

Salicylic acid-mediated drought tolerance in tea (*Camellia sinensis* L.): Integrating physiological, biochemical, and multivariate analyses with field validation

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Abstract

Drought is a major constraint to tea (*Camellia sinensis* L.) productivity, yet comparative evaluation of plant growth regulators and osmoprotectants for enhancing drought resilience remains limited. This study investigated the effectiveness of ascorbic acid (AA), glycine betaine (GB), methyl jasmonate (MeJ), cytokinin (CK), salicylic acid (SA), and kaolin in mitigating moisture-stress-induced damage in tea clones TV21 and TV25. Experiments were conducted over two consecutive years under controlled rainout-shelter conditions and subsequently validated under field rainfed conditions. Physiological, biochemical, and multivariate analyses were employed to identify key traits associated with drought tolerance.

Moisture stress markedly impaired physiological performance, reducing net photosynthetic rate (Pn) by 56% (15.47 to 6.77 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and water-use efficiency (WUE) by approximately 75–80% in TV21, while increasing electrolyte leakage to 84.21%, indicative of severe membrane damage. Among the tested treatments, SA (1.0 mM) was the most effective, increasing Pn to 28.40 and 32.27 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in TV21 and TV25, respectively, enhancing WUE to 49.14 and 68.57, and maintaining PSII efficiency ($F_v/F_m \approx 0.76\text{--}0.77$). Salicylic acid also reduced electrolyte leakage by approximately 50–60% and stimulated proline accumulation up to 463.33 $\mu\text{g g}^{-1}$, suggesting enhanced osmotic adjustment and cellular protection. Multivariate analyses revealed strong positive associations among Pn, WUE, and F_v/F_m , whereas stress-related traits such as electrolyte leakage and transpiration were negatively associated with photosynthetic performance. Clone TV25 exhibited greater physiological plasticity and responsiveness to SA than TV21. Field validation further demonstrated that SA increased green leaf yield by 10.38%, comparable to potassium application (10.70%).

Collectively, these findings demonstrate that salicylic acid enhances drought resilience in tea through coordinated regulation of photosynthetic efficiency, osmotic adjustment, and membrane stability, providing an effective physiological intervention for climate-resilient tea production under water-limited environments.

Highlights

- Drought reduced photosynthesis, water-use efficiency, and PSII efficiency while increasing membrane injury.
- Salicylic acid preserved PSII function and improved photosynthetic performance under moisture stress.
- Enhanced proline and wax accumulation contributed to osmotic adjustment and water conservation.
- Multivariate analyses identified photosynthetic competence as a key determinant of drought tolerance.
- Salicylic acid and potassium improved yield stability under rainfed field conditions.

1. Introduction

Tea (*Camellia sinensis* L.) is a globally important perennial plantation crop, whose productivity and quality are highly sensitive to environmental stresses. Among these, drought represents a major constraint, particularly under rainfed conditions where increasing climate variability intensifies moisture deficits. Drought stress adversely affects key physiological processes, including photosynthesis, stomatal conductance, and water-use efficiency (WUE), ultimately leading to reduced growth and yield (Chaves et al. 2009; Flexas et al. 2016). In tea, moisture stress has been shown to impair PSII efficiency, increase electrolyte leakage, and disrupt metabolic pathways associated with stress adaptation (Maritim et al. 2015; Li et al. 2021). These responses reflect both stomatal limitations restricting CO_2 diffusion and non-stomatal damage such as oxidative injury to photosynthetic apparatus (Lawlor and Tezara, 2009).

At the biochemical level, drought induces accumulation of osmolytes such as proline and enhances cuticular wax deposition, which contribute to osmotic adjustment and water conservation (Szabados and Saviouré 2010; Kishor et al. 2022). Recent transcriptomic and metabolomic studies further indicate that drought stress alters pathways including phenylpropanoid and flavonoid metabolism, thereby contributing to adaptive responses in tea (Li et al. 2021). However, despite these intrinsic

defense mechanisms, prolonged stress often results in irreversible physiological damage, highlighting the need for effective mitigation strategies.

Exogenous application of plant growth regulators (PGRs) and osmoprotectants has emerged as an effective approach to enhance drought tolerance. Among these, salicylic acid (SA), a key phenolic phytohormone, plays a central role in stress signaling, antioxidant regulation, and maintenance of photosynthetic efficiency (Khan et al. 2015; Nazar et al. 2015). SA regulates stomatal behavior, enhances reactive oxygen species (ROS) scavenging, and stabilizes PSII, thereby preserving cellular integrity under drought stress (Hayat, 2010; Nykiel et al. 2023). In tea, recent studies have demonstrated that SA significantly improves gas exchange, osmolyte accumulation, and antioxidant activity under moisture stress conditions (Damayanthi et al. 2023; Sheuli et al. 2025).

In addition to SA, other plant growth regulators and osmoprotectants such as glycine betaine (GB), methyl jasmonate (MeJ), and cytokinins have been reported to contribute to stress mitigation through distinct mechanisms. Glycine betaine functions as an osmoprotectant, stabilizing proteins and membranes and maintaining cellular osmotic balance under drought conditions (Ashraf and Foolad 2007). Methyl jasmonate is involved in stress signaling and defense responses, modulating antioxidant systems and secondary metabolism (Wasternack and Feussner 2018). Cytokinins play a role in delaying senescence and maintaining photosynthetic activity under stress, although their effectiveness under severe drought is often limited. Despite these advances, comparative evaluation of these regulators under controlled and field conditions in tea remains limited.

Although several studies have reported beneficial effects of salicylic acid on drought tolerance in tea, comparative evaluation against other plant growth regulators under identical stress conditions remains limited. Furthermore, the integration of physiological, biochemical, and multivariate analyses with field-scale validation has rarely been attempted in tea. Consequently, the relative importance of key traits contributing to drought resilience remains poorly understood.

Potassium nutrition also plays a critical role in drought tolerance by regulating stomatal conductance, enzyme activation, and assimilate transport (Marschner 2012; Ruan et al. 2010). The interaction between hormonal regulation (e.g., SA) and nutrient-mediated responses (e.g., potassium) provides an integrated framework for improving drought resilience, yet this interaction is not sufficiently explored in tea systems.

Importantly, drought tolerance is a complex trait governed by coordinated interactions among physiological and biochemical processes rather than individual parameters. Conventional single-trait analyses often fail to capture this complexity. Multivariate analytical approaches such as principal component analysis (PCA), clustering, and correlation heat maps offer powerful tools to identify trait networks and underlying stress-response mechanisms. However, such integrative analyses are still underutilized in tea drought research.

Genotypic variation further adds complexity to drought responses, as tea clones differ in their physiological plasticity and adaptive capacity under stress conditions (Cheruiyot et al. 2007; Upadhyaya et al. 2012). Understanding clone-specific responses under both controlled and field environments is therefore essential for developing climate-resilient management strategies.

Therefore, the present study aimed to (i) evaluate the comparative effectiveness of ascorbic acid (AA), glycine betaine (GB), methyl jasmonate (MeJ), cytokinin (CK), salicylic acid (SA), and kaoline (anti-transpirant), on physiological and biochemical processes of tea under controlled moisture stress (rainout shelter), (ii) validate their performance under field rainfed conditions, (iii) analyze trait interactions using multivariate approaches, and (iv) assess genotypic variation between tea clones (TV21 and TV25). By integrating hormonal regulation, physiological and biochemical traits, and multivariate analysis with field validation, this study provides a comprehensive framework for enhancing drought tolerance in tea.

