Postgraduate Institute of Science, University of Peradeniya, Sri Lanka

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Damayanthi, M.M.N. 

<https://orcid.org/0000-0002-1711-611X>



ABSTRACT

Salicylic acid (SA) has been known to induce drought tolerance in many plant species. In this study, we investigated the potential of exogenous application of SA to enhance drought tolerance in immature tea plants under glasshouse conditions at the Tea Research Institute in Talawakelle, Sri Lanka. One-year-old potted tea cultivars known for drought tolerance were used in the study. The plants were subjected to a drying cycle while being foliar sprayed with different concentrations of SA along with well-watered (WW), water-spray (WS) and no-spray (NS) treatments. Data were collected at 18 hours, 14 days after spraying (DAS), 21 DAS, and during the recovery after re-watering at 21 DAS. Based on the results obtained from the glasshouse study, the effective concentration of 150 mg L⁻¹ SA was selected for further testing under field conditions in Talawakelle using three-year-old tea plants. The field experiment followed a randomized complete block design (RCBD) with three blocks. When the plants reached a moderate moisture stress level, they were foliar-sprayed with 150 mg L⁻¹ SA, WS and NS treatments were included as controls. Data were collected at 7 DAS, 14 DAS, 21 DAS, and during the recovery phase after rain. The results showed that drought stress led to a decline in gas exchange parameters, relative water content, and an increase in the accumulation of osmolytes. However, the exogenous application of 150 mg L⁻¹ SA significantly improved physiological processes such as gas exchange, osmolyte accumulation, and antioxidant activity, thus effectively enhancing drought tolerance in immature tea plants.

*Corresponding author- dnalika@yahoo.com

INTRODUCTION

Drought stress is one of the most crucial environmental factors that affect plant growth, development, and plant production. In Sri Lanka, the decline in tea production due to drought was reported as approximately 4% in 1983, 26% in 1992, and 11% in 2016 compared to previous years (Wijeratne, 1996; Central Bank Annual Report, 2016). Drought influences plant physiological processes, changing cellular mechanisms and finally affecting the crop growth, productivity, and quality of tea (Das et al., 2021).

Tolerance of plants to abiotic stresses including drought can be increased by exogenous application of certain growth enhancers such as amino acids, phytohormones, soluble sugars, potassium etc. (Farooq et al., 2009). Exogenously applied phytohormones have been reported to exhibit various roles in improving drought tolerance of many plants (Pandey et al., 2003; Farooq et al., 2009; Akter et al., 2014). Most hormones generate signal transduction that leads to a series of events to achieve physiological adaptation of the plant to stress.

Salicylic acid (SA) is an endogenous growth regulator naturally produced by plants and belongs to the group of phenolic acids. It plays an important role in regulating plant growth, development, and physiological processes such as photosynthesis, and metabolism. SA also enhances plant resistance to biotic and abiotic stresses (Hassoon and Abduljabbar, 2019).

The application of SA significantly improved physiological and biochemical parameters in many plants, such as the rate of photosynthesis, chlorophyll content, relative water content, antioxidant activity, and osmolyte accumulation in plants like *Myric rubra* and *Gardenia jasminoides* (Ying et al., 2013; Yao et al., 2016). SA application has also shown positive impacts on growth parameters and yield in *Triticum aestivum* under drought stress conditions (Sohag et al., 2020; Ahmed et al., 2021). Hayat et al. (2008) reported that SA enhances the drought tolerance of plants by regulating photosynthetic parameters, membrane stability, water potential, and activity of

antioxidant enzymes. SA also enhances gas exchange processes, chlorophyll content, and antioxidant enzyme activity under drought in rubber (Nakandala et al., 2016). Furthermore, SA plays a role in rice by maintaining tissue water potential, accumulating compatible solutes, and enhancing the antioxidant system during drought stress (Farooq et al., 2009). According to Munne-Bosch and Penuelas (2003), SA plays an essential role in preventing oxidative damage and improving chlorophyll in *Phillyrea angustifolia* plants.

Exogenous SA increased flavonoid biosynthesis in *Camellia sinensis* leaves only at ambient CO₂ (Li et al., 2019). SA application too caused increasing growth rate, changing plant physiological processes, and decreased adverse effects of drought stress on sweet basil plants (Kordi et al., 2013), increased number of leaves per plant, leaf size, fresh and dry matter yield of red amaranth (Khandaker et al., 2011). SA application had a positive impact on wheat crop for enhancing its productivity (Ahmad et al., 2021).

Knowledge of plant hormonal regulation is crucial in crop management practices, particularly for mitigating the negative impacts of drought stress. While substantial progress has been made in studying different crops and minimizing drought effects through the exogenous application of SA, comprehensive information specific to tea plants remains limited. Additionally, our understanding of tea's hormonal physiology under drought stress conditions is hindered by a lack of research on hormonal effects related to gas exchange, water balance, osmolyte accumulation, and other factors. However, the potential for exogenous hormone application to alleviate drought effects on tea plants exists. Therefore, this study was conducted to investigate the relationship between the exogenous application of SA and the physiological and biochemical responses in various tea cultivars under drought stress conditions.

METHODOLOGY

Experiment 1

The experiment was conducted in a glasshouse with an average temperature of

28 °C and photosynthetically active radiation of 1200 $\mu\text{moles m}^{-2} \text{ s}^{-1}$ at the Tea Research Institute of Sri Lanka using one-year-old potted tea cultivars, namely, TRI 2025 (drought-tolerant) and TRI 2023 (drought-susceptible). The plants were brought to field capacity and then subjected to a drying cycle by withholding water until moderate moisture stress was reached (gravimetric water content around 25%). Subsequently, the plants were foliar sprayed with SA at various concentrations [0 (water-spray - WS), 50, 100, 150, and 200 mg L^{-1}], along with well-watered (WW - positive control) and no-spray (NS - negative control) treatments.

Data were collected at 18 hours, and 14 and 21 days after spraying (DAS) from randomly selected plants arranged in a Randomized Complete Block Design with 2 blocks and 24 plants per cultivar per treatment in each block. Blocking was done perpendicular to the variation of the light level on the two sides of the glasshouse. Physiological parameters (gas exchange parameters and relative water content) and biochemical parameters (total soluble sugar content, proline content, polyphenol content, antioxidant activity, and pigment concentrations) were measured from 9 am to 12.30 pm using recently matured fully-expanded leaves, along with soil moisture content.

