

Effects of Post-Harvest Environmental Conditions on GABA Accumulation and Physiological Responses in Tea Shoots

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ABSTRACT

γ -Aminobutyric acid (GABA) is a functional metabolite associated with tea quality and potential health benefits, and it is known to accumulate in plants under abiotic stress conditions. However, information regarding the physiological response and GABA accumulation in harvested tea shoots during post-harvest handling remains limited. Therefore, this study evaluates the effects of several post-harvest environmental conditions, including water status, temperature, light availability, and treatment duration, on GABA accumulation and the physiological response of harvested tea shoots under controlled conditions. The research was conducted in 2022 using tea shoots of the TTRI-12 cultivar consisting of one bud and three leaves. The shoots were harvested from the experimental garden of National Chung Hsing University and arranged in a completely randomised design with three replicates. The results showed that various post-harvest stress treatments significantly affected GABA content, proline levels, water content, and stomatal conductance. An overall increase in GABA content was observed in treatments involving soaking, high temperature (37.5°C), dark conditions, and a 2-hour treatment. Pearson correlation analysis indicated that proline was the only physiological parameter significantly associated with GABA accumulation. These findings suggest

that harvested tea shoots maintain physiological activity and metabolic activity during the post-harvest period. Overall, manipulating post-harvest environmental conditions has the potential to support the development of GABA tea and improve its quality.

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INTRODUCTION

The tea plant (*Camellia sinensis* L.) is a globally favoured beverage, recognised for its diverse bioactive constituents that enhance its quality and health benefits (Khan & Mukhtar, 2013, 2019). In addition to catechins and amino acids, gamma-aminobutyric acid (GABA) has become an important functional component in the development of tea products. GABA is a non-proteinogenic amino acid that accumulates in plant tissues due to numerous abiotic stressors (Kaspal et al., 2021; Wu et al., 2018; Yong et al., 2017). In tea products, elevated GABA levels correlate with functional value and potential health benefits, including mitigation of insomnia, depression, and anxiety, thereby reducing stress-induced increases in blood pressure (Lin et al., 2023). These beneficial effects are driving continuous initiatives to increase GABA levels in tea leaves.

Tea leaves accumulate GABA in response to abiotic stress. Previous research on diverse species of intact plants has shown that GABA enhances stress tolerance during chilling, drought, low-light, and heat stress by maintaining elevated relative water content (RWC), facilitating osmotic adjustment, stimulating proline and sugar accumulation, improving photosynthetic efficiency and stomatal conductance, and activating antioxidant defense mechanisms while reducing oxidative stress indicators (Liang et al., 2026; Malekzadeh et al., 2014; Nayyar et al., 2014; Yong et al., 2017). GABA accumulation in harvested tea plants, especially in oxygen-deficient conditions, has been studied. Post-harvest anaerobic conditions can increase tea leaf GABA concentration by eightfold, exceeding 1.5 mg/g after multiple aerobic and anaerobic incubations (Tsushida & Murai, 1987; Wu et al., 2018). The studies indicate that postharvest environmental factors significantly affect GABA accumulation in tea leaves.

In plants, GABA accumulation is associated with proline accumulation in response to several abiotic stressors. As glutamate metabolites, proline and GABA are linked to plant stress responses. Glutamate can be converted into GABA by glutamate decarboxylase (GAD) or proline by pyrroline-5-carboxylate (P5C) (Y. Wang et al., 2020). Proline accumulation can remain after rice leaves are detached from the parental plant, suggesting metabolic activity in post-harvest leaves (Chen et al., 2001). This was also shown in hypoxic post-harvest tea leaves (Siahaan & Palupi, 2025). These conditions indicate that several physiological changes may still occur during harvesting, postharvest, and before tea processing.

Post-harvest leaf physiology affects stomatal behaviour. Physiological indicators such as stomatal conductance are affected by environmental factors including light, water availability, temperature, CO₂ concentration, and abiotic stress (Faralli et al., 2019; Xu & Zhou, 2008). In general, harvested leaves degrade slowly rather than immediately reaching minimum stomatal conductance (Duursma et al., 2018). Given that stomata regulate gas exchange, including oxygen diffusion, changes in stomatal conductance may influence GABA accumulation during post-harvest stress (Lawson & Blatt, 2014; X. Mei et al., 2016).

Previous studies have extensively discussed the role and accumulation of GABA in various plant species experiencing abiotic stress (Liang et al., 2026; Malekzadeh et al., 2014; Nayyar et al., 2014; Yong et al., 2017). Additionally, increased GABA accumulation in tea leaves due to anaerobic treatment and its correlation with various plant physiological responses have been reported (Liao et al., 2017; Tsushida & Murai, 1987; Wu et al., 2018). However, Research on GABA accumulation and physiological responses of tea shoots under post-harvest abiotic stress remains limited, despite the known influence of pre- and post-harvest stress on metabolites related to tea quality (Shao et al., 2021).