2. Materials and Methods

2.1 Experimental site

The study was conducted at the Tocklai Tea Research Institute, Tea Research Association, Jorhat, Assam, India. The experiment was conducted in two phases (i) a study inside rainout shelter during the first year in controlled environment (ii) a field experiment in rainfed conditions during the second year. Experimental details are presented in section 2.2 and 2.3. Two commercially important tea clones, TV21 and TV25, were selected, and uniform, healthy bushes of comparable age and vigor were used throughout the study.

2.2 Phase I: Rainout shelter experiment

First phase of the study was conducted inside a rain-out shelter in pots. The experiment was conducted in a rainout shelter at the Tocklai Tea Research Institute, Tea Research Association, Jorhat, Assam, India (26.75° N, 94.22° E; 96 m above mean sea level). The shelter measured 15 m × 10 m × 4 m (length × width × height) and was covered with transparent polyethylene film (200 µm thickness), transmitting approximately 82% of photosynthetically active radiation (PAR) and 65% of diffused light. The roof was designed in a dome-shaped configuration to facilitate rapid drainage of rainwater and ensure uniform light distribution within the shelter. The structure remained open on all sides to maintain natural air circulation and minimize microclimatic deviations from ambient field conditions while excluding rainfall.

One-year-old tea clones TV21 (drought-susceptible) and TV25 (drought-tolerant) were transplanted into plastic pots (40 cm diameter × 30 cm height) containing a uniform growth medium. Plants were maintained under well-watered conditions for two months to ensure uniform establishment before treatment imposition.

The experiment was arranged in a completely randomized design (CRD) comprising two tea clones and eight treatments, with three replications per treatment and twenty plants per replication. Foliar application of treatments was initiated in mid-December and continued until mid-March at 30-day intervals. Drought stress was imposed by withholding irrigation, whereas control plants were irrigated regularly throughout the experimental period. Morphological, physiological, and biochemical parameters were recorded 15 days after each treatment application to assess treatment effects on drought tolerance.

The rainout shelter provided a semi-controlled environment that enabled precise exclusion of natural rainfall while maintaining near-ambient environmental conditions, thereby facilitating reliable evaluation of drought responses and mitigation strategies in tea. Treatments comprising of ascorbic acid (AA), glycine betain (GB), methyl jasmonate (MeJ), cytokinin (CK), salicylic acid (SA) and kaoline (anti-transperant), with three different concentrations were foliar applied in two tea clones TV21 and TV25 under stress. Each treatment was replicated three times in a Completely Randomized Design (CRD). For comparisons one control set was planted which were irrigated at regular intervals and another set of plants were kept under stress condition where moisture stress was created by withholding irrigation. The effect of treatments were monitored by assessing various physiological parameters such as net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (e) leaf temperature (l_t), water use efficiency (WUE), carboxylation efficiency (ci/ca), maximum quantum yield of PSII and electrolyte leakage (EL). Net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) were measured using a portable infrared gas analyzer (IRGA; CIRAS II, USA). Chlorophyll fluorescence (F_v/F_m) was measured using a portable pulse-amplitude modulated fluorometer (Junior PAM, Heinz Walz GmbH, Germany). Leaf temperature was recorded using a handheld infrared thermometer (Fluke 62 MAX, USA). Water-use efficiency (WUE) was calculated as the ratio of photosynthesis to transpiration ($WUE = P_n/E$), and carboxylation efficiency was expressed as the ratio of intercellular to ambient CO_2 concentration (C_i/C_a). PSII efficiency was calculated using the relation $F_v/F_m = (F_m - F_o)/F_m$. Electrolyte leakage (EL%) was estimated as $EL (\%) = (EC_1/EC_2) \times 100$, where EC_1 represents initial conductivity and EC_2 represents conductivity after boiling, measured using a digital conductivity meter. Electrical conductivity was measured using a digital conductivity meter (HI 99300, Hanna Instruments, Woonsocket, RI, US).

2.3 Phase II: Field experiment

Field validation was carried out in natural rainfed conditions in the New Botanical Area of TRA, Tocklai, in an unpruned section. Based on the first-year results, selected treatments included glycine betaine (100 mM), ascorbic acid (200 ppm), salicylic acid (1.0 mM), muriate of potash (MOP @ 2%), and cytokinin (40 µM), along with an untreated control. Foliar sprays were applied from mid-November to mid-March at 21 day interval.

Physiological parameters (P_n , g_s , E , LT , WUE , C_i/C_a , mesophyll efficiency, and transpiration ratio) were measured using the same instruments as described above. Chlorophyll fluorescence (F_v/F_m) was measured using the PAM fluorometer. Biochemical parameters including proline content were estimated following Bates et al. (1973), while chlorophyll content was measured using a SPAD meter (SPAD-502 Plus, Konica Minolta, Japan). Soil moisture content was measured using a soil moisture probe (ThetaProbe ML2x, Delta-T Devices, UK), and leaf water potential was measured using a pressure chamber (Model 600, PMS Instrument Company, USA).

Green leaf yield was recorded on a plot basis during the harvesting period.

2.4 Statistical and multivariate analysis

Data were analyzed using analysis of variance (ANOVA) under the randomized block design using SPSS (version 25.0, IBM Corp., USA). Treatment means were compared at $P \leq 0.05$. Correlation analysis was performed to evaluate relationships among traits. Multivariate analyses, including principal component analysis (PCA), hierarchical clustering, and heatmap visualization, were performed using R software (version 4.2.0; packages: ggplot2, pheatmap, and factoextra).

3. Results

3.1 Rain-out shelter experiment

3.1.1 Physiological responses

Moisture stress (T2) significantly reduced physiological performance in both tea clones (TV21 and TV25) ($P \leq 0.05$) (Table 1; Table 2). In TV21, F_v/F_m declined from 0.74 (control) to 0.35, while net photosynthetic rate (P_n) decreased from 15.47 to 6.77 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Water use efficiency (WUE) declined from 35.64 to 7.80, whereas transpiration rate increased from 0.47 to 0.95 $\text{mmol m}^{-2} \text{s}^{-1}$. Electrolyte leakage (EL) increased from 61.69% to 84.21%, indicating severe membrane damage.

Similarly, in TV25 (Table 2), F_v/F_m decreased from 0.77 to 0.37, P_n from 21.83 to 9.03 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and WUE from 40.21 to 9.64. Transpiration increased from 0.57 to 1.07 $\text{mmol m}^{-2} \text{s}^{-1}$, while EL increased from 57.85% to 67.74%.

Among treatments, salicylic acid (SA @ 1.0 mM; T16) recorded the highest physiological performance (Table 1; Table 2). In TV21, P_n increased to 28.40 $\mu\text{mol m}^{-2} \text{s}^{-1}$, WUE to 49.14, and F_v/F_m to 0.77, while EL decreased to 35.28%. In TV25, P_n reached 32.27 $\mu\text{mol m}^{-2} \text{s}^{-1}$, WUE 68.57, and F_v/F_m 0.76, with EL reduced to 30.20%.

Intermediate improvements were observed under glycine betaine (GB) and methyl jasmonate (MeJ) treatments, while cytokinin treatments showed moderate effects on physiological traits.

3.1.2 Biochemical responses

Moisture stress significantly increased proline and wax accumulation in both clones (Table 1; Table 2). In TV21, proline increased from 38.33 to 102.17 $\mu\text{g g}^{-1}$, whereas in TV25 it increased from 68.33 to 261.67 $\mu\text{g g}^{-1}$ under stress conditions.