A portable photosynthesis system (model: LI-6400XT, Li-Cor Inc., USA) was used to determine the net photosynthetic rate, stomatal conductance, and transpiration rate. Dark respiration was also measured in another set of similar maturity leaves by covering the leaves with aluminum foil for 30 minutes prior to the gas exchange measurements. At 21 DAS, the plants were re-watered up to field capacity, and the recovery of plants was visually assessed after another 7 days.

Statistical analysis was performed using ANOVA, and mean separation was done by Duncan New Multiple Range Test using the SAS statistical package. The drought tolerance index (DTI) for each parameter was calculated by taking relative values for each treatment using the corresponding values of

well-watered plants as the reference (Equation 1) using SAS statistical software, following the method described by Al-Azab et al. (2022).

Example:

$$DTI_p = \frac{X_p}{W_p} \quad \text{Equation 1}$$

Where DTI_p = Drought Tolerance Index for Photosynthetic Rate, X_p = Photosynthetic Rate of a specific treatment at a particular measurement interval, W_p = Corresponding Photosynthetic Rate of well-watered (WW) condition at the same measurement interval. The experiment was repeated twice to confirm the trends of the results. The average temperature was 28 °C, the average relative humidity was 50%, and the Photosynthetically Active Radiation was 1200 $\mu\text{moles m}^{-2} \text{ s}^{-1}$.

Experiment 2

This experiment was conducted at the Field No 08NC (elevation: 1440 m amsl, latitude: 6° 53' 6.84" N, longitude: 80° 40' 17.95" E) at the Fairfield Division, Barewell Estate, Talawakelle (agro-ecological region WU2) using three-year-old cultivar TRI 4078 (drought tolerant). The average annual rainfall in this region is 2250 mm and maximum and minimum temperatures were 22.5 °C and 14 °C, respectively. The experiment was arranged in a Randomized Complete Block Design (RCBD) with 3 replicates. When plants reached a moderate moisture stress (gravimetric water content - 28.93%), they were foliar sprayed with SA in selected concentration (150 mg L^{-1}) based on the results of the glasshouse studies along with water-spray (WS) and no-spray (NS) treatments.

Data were collected from randomly selected plants at 7, 14, 21 days after spraying (DAS) and during the recovery after rain (75 DAS). Soil moisture content in the active root zone depth was measured using the gravimetric method, along with the physiological and biochemical parameters. Gas exchange parameters were measured as described in the previous experiment.

Leaf relative water content (RWC) was measured using the method described by

Lafitte (2002). Antioxidant activity of leaves was measured using the DPPH radical scavenging method (Prieto, 2012) and FRAP assay (Benzie and Strain, 1996). Leaf total soluble sugar content was measured according to Dubois et al. (1956), and leaf proline content was measured according to Bates et al. (1973) using oven-dried, powdered leaf samples from both experiments. Leaf polyphenol content was measured according to ISO14502-1 (2005). Chlorophyll a, b, and total carotenoid concentrations were also measured according to Lichtenthaler and Welburn (1983).

Data were statistically analyzed using Analysis of Variance (ANOVA) procedure for each cultivar and for each measurement interval separately. Means were separated using Duncan's Multiple Range test.

RESULTS AND DISCUSSION

Experiment 1

The mean soil moisture content in the glasshouse trial, given as the volumetric water content averaged across all treatments (except well-watered plants), was 25.23%, 8.21%, and 6.51% at 18 hours, 14 days, and 21 days after spraying (DAS), respectively. Drought stress led to a decline in leaf relative water content (RWC) in both tea cultivars. Cultivar TRI 2025 generally maintained a higher RWC compared to TRI 2023 cultivar on most occasions (Data not shown). The application of SA resulted in a slower reduction of RWC in both cultivars as drought stress progressed. At 21 DAS, the maintenance of a comparatively higher Drought Tolerance Index (DTI) for RWC was observed in plants sprayed with 150 mg L⁻¹ and 200 mg L⁻¹ SA, compared to the water-spray (WS) and no-spray (NS) conditions in both cultivars (Figure 1).

In TRI 2025, even at 18 hours after spraying SA at concentrations of 100, 150, and 200 mg L⁻¹, the plants exhibited a significantly higher Drought Tolerance Index (DTI) for photosynthetic rate ($P < 0.05$). However, in cultivar TRI 2023, only the plants sprayed with 150 mg L⁻¹ of SA showed the highest DTI ($P < 0.05$) for photosynthetic rate at 18 hours

after spraying. Spraying SA at concentrations of 150 and 200 mg L⁻¹ resulted in a higher DTI ($P < 0.05$) for photosynthetic rate even at later stages (14 DAS and 21 DAS) in both cultivars (Figure 2).

As drought progressed, stomatal conductance gradually declined. Similar to the rate of photosynthesis, the application of SA resulted in a much slower reduction of stomatal conductance in both cultivars. In cultivar TRI 2025, significantly higher Drought Tolerance Index (DTI) for stomatal conductance was observed in plants sprayed with 150 and 200 mg L⁻¹ of SA throughout the measurement intervals (Figure 3). On the other hand, in cultivar TRI 2023, there were no significant differences ($P > 0.05$) initially observed for the application of SA at different concentrations. However, spraying SA at concentrations of 150 and 200 mg L⁻¹ resulted in a significantly higher DTI ($P < 0.05$) for stomatal conductance at latter stages (14 DAS and 21 DAS).

As drought progressed, the rate of transpiration gradually declined in both cultivars to varying degrees. At 14 DAS, TRI 2025 cultivars treated with 200 mg L⁻¹ of SA exhibited a significantly higher Drought Tolerance Index (DTI) for the rate of transpiration, followed by the 150 mg L⁻¹ treatment (Figure 4). At a later stage (21 DAS), the 150 mg L⁻¹ SA-treated plants showed a higher DTI ($P < 0.05$) for the rate of transpiration in the TRI 2025 cultivar, followed by the 200 mg L⁻¹ SA-treated plants. The lowest DTI was observed in the no-spray (NS) treatments in both cultivars at the later stages. However, the application of SA at concentrations of 150 and 200 mg L⁻¹ resulted in relatively higher DTI for the rate of transpiration at 14 DAS and 21 DAS in the TRI 2023 cultivar.