Application of SA (1.0 mM) resulted in maximum proline accumulation, reaching 403.33 $\mu\text{g g}^{-1}$ (TV21) and 463.33 $\mu\text{g g}^{-1}$ (TV25). Wax content also increased substantially under SA (1.0 mM), from 16.41 to 47.38 (TV21) and 14.30 to 61.17 (TV25).

GB treatments also enhanced biochemical traits, whereas MeJ and cytokinin treatments showed moderate responses compared to stress conditions.

Table 1

Effects of exogenous application of plant growth regulators and osmoprotectants on physiological and biochemical traits of tea clone TV21 under moisture stress conditions in a rainout shelter experiment.

Treatment	Fv/Fm	Pn ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	WUE ($\mu\text{mol CO}_2\text{ mmol}^{-1}\text{H}_2\text{O}$)	Ci/Ca	E ($\text{mmol m}^{-2}\text{s}^{-1}$)	Root:shoot ratio	EL (%)	Epicuticular wax ($\mu\text{g cm}^{-2}$)	Proline ($\mu\text{g g}^{-1}\text{FW}$)	E/P
Control	0.74 ^b	15.47 ^b	35.64 ^c	0.73 ^a	0.47 ^c	0.76 ^b	61.69 ^d	16.41 ^d	38.33 ^d	30.55 ^d
Water stress	0.35 ^d	6.77 ^d	7.80 ^f	0.39 ^d	0.95 ^a	0.81 ^b	84.21 ^a	37.51 ^a	102.17 ^b	158.39 ^a
AA (100 ppm)	0.72 ^b	13.77 ^c	16.17 ^e	0.32 ^e	0.85 ^a	0.57 ^c	74.30 ^b	39.78 ^a	173.33 ^a	62.96 ^c
AA (200 ppm)	0.61	16.93 ^b	31.76 ^d	0.31 ^e	0.53 ^b	0.73 ^b	62.30 ^d	38.50 ^b	155.00 ^a	31.52 ^d
AA (300 ppm)	0.54 ^c	13.47 ^c	32.72 ^d	0.59 ^b	0.43 ^c	0.80 ^b	58.94 ^d	41.14 ^a	144.17 ^a	31.52 ^d
GB (50 mM)	0.56 ^c	13.30 ^c	31.26 ^d	0.43 ^d	0.45 ^c	0.81 ^b	68.44 ^c	44.19 ^a	183.33 ^a	34.22 ^d
GB (100 mM)	0.68 ^b	15.20 ^b	36.25 ^c	0.53 ^c	0.46 ^c	0.79 ^b	55.94 ^d	44.08 ^a	140.17 ^a	31.09 ^d
GB (200 mM)	0.53 ^c	14.70 ^c	17.27 ^e	0.37 ^d	0.85 ^a	0.96 ^a	54.25 ^d	40.71 ^a	196.42 ^a	58.67 ^c
MeJ (25 μM)	0.53 ^c	13.88 ^c	39.56 ^b	0.31 ^e	0.47 ^c	0.76 ^b	52.54 ^d	43.06 ^a	147.17 ^a	36.76 ^d
MeJ (50 μM)	0.59 ^c	15.50 ^b	22.85 ^e	0.26 ^f	0.76 ^b	1.26 ^a	53.49 ^d	45.38 ^a	130.00 ^a	47.16 ^c
MeJ (100 μM)	0.55 ^c	16.17 ^b	25.93 ^e	0.50 ^c	0.70 ^b	0.57 ^c	50.03 ^d	42.81 ^a	169.33 ^a	44.13 ^c
CK (20 μM)	0.50 ^c	14.17 ^c	16.43 ^e	0.45 ^d	0.85 ^a	0.92 ^a	49.92 ^d	41.29 ^a	191.50 ^a	77.13 ^b
CK (40 μM)	0.44 ^d	15.37 ^b	31.16 ^d	0.65 ^b	0.51 ^b	0.49 ^c	35.45 ^e	41.44 ^a	201.67 ^a	33.65 ^d
CK (60 μM)	0.51 ^c	13.73 ^c	30.76 ^d	0.35 ^d	0.50 ^b	0.55 ^c	34.23 ^e	41.78 ^a	223.33 ^a	35.97 ^d
SA (0.5 mM)	0.71 ^b	23.10 ^a	19.80 ^e	0.42 ^d	0.42 ^c	0.90 ^a	39.37 ^e	46.37 ^a	260.00 ^a	21.39 ^d
SA (1.0 mM)	0.77 ^a	28.40 ^a	49.14 ^a	0.55 ^c	0.59 ^b	1.65 ^a	35.28 ^e	47.38 ^a	403.33 ^a	22.47 ^d
SA (1.5 mM)	0.55 ^c	19.50 ^b	28.87 ^d	0.77 ^a	0.50 ^b	0.82 ^b	40.56 ^e	45.34 ^a	263.33 ^a	25.76 ^d

Values represent means of three replications. Different letters within a column indicate significant differences among treatments at $P \leq 0.05$ according to the LSD test. Fv/Fm = maximum quantum yield of PSII; Pn = net photosynthetic rate; WUE = water-use efficiency; Ci/Ca = intercellular to ambient CO_2 ratio; E = transpiration rate; R:S = root-to-shoot ratio; EL = electrolyte leakage; E/P = epicuticular wax content.

Table 2

Effects of exogenous application of plant growth regulators and osmoprotectants on physiological and biochemical traits of tea clone TV25 under moisture stress conditions in a rainout shelter experiment.

Treatment	Fv/Fm	Pn ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	WUE ($\mu\text{mol CO}_2\text{ mmol}^{-1}\text{H}_2\text{O}$)	Ci/Ca	E ($\text{mmol m}^{-2}\text{s}^{-1}$)	Root:shoot ratio	EL (%)	Epicuticular wax ($\mu\text{g cm}^{-2}$)	Proline ($\mu\text{g g}^{-1}\text{FW}$)	E/P
Control	0.77 ^b	21.83 ^c	40.21 ^d	0.65 ^b	0.57 ^c	0.43 ^c	57.85 ^d	14.30 ^d	68.33 ^d	25.79 ^d
Water stress	0.37 ^d	9.03 ^e	9.64 ^g	0.25 ^e	1.07 ^a	0.55 ^c	67.74 ^c	46.85 ^b	261.67 ^b	122.19 ^a
AA (100 ppm)	0.45 ^c	21.73 ^c	58.78 ^c	0.47 ^c	0.42 ^c	0.47 ^c	63.54 ^d	49.71 ^b	230.00 ^c	19.01 ^e
AA (200 ppm)	0.37 ^c	21.17 ^c	36.61 ^d	0.59 ^b	0.60 ^b	0.51 ^c	59.42 ^d	44.47 ^c	240.00 ^c	29.62 ^d
AA (300 ppm)	0.35 ^c	21.00 ^c	50.14 ^c	0.59 ^b	0.44 ^c	0.40 ^d	55.54 ^d	45.43 ^c	233.33 ^c	22.30 ^e
GB (50 mM)	0.25 ^d	15.60 ^d	37.00 ^d	0.69 ^a	0.47 ^c	0.57 ^c	58.97 ^d	47.41 ^b	245.00 ^c	31.29 ^d
GB (100 mM)	0.52 ^c	20.13 ^c	44.99 ^d	0.48 ^c	0.46 ^c	0.42 ^c	57.25 ^d	49.68 ^b	266.67 ^c	23.51 ^e
GB (200 mM)	0.38 ^c	24.43 ^b	48.33 ^d	0.61 ^b	0.52 ^b	0.52 ^c	53.71 ^d	42.75 ^c	266.67 ^c	20.96 ^e
MeJ (25 μM)	0.23 ^d	22.87 ^c	58.09 ^c	0.51 ^c	0.46 ^c	0.53 ^c	51.50 ^d	45.39 ^c	313.33 ^b	20.00 ^e
MeJ (50 μM)	0.36 ^c	18.53 ^d	35.56 ^d	0.62 ^b	0.65 ^b	0.45 ^c	50.16 ^d	46.25 ^c	301.67 ^b	36.18 ^d
MeJ (100 μM)	0.37 ^c	18.50 ^d	49.28 ^c	0.62 ^b	0.50 ^b	0.49 ^c	32.71 ^e	48.79 ^b	243.63 ^c	28.32 ^d
CK (20 μM)	0.49 ^c	14.77 ^d	55.33 ^c	0.47 ^c	0.82 ^b	0.39 ^d	34.95 ^e	47.04 ^b	241.67 ^c	57.63 ^c
CK (40 μM)	0.17 ^e	15.27 ^d	32.23 ^d	0.66 ^a	0.51 ^b	0.40 ^d	31.33 ^e	43.54 ^c	318.33 ^b	34.49 ^d
CK (60 μM)	0.43 ^c	13.37 ^d	45.43 ^d	0.52 ^c	0.54 ^b	0.50 ^c	33.67 ^e	47.55 ^b	266.67 ^c	40.24 ^d
SA (0.5 mM)	0.48 ^c	24.43 ^b	47.75 ^d	0.65 ^a	0.53 ^b	0.61 ^b	32.50 ^e	46.61 ^b	388.33 ^a	21.59 ^e
SA (1.0 mM)	0.76 ^a	32.27 ^a	68.57 ^a	0.74 ^a	0.48 ^c	1.82 ^a	30.20 ^e	61.17 ^a	463.33 ^a	14.90 ^e
SA (1.5 mM)	0.35 ^c	21.80 ^c	53.28 ^c	0.67 ^a	0.50 ^b	0.89 ^b	39.38 ^d	53.21 ^b	445.00 ^a	23.51 ^e

Values represent means of three replications. Different letters within a column indicate significant differences among treatments at $P \leq 0.05$ according to the LSD test. Fv/Fm = maximum quantum yield of PSII; Pn = net photosynthetic rate; WUE = water-use efficiency; Ci/Ca = intercellular to ambient CO_2 ratio; E = transpiration rate; R:S = root-to-shoot ratio; EL = electrolyte leakage; E/P = epicuticular wax content.

3.1.3 Multivariate analysis

Multivariate analyses revealed distinct treatment-dependent patterns in both tea clones, reflecting substantial variation in physiological and biochemical responses to moisture stress and mitigation treatments (Fig. 1; Fig. 2). Heatmap visualization identified two contrasting response groups. Moisture-stressed plants were characterized by elevated electrolyte leakage, transpiration-related traits, and proline accumulation, whereas treatments associated with improved drought tolerance exhibited higher photosynthetic activity, water-use efficiency, and PSII performance. Salicylic acid-treated plants consistently formed a separate cluster, indicating a distinct physiological response under drought conditions.

Hierarchical clustering further differentiated treatments into stress-sensitive and stress-mitigated groups, demonstrating clear divergence in adaptive responses. Principal component analysis (PCA) supported these patterns and identified the major sources of variation among treatments. In clone TV21, PC1 explained 54.3% of the total variance and showed strong positive loadings for net photosynthetic rate (Pn), water-use efficiency (WUE), and maximum quantum yield of PSII (Fv/Fm), indicating that photosynthetic competence was the primary determinant of treatment differentiation. In contrast, PC1 was negatively associated with electrolyte leakage and transpiration-related traits, suggesting that reduced cellular injury and improved water-use regulation contributed to enhanced drought tolerance. PC2 accounted for 20.1% of the total variance and was primarily influenced by proline accumulation, wax deposition, and Ci/Ca, reflecting metabolic and osmotic adjustments associated with stress adaptation.

A comparable trend was observed in clone TV25, where PC1 was dominated by traits related to photosynthetic efficiency and water-use regulation, whereas PC2 was associated with biochemical indicators of stress response. The separation of salicylic acid-treated plants from moisture-stressed treatments along the principal component axes indicates a marked shift in physiological status from stress-associated traits towards improved photosynthetic and metabolic performance. Scree plot analysis confirmed that the first two principal components captured the majority of total variation, demonstrating that a relatively small number of integrated physiological and biochemical traits effectively explained treatment responses under drought conditions.

3.1.4 Genotypic variation

TV25 exhibited higher physiological and biochemical performance compared to TV21 under both control and treatment conditions (Table 1; Table 2). Under control conditions, Pn was **21.83 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (TV25)** compared to **15.47 (TV21)**. Under SA treatment, TV25 showed higher Pn (**32.27**) and WUE (**68.57**) than TV21 (**28.40 and 49.14**, respectively). Proline accumulation was also higher in TV25.

3.2 Field experiment under rainfed conditions

3.2.1 Physiological performance

Field evaluation under rainfed conditions showed significant treatment effects (Fig. 3). The highest net photosynthetic rate was recorded in T4 (SA @ 1.0 mM), showing a 40.60% increase over control, followed by T5 (MOP @ 2%).

PSII efficiency and mesophyll efficiency were highest under SA (1.0 mM) treatment, while transpiration ratio was lowest (97.9), indicating improved water-use regulation. Electrolyte leakage was lowest in T5 (MOP) followed by SA (T4) and GB (T2).

3.2.2 Yield response

Significant variation in yield was observed among treatments (Fig. 4). The highest yield was recorded in T5 (MOP @ 2%), followed by T4 (SA @ 1.0 mM) and T2 (GB @ 100 mM).

Yield increase over control was 10.70% in T5 and 10.38% in T4, with no significant difference between them. Moderate increases were observed under ascorbic acid and cytokinin treatments.

3.2.3 Trait relationships under field conditions

Multivariate analyses revealed distinct associations among physiological traits under rainfed field conditions (Fig. 5). Correlation analysis demonstrated strong positive relationships among net photosynthetic rate (Pn), water-use efficiency (WUE), mesophyll efficiency (ME), and maximum quantum yield of PSII (Fv/Fm), indicating coordinated regulation of photosynthetic performance under moisture-limited environments. In contrast, transpiration rate (E), transpiration ratio (TR), and electrolyte leakage (EL) exhibited negative correlations with photosynthetic traits, suggesting their association with drought-induced stress and membrane instability. Hierarchical clustering classified the measured variables into three major functional groups corresponding to photosynthetic efficiency, water-loss regulation, and internal CO₂ dynamics. Principal component analysis (PCA) further confirmed that variation among treatments was primarily explained by traits related to photosynthetic capacity and water-use efficiency, highlighting their central role in drought adaptation and productivity maintenance in tea.

4. Discussion

Drought-induced reductions in photosynthetic efficiency, water-use efficiency, and membrane stability observed in the present study demonstrate the multifaceted impact of moisture deficit on tea physiology. The marked decline in Pn, WUE, and Fv/Fm, together with increased electrolyte leakage, indicates that drought imposed both stomatal and metabolic constraints on photosynthesis. Reduced CO₂ availability resulting from stomatal closure limits carbon assimilation, whereas impairment of PSII function and membrane integrity further restricts photosynthetic performance. Such responses are characteristic of drought-stressed tea plants and have been widely associated with oxidative damage, disruption of electron transport processes, and loss of cellular homeostasis (Chaves et al. 2009; Lawlor and Tezara 2009; Li et al. 2021).