Moisture stress led to a significantly higher dark respiration ($P < 0.05$) compared to the well-watered treatments at 10 DAS. The maintenance of significantly lower dark respiration was observed in plants sprayed with SA at concentrations of 150 and 200 mg L⁻¹ in the TRI 2025 cultivar and at a concentration of 200 mg L⁻¹ in the TRI 2023 cultivar, compared to other treatments (Table 1)

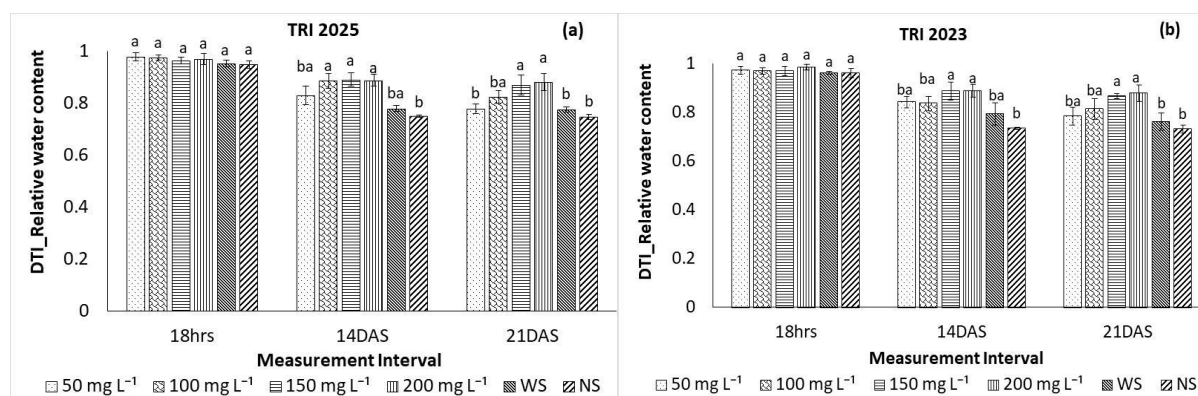


Figure 1: Variation of drought tolerance index (DTI) for relative water content (RWC) of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing plants to drought stress. Bars with different lowercase letters are significantly different at $p < 0.05$ in each measurement interval (18hrs: 18 hours after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

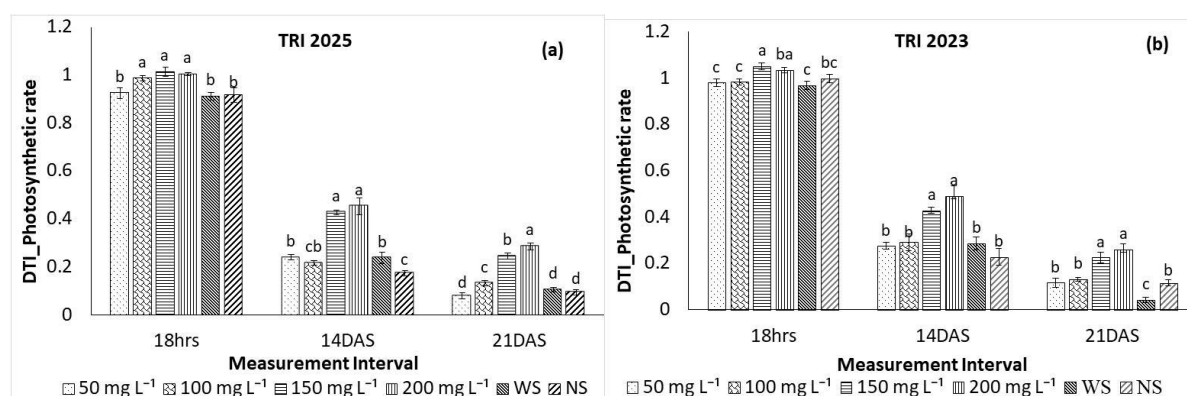


Figure 2: Variation of drought tolerance index (DTI) for photosynthetic rate of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing the plants to drought stress. Bars with different lowercase letters are significantly different at $p < 0.05$ in each measurement interval (18hrs: 18 hours after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

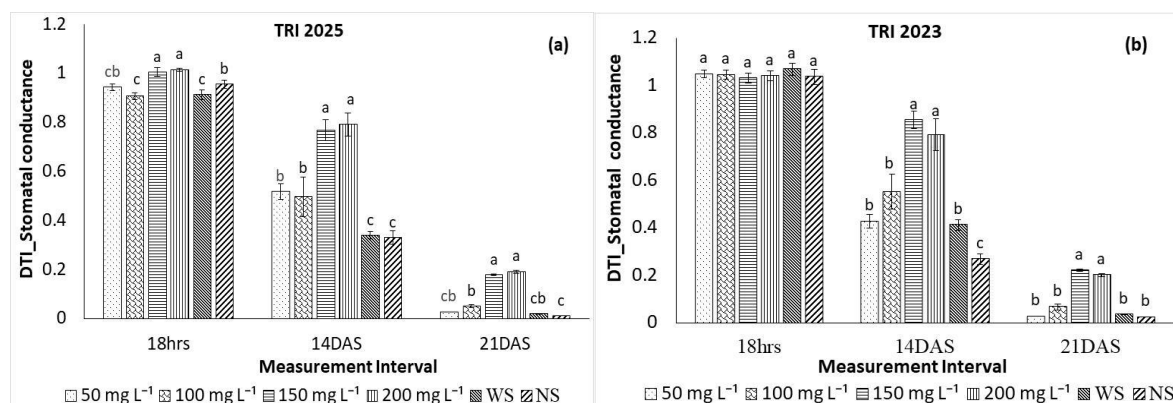


Figure 3: Variation of drought tolerance index (DTI) for stomatal conductance of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing plants to drought stress. Bars with different lowercase letters are significantly different at $p < 0.05$ in each measurement interval (18hrs: 18 hours after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