Among the evaluated treatments, salicylic acid consistently exhibited the strongest protective effect against drought-induced physiological dysfunction. The recovery of Pn and Fv/Fm under SA treatment suggests preservation of PSII photochemistry and maintenance of photosynthetic activity under moisture stress. Salicylic acid functions as an important signaling molecule involved in stress adaptation and has been reported to enhance tolerance through modulation of redox balance, stabilization of chloroplast structures, and regulation of antioxidant defense pathways (Khan et al. 2015; Nazar et al. 2015; Nykiel et al. 2023). The substantial reduction in electrolyte leakage observed in the present study further indicates improved membrane stability and reduced cellular injury. Although antioxidant enzyme activities were not assessed, the physiological responses observed are consistent with reduced oxidative damage and enhanced cellular protection reported previously in tea subjected to drought stress (Damayanthi et al. 2023; Sheuli et al. 2025). These findings suggest that maintenance of photosynthetic integrity represents a major component of SA-mediated drought tolerance.

The significant accumulation of proline following SA application provides additional evidence of enhanced stress adaptation. Proline serves multiple protective functions under drought, including osmotic adjustment, stabilization of proteins and membranes, protection of photosynthetic machinery, and maintenance of cellular redox status (Szabados and Saviouré 2010; Kishor et al. 2022). Increased proline accumulation, coupled with greater wax deposition, indicates activation of complementary mechanisms that improve water retention and reduce cellular dehydration. Wax accumulation may strengthen the cuticular barrier and reduce non-stomatal water loss, thereby contributing to improved plant water status during drought. Together, these responses suggest that SA-mediated drought tolerance involves coordinated regulation of both physiological and biochemical defense mechanisms rather than a single protective pathway.

The multivariate analyses provided valuable insight into the complex relationships among drought-responsive traits. Strong positive associations among Pn, WUE, and Fv/Fm indicate that maintenance of photosynthetic efficiency is a key determinant of drought resilience in tea. In contrast, the negative relationship between electrolyte leakage and photosynthetic traits highlights the importance of membrane stability for sustaining physiological activity under stress. The separation of treatments in PCA and hierarchical clustering analyses further demonstrates that drought adaptation is governed by integrated trait networks involving photosynthetic performance, water-use regulation, and cellular protection. These findings reinforce the utility of multivariate approaches for identifying key physiological indicators associated with stress tolerance and for improving trait-based selection strategies in tea.

Distinct genotypic differences were observed between TV21 and TV25, with TV25 exhibiting greater physiological resilience under both stress and mitigation treatments. Higher photosynthetic capacity, improved water-use efficiency, and greater accumulation of protective metabolites in TV25 indicate superior adaptive plasticity under drought conditions. Such differences likely reflect variation in the efficiency of stress perception, osmotic adjustment, maintenance of photosynthetic machinery, and cellular protection mechanisms. Previous studies have similarly documented substantial variability among tea genotypes in their response to water deficit (Cheruiyot et al. 2007; Maritim et al. 2015; Upadhyaya et al. 2012). The superior performance of TV25 highlights its potential value as a genetic resource for drought-tolerance improvement programs.

Field validation confirmed that the physiological advantages conferred by SA under controlled conditions translated into improved productivity under rainfed environments. Yield responses under SA were comparable to those obtained with potassium application, emphasizing the importance of both hormonal regulation and mineral nutrition in drought adaptation. Potassium is known to support stomatal regulation, osmotic balance, enzyme activation, and assimilate transport, thereby contributing to maintenance of photosynthetic activity under moisture stress (Marschner 2012; Ruan et al. 2010). The comparable effectiveness of SA and potassium suggests that successful drought mitigation requires the integration of signaling, metabolic, and nutritional processes that collectively sustain physiological function and yield stability.

Overall, the results demonstrate that salicylic acid enhances drought tolerance in tea through coordinated regulation of photosynthetic performance, osmotic adjustment, and membrane stability. By maintaining PSII functionality, promoting accumulation of protective metabolites, and reducing cellular injury, SA effectively mitigates the adverse effects of moisture stress. The integration of physiological measurements, biochemical responses, multivariate analyses, and field validation provides a comprehensive framework for understanding drought adaptation in tea and supports the use of salicylic acid as a practical strategy for improving climate resilience in tea production systems.

Conclusion

This study demonstrates that moisture stress severely impairs photosynthetic efficiency, water-use traits, and membrane stability in tea, leading to substantial reductions in physiological performance and yield. The consistent superiority of salicylic acid (1.0 mM) across both controlled and field conditions highlights its central role in enhancing drought tolerance through coordinated regulation of PSII activity, osmotic adjustment, and cellular integrity.

The integration of multivariate analysis revealed that drought resilience in tea is governed by tightly linked physiological and biochemical traits, particularly the positive coupling of photosynthetic efficiency (P_n , WUE, F_v/F_m) and the negative association with stress indicators (electrolyte leakage and transpiration). These findings emphasize that trait integration, rather than single-parameter assessment, is critical for understanding and improving stress tolerance.

Field validation further established the complementary role of potassium nutrition (MOP @ 2%) in enhancing yield and maintaining physiological stability under moisture-limited conditions. The combined application of salicylic acid and potassium thus represents a practical and scalable strategy for improving drought resilience in tea plantations.

Overall, the study provides a robust physiological and analytical framework for developing climate-resilient tea production systems and offers actionable insights for both agronomic management and future breeding programs targeting drought tolerance.

Abbreviations

Abbreviation	Full Form
AA	Ascorbic Acid
ANOVA	Analysis of Variance
CK	Cytokinin
Ci/Ca	Intercellular to Ambient Carbon Dioxide Ratio
CRD	Completely Randomized Design
E	Transpiration Rate
EL	Electrolyte Leakage
E/P	Epicuticular Wax to Proline Ratio
Fo	Minimum Fluorescence Yield
Fm	Maximum Fluorescence Yield
Fv/Fm	Maximum Quantum Yield of Photosystem II
GB	Glycine Betaine
gs	Stomatal Conductance
IRGA	Infrared Gas Analyzer
LT	Leaf Temperature
LSD	Least Significant Difference
ME	Mesophyll Efficiency
MeJ	Methyl Jasmonate
MOP	Muriate of Potash
PCA	Principal Component Analysis
PGRs	Plant Growth Regulators
Pn	Net Photosynthetic Rate
PSII	Photosystem II
ROS	Reactive Oxygen Species
R:S	Root-to-Shoot Ratio
SA	Salicylic Acid
SE	Standard Error
SPAD	Soil Plant Analysis Development
TRA	Tea Research Association
TR	Transpiration Ratio
TV21	Tocklai Vegetative Clone 21
TV25	Tocklai Vegetative Clone 25
WUE	Water-Use Efficiency

Declarations

Author Contributions

B.G. Conceptualization, Methodology, Supervision, Project administration, Data curation, Writing and review.

B. Das: Resources, Field experimentation, Validation

R. S.: Data collection field as well as laboratory

S. K. S.: Investigation, Data collection

P. K. P.: Investigation, Data curation, Formal analysis, Writing – original draft

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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No new data were generated or analyzed in support of this research.

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This research article does not involve human participants, animals, or experimental studies requiring ethical approval.