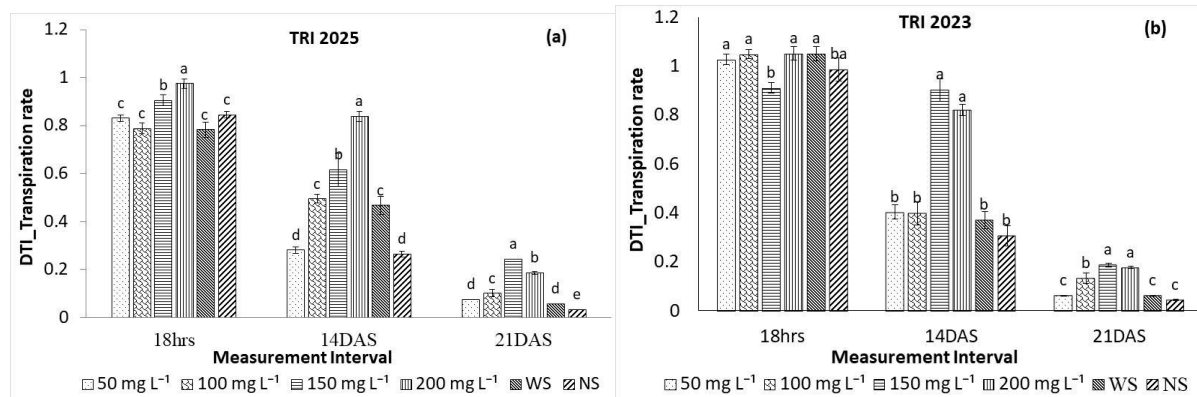


Figure 4: Variation of drought tolerance index (DTI) for transpiration rate of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing plants to drought stress. Bars with different lowercase letters are significantly different at $p < 0.05$ in each measurement interval (18hrs: 18 hours after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

Table 1: Variation of dark respiration of cultivar TRI 2025 and TRI 2023 at 10 days after spraying of salicylic acid (SA) and exposing plants to drought stress.

Treatment	Dark respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	
	TRI 2025	TRI 2023
SA@50 mg L ⁻¹	4.31 ^c	4.57 ^c
SA@100 mg L ⁻¹	3.80 ^c	4.54 ^c
SA@150 mg L ⁻¹	2.28 ^b	4.51 ^c
SA@200 mg L ⁻¹	2.47 ^b	4.08 ^b
Water-spray	4.08 ^c	4.91 ^d
No-spray	4.01 ^c	5.82 ^e
Well-watered	1.61 ^a	2.22 ^a
P value	<.0001	<.0001

Numbers with different lowercase letters are significantly different at $p = 0.05$ in each measurement interval; SA = salicylic acid.

Drought stress resulted in increased accumulation of osmolytes, including total soluble sugars and proline, in both cultivars. At 21 DAS, plants treated with SA at 200 mg L⁻¹ exhibited the highest Drought Tolerance Index (DTI) for total soluble sugars, followed by the 150 mg L⁻¹ treatment (Figure 5). The SA-treated plants, particularly at concentrations of 150 and 200 mg L⁻¹, showed a significantly higher DTI ($P < 0.05$) for sugars at 14 DAS and 21 DAS compared to the water-spray (WS) treatment in the TRI 2025 cultivar. With the progression of drought stress and the application of SA,

especially at concentrations of 150 and 200 mg L⁻¹, the accumulation of total soluble sugars increased in the TRI 2023 cultivar. The WS treatment exhibited the lowest accumulation of total soluble sugar in both cultivars at 14 DAS and 21 DAS.

Moisture stress caused the accumulation of proline in all treatments at varying concentrations. In the TRI 2025 cultivar, initially the no-spray (NS) treatment showed the highest DTI for proline ($P < 0.05$). However, as drought progressed, at 14 DAS and 21 DAS, plants treated with 150 mg L⁻¹ of

SA accumulated more proline and exhibited a higher DTI for proline, followed by the 200 mg L⁻¹ treatment. The lowest DTI was observed in plants treated with 50 mg L⁻¹ of SA (Figure 6). Initially (18 hours after spraying), the 50 mg L⁻¹ and NS treatments showed a significantly higher DTI for proline in the TRI 2023 cultivar. At 21 DAS, the SA-treated plants and NS treatments exhibited the highest DTI for proline, while the WS treatment showed the lowest DTI.

In contrast to the accumulation of osmolytes, there was a decrease in starch content in both cultivars. Although no clear pattern or significant variation was observed, there was a tendency for SA-treated plants to exhibit a comparatively higher DTI for starch content in both cultivars compared to the other treatments (Data not shown).

As drought progressed, there was a tendency for a decrease in polyphenol content in both cultivars. The DTI for polyphenol content also tended to decrease with the progression of drought in the TRI 2025 cultivar. However, with the application of 100, 150, and 200 mg L⁻¹ of SA, these plants maintained a higher DTI ($P < 0.05$) for polyphenol content as drought progressed, especially at 14 DAS and 21 DAS (Figure 7).

Similarly, in the TRI 2023 cultivar, the DTI for polyphenol content also decreased with the progression of drought. Unlike in the TRI 2025 cultivar, SA sprays at 100, 150, and 200 mg L⁻¹ to plants in the TRI 2023 cultivar maintained a higher DTI ($P < 0.05$) for polyphenol content throughout the measurement period as drought progressed, including 18 hours after spraying.

Total chlorophyll content also decreased with increasing water deficit. As drought progressed, the DTI for total chlorophyll content decreased in both cultivars. In the TRI 2025 cultivar, SA spraying at 100, 150, and 200 mg L⁻¹ showed a higher DTI ($P < 0.05$) for leaf chlorophyll content with the progression of drought at 14 DAS. Although no clear pattern of variation was observed in the DTI for chlorophyll a, chlorophyll b, and carotenoid contents, the SA-treated plants exhibited a comparatively higher DTI for

chlorophyll a, chlorophyll b, and carotenoid in both cultivars compared to the no-spray treatment (Data not shown).

Moisture stress led to a reduction in the DPPH EC 50 value, indicating an increase in antioxidant activity in all treatments. In the TRI 2025 cultivar, the application of SA resulted in a comparatively lower DTI for the DPPH EC 50 value (higher DTI for antioxidant activity) (Figure 8) at later stages (14 DAS and 21 DAS). In the TRI 2023 cultivar, SA treatment at 100 and 150 mg L⁻¹ resulted in a lower DTI ($P < 0.05$) for the DPPH EC 50 value (higher DTI for antioxidant activity) at 18 hours after spraying. At later stages (14 DAS and 21 DAS), the plants treated with 100, 150, and 200 mg L⁻¹ of SA showed a lower DTI ($P < 0.05$) for the DPPH EC 50 value (higher DTI for antioxidant activity) in the TRI 2023 cultivar.

The highest DTI ($P < 0.05$) for antioxidant activity, as measured by the FRAP assay, was observed in plants treated with 200 mg L⁻¹ of SA in the TRI 2025 cultivar during the later stages (14 DAS and 21 DAS) as drought progressed. In the TRI 2023 cultivar, plants treated with 200 mg L⁻¹ of SA exhibited the highest DTI ($P < 0.05$) for antioxidant activity based on the FRAP assay throughout the entire experiment (Figure 9).

In both cultivars, the well-watered plants remained unaffected and did not show any signs of wilting throughout the experiment. Re-watering at 21 DAS and observations on the recovery after another 7 days indicated that the application of SA resulted in higher percentages of recovery in tea plants (Table 2).

Experiment 2

In the field experiment, the average moisture content across all treatments was 28.36%, 24.87%, 22.47%, and 30.2% at 7 DAS, 14 DAS, 21 DAS, and recovery after rain, respectively. As drought progressed, the relative water content (RWC) declined. SA-treated plants exhibited significantly higher RWC, while WS and NS-treated plants showed the lowest RWC at 14 DAS, 21 DAS, and recovery after rain (Figure 10).

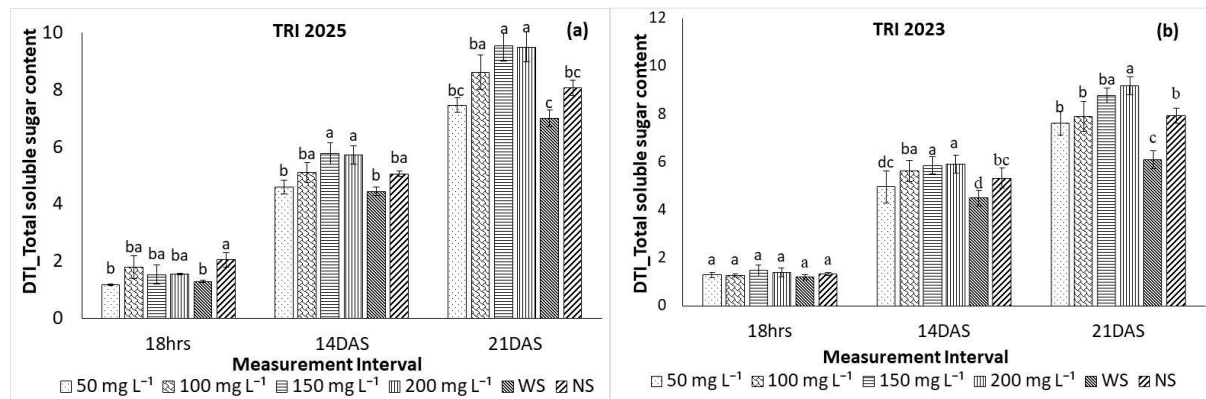


Figure 5: Variation of drought tolerance index (DTI) for total soluble sugar of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing plants to drought stress. Bars with different lowercase letters are significantly different at $p < 0.05$ in each measurement interval (18hrs: 18 hours after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

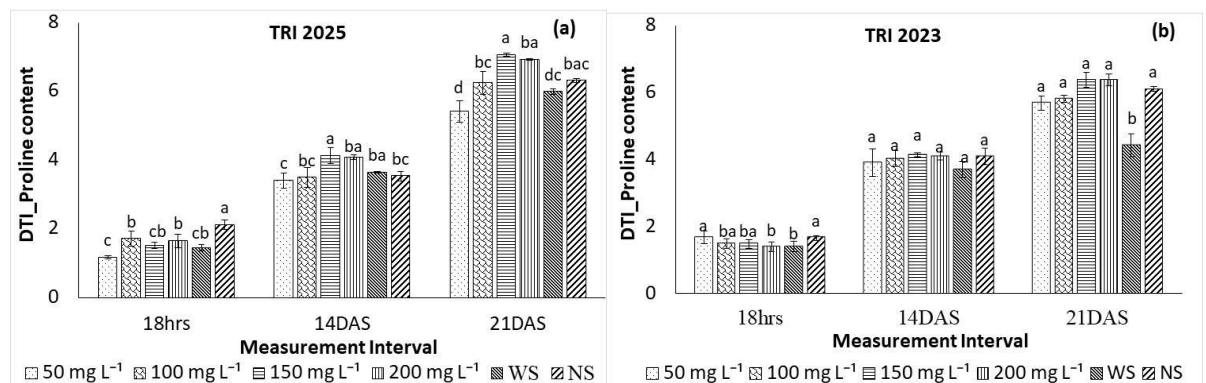


Figure 6: Variation of drought tolerance index (DTI) for proline content of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing plants to drought stress. Bars with different lowercase letters are significantly different at $p < 0.05$ in each measurement interval (18hrs: 18 hours after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

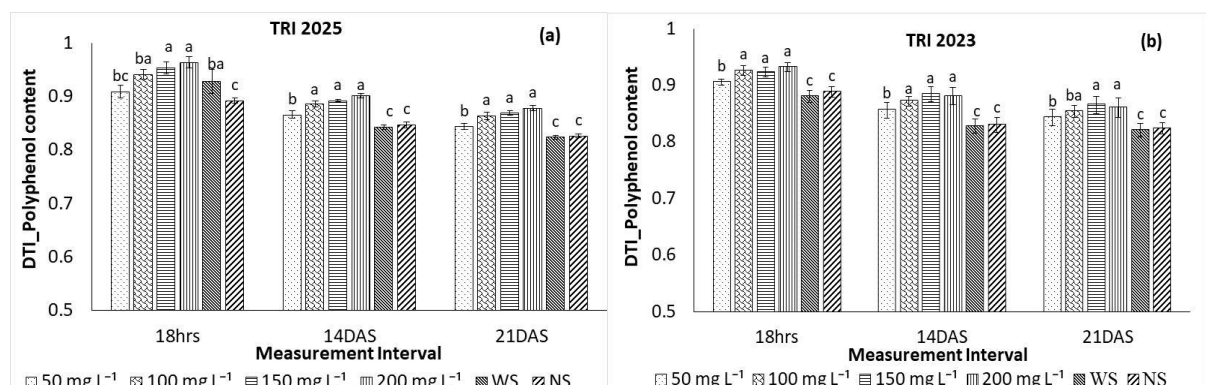


Figure 7: Variation of drought tolerance index (DTI) for polyphenol content of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing plants to drought stress. Bars with different lowercase letters are significantly different at $p < 0.05$ in each measurement interval (18hrs: 18 hours after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