Clinical trial number: Not applicable

Consent for Publication: Not applicable

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Figures

Fig. 1. TV21

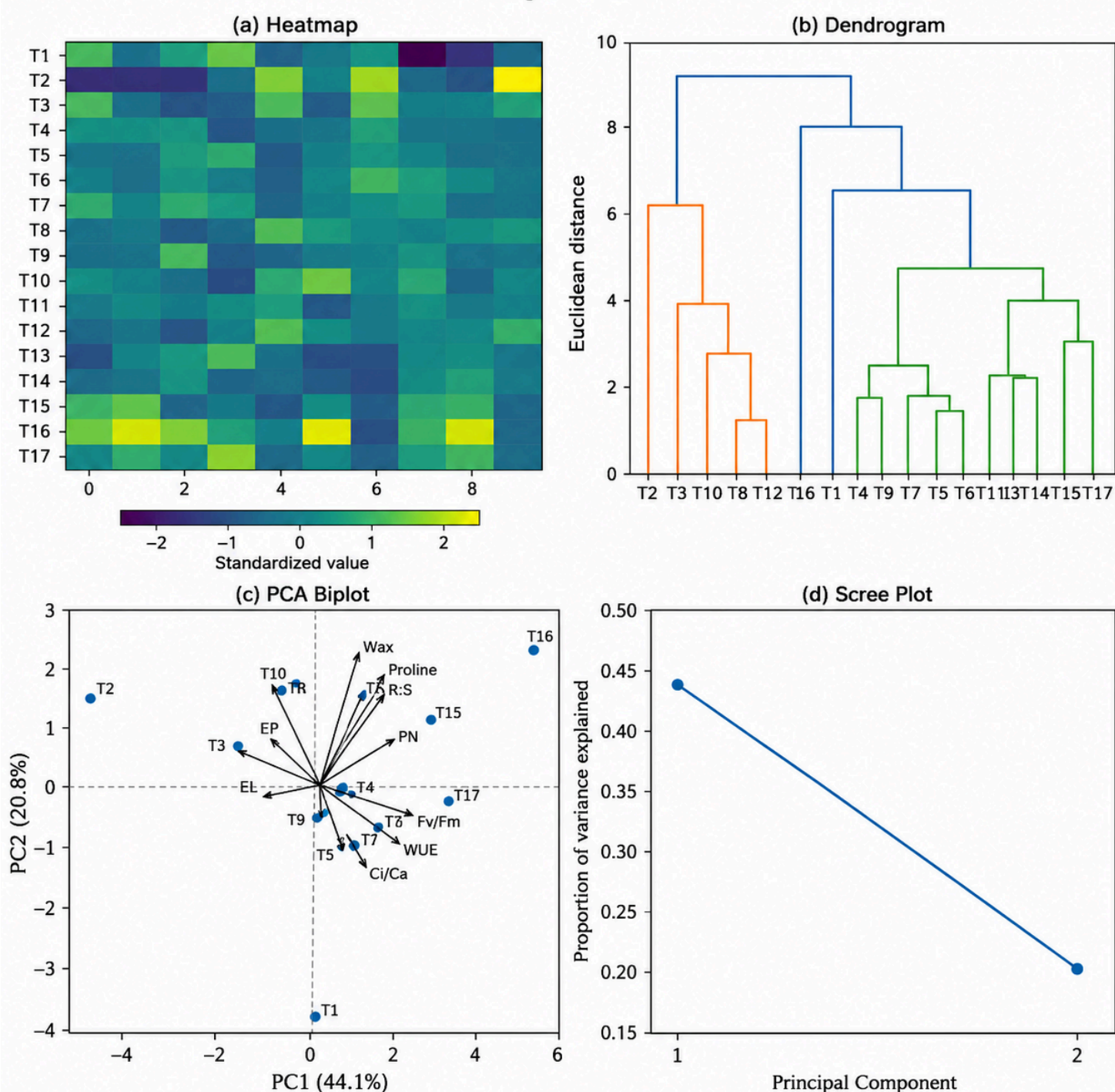


Figure 1

Multivariate analysis of physiological and biochemical responses of tea clone TV21 under moisture stress and mitigation treatments. (a) Heatmap showing standardized trait variation among treatments; (b) hierarchical clustering dendrogram illustrating treatment similarity based on physiological and biochemical attributes; (c) principal component analysis (PCA) biplot showing relationships among treatments and measured traits; and (d) scree plot indicating the percentage variance explained by principal components. Treatments are abbreviated as T1–T17 according to the experimental design

Fig. 2. TV25

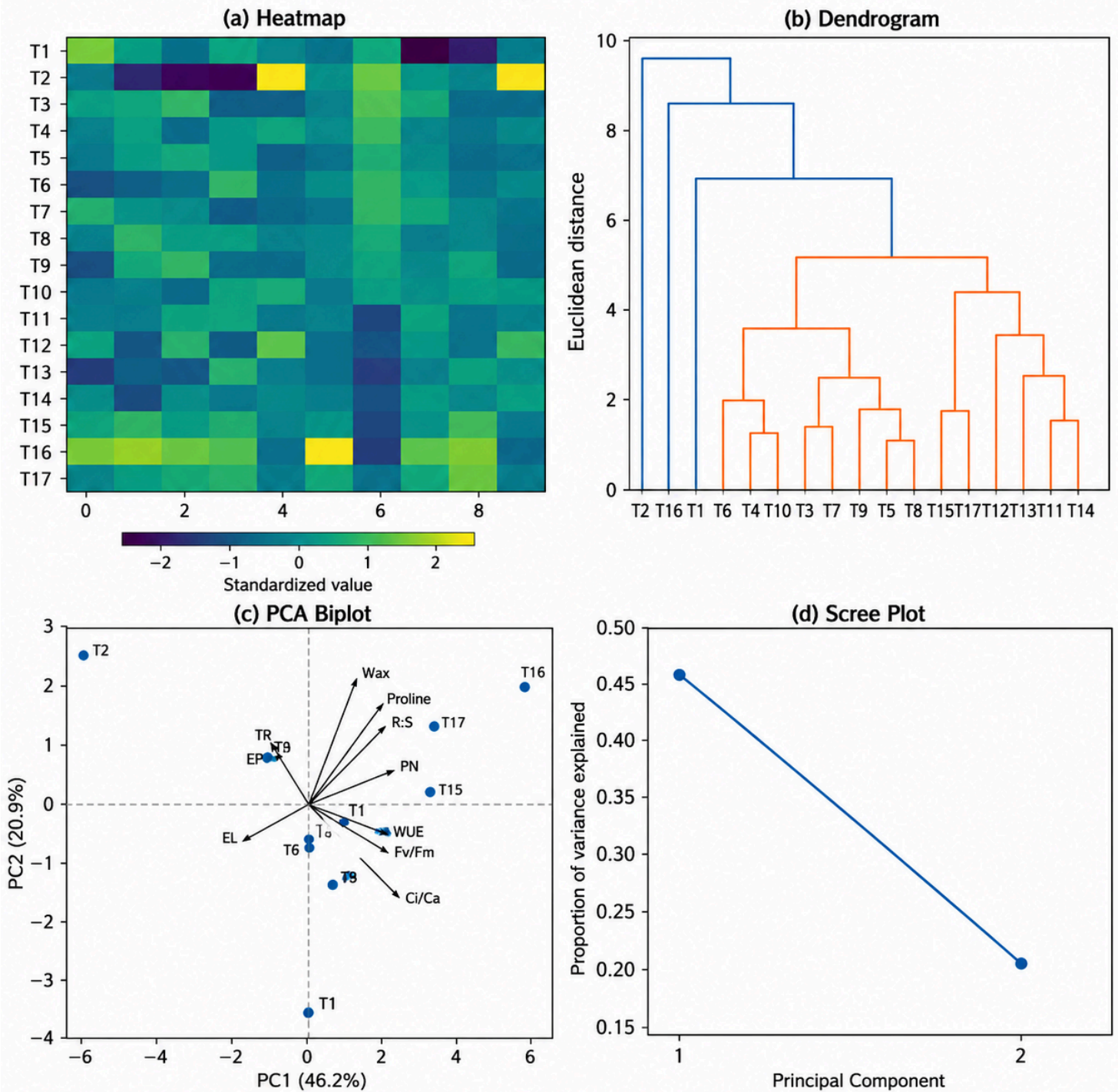


Figure 2

Multivariate analysis of physiological and biochemical responses of tea clone TV25 under moisture stress and mitigation treatments. (a) Heatmap showing standardized trait variation among treatments; (b) hierarchical clustering dendrogram illustrating treatment similarity; (c) PCA biplot depicting trait–treatment associations; and (d) scree plot showing variance explained by principal components.