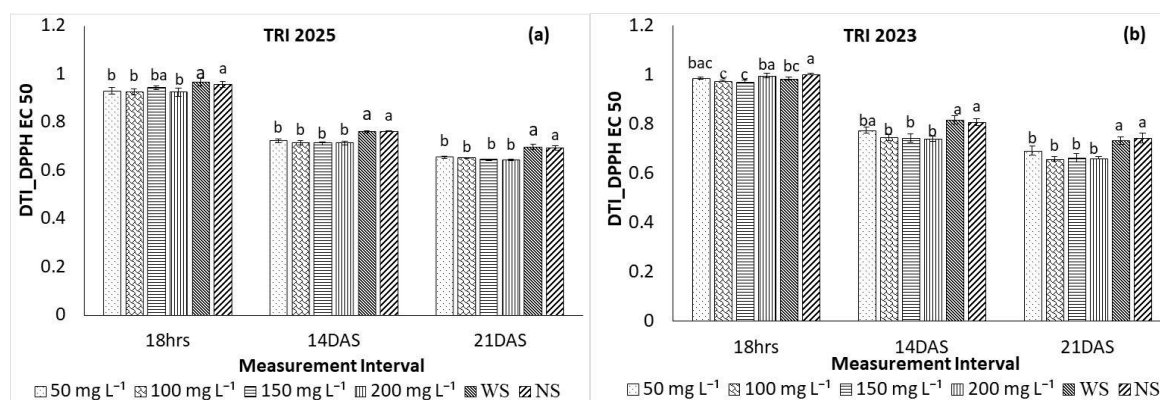


Figure 8: Variation of drought tolerance index (DTI) for DPPH EC 50 value of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing plants to drought stress. Bars with different lowercase letters are significantly different at $p<0.05$ in each measurement interval (18hrs: 18 hours after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

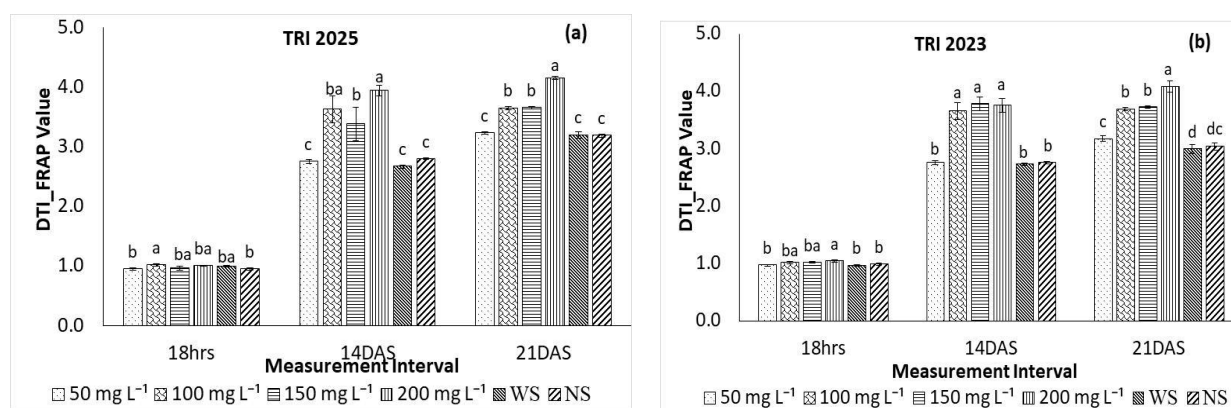


Figure 9: Variation of drought tolerance index (DTI) for FRAP value of cultivar (a) TRI 2025 and (b) TRI 2023 after spraying of salicylic acid and exposing plants to drought stress. Bars with different lowercase letters are significantly different at $p<0.05$ in each measurement interval (18hrs: 18 hours after spraying, 3DAS: 3 days after spraying, 14DAS: 14 days after spraying, 21DAS: 21 days after spraying, NS: no-spray, WS: water-spray). Vertical lines represent the standard error of means.

Table 2: Variation of recovery percentage of immature tea plants of cultivar TRI 2025 and TRI 2023 after 7 days of re-watering.

Treatment	Recovery percentage (%)	
	TRI 2025	TRI 2023
SA @ 50 mg L ⁻¹	34.29	28.57
SA @100 mg L ⁻¹	69.05	64.29
SA @150 mg L ⁻¹	80.36	77.50
SA @200 mg L ⁻¹	85.71	80.36
Water-spray	28.57	12.50
No -spay	14.29	7.14
Well-watered	100.00	100.00

At 7 DAS, 14 DAS, and 21 DAS, SA-treated plants demonstrated higher photosynthetic rates compared to the WS treatment. The photosynthetic rate was lowest in the WS treatment at 14 DAS and 21 DAS. There were no significant differences among treatments at the recovery after rain. Similarly, SA-treated plants exhibited significantly higher ($P<0.05$) stomatal conductance at 7 DAS and 14 DAS compared to the WS treatments.

Initially (7 DAS), SA-treated plants displayed significantly higher transpiration rates.

Furthermore, at a later stage (21 DAS), WS plants maintained a comparatively higher transpiration rate. There were no significant differences among treatments during the recovery after the rain (Figure 10).

Moisture stress led to the accumulation of total soluble sugars and proline in all treatments, albeit at varying concentrations. The SA-treated plants exhibited the highest accumulation of total soluble sugars at 14 DAS and proline at 21 DAS (Figure 12).

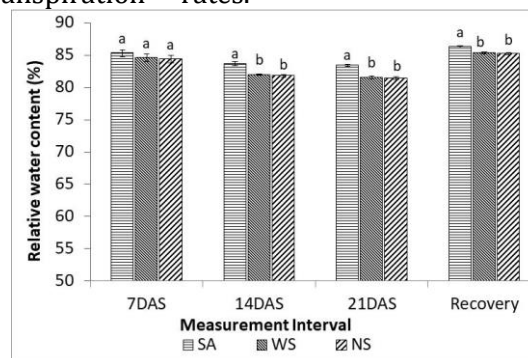


Figure 10: Variation of relative water content of immature tea plants during progressive drought stress and recovery after rain at Barewell Estate (7DAS, 14DAS and 21DAS denoted 7, 14 and 21 days after spraying respectively, NS: no-spray, WS: water-spray). Bars with different lowercase letters are significantly different at $p<0.05$ in each measurement interval. Vertical lines represent the standard error of means.