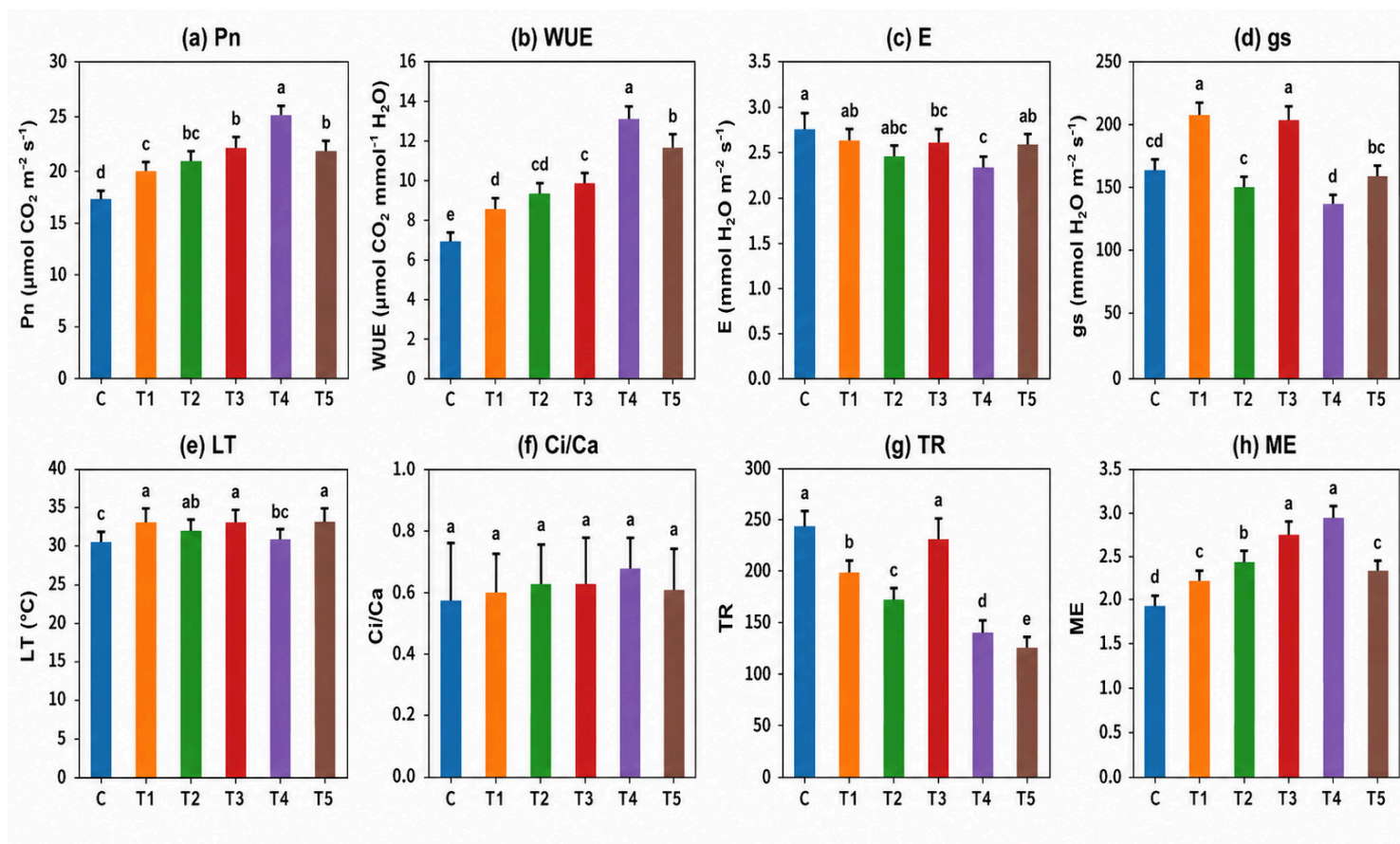


Figure 3

Effects of selected drought-mitigating treatments on physiological traits of tea under rainfed field conditions. Panels show (a) net photosynthetic rate (Pn), (b) water-use efficiency (WUE), (c) transpiration rate (E), (d) stomatal conductance (gs), (e) leaf temperature (LT), (f) intercellular to ambient CO₂ ratio (Ci/Ca), (g) transpiration ratio (TR), and (h) mesophyll efficiency (ME). Bars represent mean $\pm$ SE (n = 3). Different lowercase letters above bars indicate significant differences among treatments according to the LSD test at $P \leq 0.05$. Treatments: C, control; T1, ascorbic acid (200 ppm); T2, glycine betaine (100 mM); T3, cytokinin (40 μM); T4, salicylic acid (1.0 mM); and T5, MOP (2%).

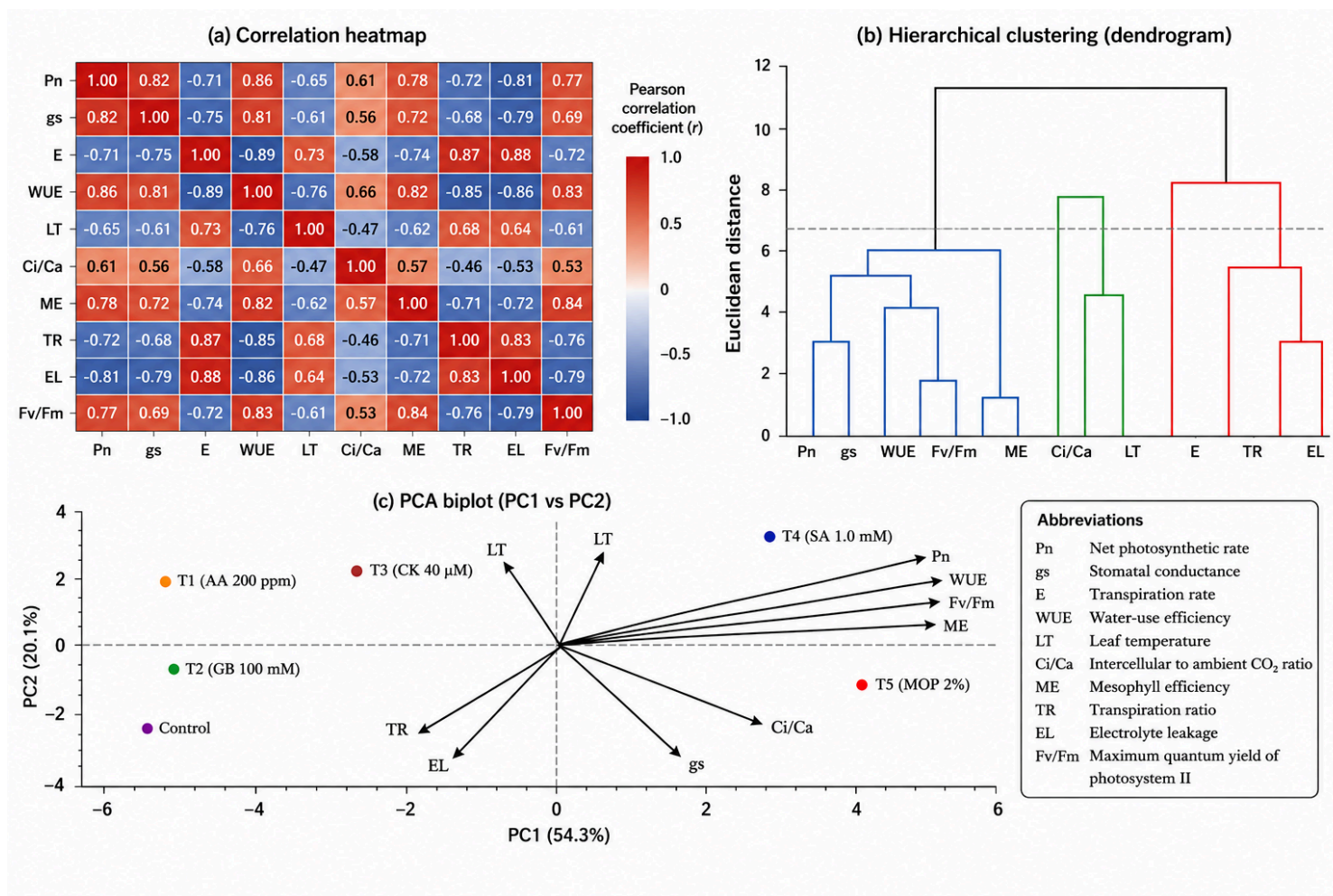


Figure 4

Effect of selected drought-mitigating treatments on green leaf yield of tea under rainfed field conditions. Bars represent mean values $\pm$ SE ($n = 3$). Different lowercase letters above bars indicate significant differences among treatments according to the LSD test at $P \leq 0.05$. Treatments: T1, ascorbic acid (200 ppm); T2, glycine betaine (100 mM); T3, cytokinin (40 μ M); T4, salicylic acid (1.0 mM); and T5, MOP (2%).

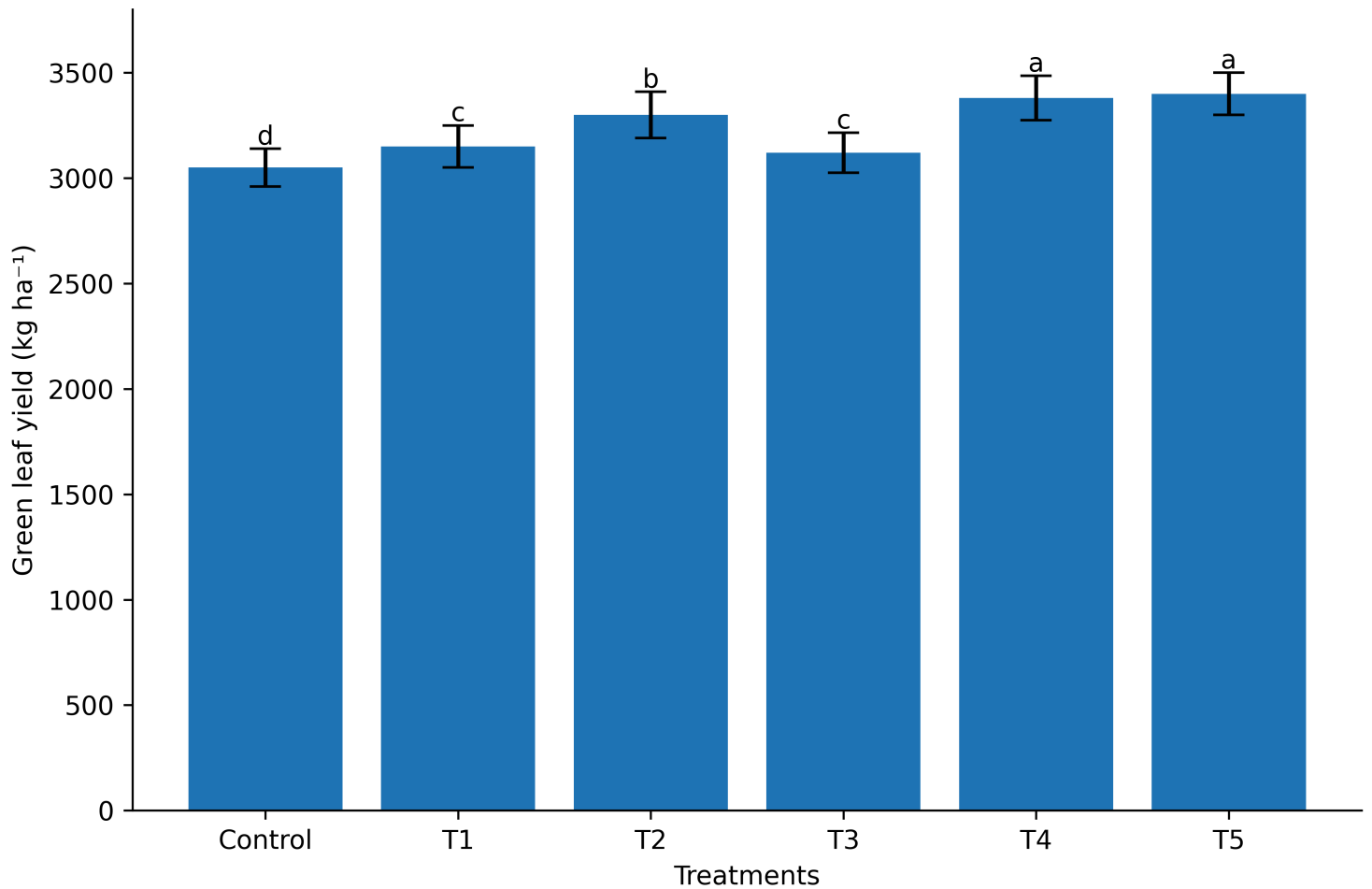


Figure 5

Multivariate analysis of physiological traits of tea under rainfed field conditions. **(a)** Pearson correlation heatmap illustrating pairwise relationships among physiological parameters. Red and blue color gradients indicate positive and negative correlations, respectively, with correlation coefficients (r) displayed within cells. **(b)** Hierarchical clustering dendrogram based on Euclidean distance showing the grouping of physiological traits according to similarity in their response patterns. **(c)** Principal component analysis (PCA) biplot showing treatment scores and trait loadings on the first two principal components. PC1 and PC2 explained **54.3%** and **20.1%** of the total variance, respectively, accounting for **74.4%** of the cumulative variation. Traits associated with photosynthetic performance and physiological efficiency, including net photosynthetic rate (P_n), water-use efficiency (WUE), maximum quantum yield of PSII (F_v/F_m), and mesophyll efficiency (ME), exhibited strong positive loadings on PC1, whereas stress-related traits such as transpiration rate (E), transpiration ratio (TR), and electrolyte leakage (EL) were negatively associated. The multivariate analyses identify photosynthetic competence, water-use regulation, and membrane stability as the principal determinants of drought tolerance in tea under field conditions.

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