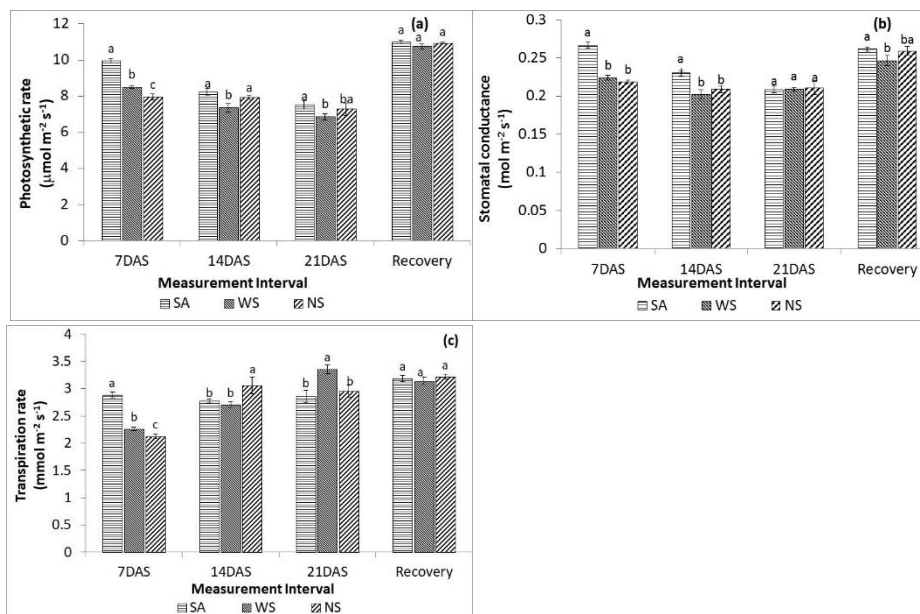


Figure 11: Variation of (a) photosynthetic rate, (b) stomatal conductance and (c) transpiration rate of immature tea plants during progressive drought stress and recovery after rain at Barewell Estate (7DAS, 14DAS and 21DAS denoted 7, 14 and 21 days after spraying respectively, NS: no-spray, WS: water-spray). Bars with different lowercase letters are significantly different at $p<0.05$ in each measurement interval. Vertical lines represent the standard error of means.

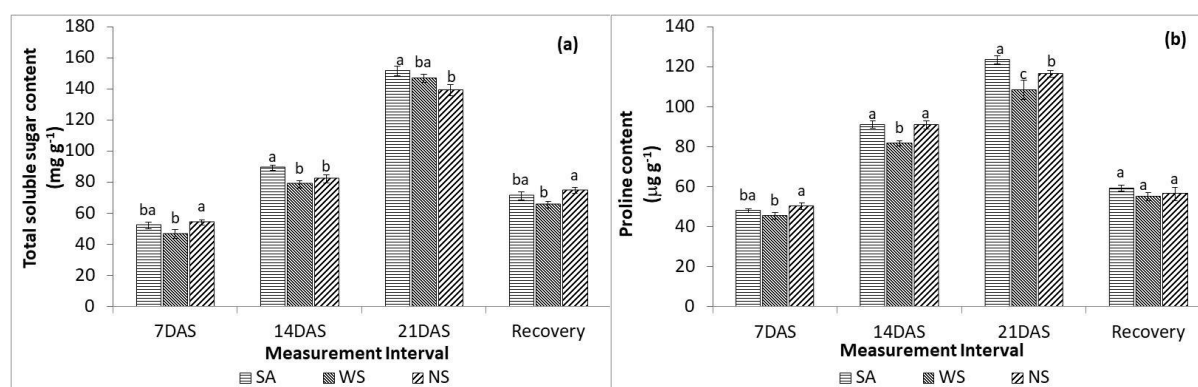


Figure 12: Variation of (a) total soluble sugar content and (b) proline of immature tea plants during progressive drought stress and recovery after rain at Barewell Estate (7DAS, 14DAS and 21DAS denoted 7, 14 and 21 days after spraying respectively, NS: no-spray, WS: water-spray). Bars with different lowercase letters are significantly different at $p < 0.05$ in each measurement interval. Vertical lines represent the standard error of means.

Drought is a significant abiotic stress that negatively affects the physiological and biochemical characteristics of plants. Salicylic acid (SA) is an important signaling molecule involved in plant defense and has been shown to play a role in local and systemic resistance to fungal pathogens (Hayat et al., 2012). Numerous studies have demonstrated that exogenous application of SA can enhance the tolerance of plants to various abiotic stresses, including drought, by improving physiological processes (photosynthesis, stomatal conductance, and transpiration). The interaction between SA and drought has been reported in many plant species. Therefore, the positive effects of SA on the physiological performance of tea plants under drought conditions observed in this experiment align with findings reported by Yao et al. (2016) on *G. jasminoides*, Ying et al. (2013) on *M. rubra*, Hayat et al. (2008) on *Lycopersicon esculentum*, Jesus et al. (2015) on *Eucalyptus globulus*, and Munne-Bosch and Penuelas (2003) on *Phillyrea angustifolia*. The positive effects of SA observed in previous research confirm that SA plays a crucial role in regulating important processes and promoting plant growth.

The application of exogenous SA aids in better maintenance of the DTI for RWC during the latter stages of the drying cycle (21 DAS). The glasshouse study

demonstrated that applying SA at concentrations of 150 mg L⁻¹ and 200 mg L⁻¹ had a positive impact on the DTI for RWC in plants. These findings are supported by field investigations where the exogenous application of 150 mg L⁻¹ SA improved RWC in water-stressed immature tea plants. The results regarding RWC align with previous studies conducted on *M. rubra*, *G. jasminoides*, and other plant species (Ying et al., 2013; Askari and Ehsanzadeh, 2015; Manzoor et al., 2015; Yao et al., 2016). RWC is considered a measure of internal plant water status and reflects the metabolic activity of tissues (Anjum et al., 2011). Therefore, the maintenance of higher RWC in leaves can be an indication of greater tolerance to drought.

Photosynthesis is the primary determinant of plant productivity. The application of SA at concentrations of 150 and 200 mg L⁻¹ resulted in a significantly higher DTI for the photosynthetic rate compared to the no-spray treatment in both drought-tolerant and drought-susceptible cultivars under glasshouse conditions. Gas exchange data collected from field-grown immature tea plants also exhibited similar trends, with higher photosynthetic rates and stomatal conductance observed in SA-treated plants compared to the WS treatment. These findings align with the results of studies conducted by Yao et al. (2016) on *G.*

jasminoides plants, Jesus et al. (2015) on *E. globulus* plants, and Khalvandi et al. (2021) on *T. aestivum*. The maintenance of a relatively higher photosynthetic rate under moisture stress conditions serves as an indicator of increased productivity under such conditions. Stomatal regulation is another crucial physiological process involved in maintaining photosynthesis. The present study's results demonstrate a significant reduction in stomatal conductance under drought stress compared to well-watered plants. However, the application of SA at concentrations of 150 and 200 mg L⁻¹ resulted in a minimal reduction of stomatal conductance in drought-stressed plants compared to the no-spray treatment, in both cultivars. The enhancement of stomatal conductance using SA has also been observed in *H. brasiliensis* (Nakandala et al., 2016) and *M. rubra* plants (Ying et al., 2013). This beneficial regulation of stomatal function may have contributed to the observed increase in photosynthesis rate in SA-sprayed plants at concentrations of 150 and 200 mg L⁻¹ compared to the no-spray plants. The transpiration rate decreased with the progression of drought in all plants compared to the well-watered plants. However, plants treated with SA at concentrations of 150 and 200 mg L⁻¹ exhibited a relatively higher DTI for transpiration rate, while a comparatively stable transpiration rate was observed in SA-treated field-grown plants. The maintenance of a higher transpiration rate under moisture stress has been reported in *G. jasminoides* plants (Yao et al., 2016), which is important for plant metabolic processes, leaf cooling, and osmotic balance within cells.

The application of SA increased the accumulation of osmolytes, such as total soluble sugar and proline, with varying amounts observed between the two cultivars and among the treatments in our glasshouse study. SA-treated plants exhibited the highest accumulation of total soluble sugar at 14 DAS and proline at 21 DAS in field-grown plants as well. The promotion of osmolyte accumulation using SA has been reported in *G. jasminoides*, *E. globulus*, *T. aestivum*, *Zea mays*, and *L. esculentum* plants (Hayat et al., 2008; Jesus et al., 2015; Manzoor et al., 2015; Marcinska et al., 2015; Yao et al., 2016). The

SA-induced sugar accumulation partially contributes to osmotic adjustment, aiding in better survival under drought conditions. The osmotic adjustment reduces cell osmotic potential, helping to maintain cell turgor and physiological processes during short-term drought stress conditions (Serraj and Sinclair, 2002). Furthermore, Sinay and Karuwal (2014) reported that increased total soluble sugar supports improved osmotic adjustment, decreased osmotic potential, and enhanced cell turgidity, enabling plant cells to function under abiotic stress conditions.

Furthermore, proline acts as both a scavenger for reactive oxygen species and an osmolyte, thereby improving growth characteristics and yield production under drought conditions (Abdelaal et al., 2020). Proline also serves as a nitrogen reservoir during restricted growth stages, a signaling compound to regulate mitochondrial function, and influences cell proliferation, all of which are essential for stress recovery under drought conditions. An increase in proline content helps protect membrane integrity by reducing lipid oxidation, maintaining cellular redox potential, and scavenging free radicals.

With the progression of drought, polyphenol content tended to decrease. However, the exogenous application of SA improved the total polyphenol levels in both cultivars. The improvement of polyphenol contents using SA has also been reported in *Citrus limon* plants (Siboza et al., 2014). Polyphenols act as antioxidants, radical scavengers, and photoprotectors by limiting light penetration into the mesophyll cells and reducing the excitation of chlorophyll during moisture stress.

According to the results obtained from the present study, the DTI for total chlorophyll content decreased with an increase in water deficit. However, the SA application improved the DTI for total chlorophyll content in both cultivars compared to the no-spray treatment. The reduction of chlorophyll content under drought stress could be attributed to various factors, including the degradation of chloroplast and photosynthetic apparatus, chlorophyll photooxidation, destruction of chlorophyll

substrate, inhibition of chlorophyll biosynthesis, and an increase in chlorophyllase activity. Reduction in carotenoid content under drought stress has been associated with the degradation of β -carotene (Kabiri, 2014). Application of SA in various plants, such as *Nigella sativa* (Kabiri, 2014), *H. brasiliensis* (Nakandala et al., 2016), *G. jasminoides* (Yao et al., 2016), *Achillea millefolium* (Gorni and Pacheco, 2016), and *Glycine max* (Razmi et al., 2017), has shown an increase in chlorophyll content. The degradation of total chlorophyll content was lower in more drought-tolerant plants (Arunyanark et al., 2008). Therefore, the externally-applied SA can induce drought tolerance in plants by improving the total chlorophyll content.

This study also demonstrated that the application of SA had a positive effect on dark respiration in both tea cultivars. Dark respiration rates generally increase during drought stress to varying degrees among different cultivars. According to Ribas-carbo et al. (2005), this increase is due to the shift of electrons from cytochrome to the alternative pathway, which helps maintain high respiration rates. The decline in dark respiration contributes to drought tolerance by reducing the consumption of soluble sugars, which play a crucial role as osmoprotectants during water deficit stress (Guez-calcerrada, 2011).

Drought-induced generation and accumulation of reactive oxygen species have been reported in many plants. Changes in antioxidant levels in plants regulate the scavenging activity of reactive oxygen species. In this study, based on the results of DPPH and FRAP antioxidant assays, the exogenous application of SA improved the DTI for antioxidants. According to Shamili et al. (2021), SA improved the antioxidant ability of salt-exposed guava plants, suggesting that SA application could be a potential, simple, and efficient approach to reduce oxidative stress injury in salt-exposed *Psidium guajava*. Previous findings have also reported that SA application increased the activity of antioxidant enzymes such as peroxidase and superoxide dismutase (Hayat et al., 2008). Promotion of antioxidant activity

using SA has been reported in various plants, including *Capsicum annuum* (khazaei and Estaji, 2020), *P. guajava* (Shamili et al., 2021), *Aristotelia chilensis* (Gonzalez-villagra et al., 2022), and barley (Fayez and Bazaid, 2013).

It has been observed that plants treated with SA generally exhibit better recovery after drought upon re-watering. The ability to maintain photoprotective and antioxidative mechanisms during drought is often associated with a rapid recovery of plants (Munne-Bosch and Alegre, 2000; Munne-Bosch and Penuelas, 2003).

CONCLUSIONS

The exogenous application of SA at concentrations of 150 and 200 mg L⁻¹ significantly improved physiological processes (photosynthesis, stomatal conductance, and transpiration), osmolyte accumulation, and antioxidant activity, thereby enhancing the drought tolerance index for immature tea plants. Considering the environmental impact and cost-effectiveness, the application of SA at the lowest effective concentration, i.e., 150 mg L⁻¹, would reduce the impact of drought on immature tea plants. The field study further confirmed that the exogenous application of 150 mg L⁻¹ SA under drought conditions was beneficial in enhancing drought tolerance in field-grown immature tea plants.

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