Water status affects tissue hydration (da S. Lopes et al., 2024); temperature affects cellular metabolism (Marchin et al., 2022; Priya et al., 2019); light exposure during tea shoot harvesting and storage can alter stomatal regulation and leaf physiological activity (Ye et al., 2021); and stress duration affects metabolic responses. These changes may trigger physiological and biochemical responses associated with GABA accumulation in tea shoots during post-harvest processing. This study examines how post-harvest environmental conditions, including water status, temperature, light availability, and treatment duration, affect GABA, proline accumulation, water content, and stomatal conductance in tea shoots, as well as the correlations among these parameters. Field conditions during tea harvesting determined treatment considerations. The results of this research are expected to contribute to a deeper understanding of post-harvest stress physiology in tea shoots and provide useful information for the development of GABA tea processing.

MATERIALS AND METHODS

This experiment was conducted from May to December 2022 at the Agronomy Department Lab of National Chung Hsing University (NCHU) in Taiwan. The tea leaves cultivar TTRI-12 were used as the sample for this experiment and were obtained from the NCHU practice field. Although the ages of individual plants in the field varied, the sample selection was based on uniform tip maturity and consistent harvesting standards to minimise physiological variation among samples. The harvesting criteria used for the samples in this study consisted of one bud and three leaves to mimic the actual condition of tea leaves typically used in a tea beverage product (Aaqil et al., 2023).

Experimental Design and Sample Preparation

The experiment was conducted as a factorial experiment arranged in a completely randomised design (CRD). The treatment factors were leaf water status (soaking and withering); treatment duration (2, 4, and 8 hours); treatment temperature (25 °C and 37.5 °C); and light conditions (light at 94 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and dark). The initial sample was used as the control before treatment and served as a baseline for evaluating physiological changes after treatment application. After harvesting, fresh tea shoots were divided into three groups: control, soaking, and withering. In the soaking treatment, tea shoot stems

were soaked in water to maintain freshness and turgidity during storage, whereas in the withering treatment, they were only placed in trays. Both soaking and withering treatments were applied under combinations of two temperatures (25 °C and 37.5 °C), two light conditions (light and dark), and three treatment durations (2, 4, and 8 hours). At the end of each treatment duration, samples were immediately stored at -20 °C until further simultaneous physiological analysis. Storage at -20 °C for both the control and other treatments was intended to ensure that all treatments were analysed together under the same conditions, thereby minimising inter-analysis variation and maintaining consistency among measurements. The sample size per treatment was 4-5 tea shoots, and the treatment was conducted in a growth chamber with 3 replicates.

Determination of Water Content

For water content determination, the samples were ground in liquid nitrogen and stored at -20 °C until analysis. Approximately 0.3-0.5 g of the sample mass was transferred into an Eppendorf tube and dried in an oven at 70 °C for 48 hours.

Determination of Stomatal Conductance

Stomatal conductance was measured with a leaf porometer (SC-1, METER Group, Inc., Pullman, WA, USA) on the abaxial surface of the tea leaf. For the control group, measurements were taken immediately after harvest; for the tea groups exposed to water status, duration, temperature, and light conditions, checks were conducted immediately after treatment completion.

Determination of Proline Content

For proline determination, samples were ground with liquid nitrogen, placed into tubes, and stored at -20 °C. After all the samples were ground, between 0.3-0.5 g of each sample was placed in an Eppendorf tube. Proline analysis was based on the procedure from Bates et al. (1973) with some modifications. A total of 1.5 mL of Sulfosalicylic acid 3% (w/v) was added to the sample, which was then centrifuged for 20 minutes at 25 °C and 10,000 g. For the analysis, a total of 0.5 mL of the supernatant was used for the proline assay. The ninhydrin reagent was prepared by dissolving 0.25 g of ninhydrin in a mixture of 6 mL of acetic acid and 4 mL of 6 M H₃PO₄. A total of 0.5 mL of filtrate was reacted with 0.5 mL of ninhydrin acid solution and 0.5 mL of acetic acid, then incubated for 1 hour at 100 °C, and finally immersed in an ice bath to stop the reaction. Then the mixture was extracted with 2 mL of toluene and vigorously shaken in a vortex mixer for 15-20 seconds. The absorbance was measured at 520 nm with the spectrophotometer in cuvette mode (SpectraMax M2, Molecular Devices, San Jose, CA, USA). Toluene was used for the blank. A standard curve was generated using known concentrations of pure proline for quantification.

Determination of GABA Content

For GABA determination, samples were ground in liquid nitrogen, placed in tubes, and stored at 20 °C. After all the samples were ground, 0.3-0.5 g of each sample was placed in an Eppendorf tube. For the GABA extraction, the tea leaf samples were mixed with 1 mL of hot water and allowed to stand for 6 minutes. A 250 µl reagent solution containing 300 mL of o-phthalaldehyde (OPA) stock solution, 20 µl of 3-mercaptopropionic acid, and 4 mL of borate buffer was added to a 50 µl aliquot of the supernatant. The mixture was then filtered using a syringe filter, and 20 µl was injected into an HPLC (High-Performance Liquid Chromatography) system (Model LC-10AD VP, Shimadzu, Japan). For the HPLC analysis, mobile phase I consisted of 90 mL of phosphate buffer mixed with 370 mL of ddH₂O, and mobile phase II consisted of acetonitrile (HPLC grade). The stock solution of OPA was prepared by dissolving 100 mg OPA in 3 mL of methanol. The borate buffer was prepared by dissolving 4.77 g of boric acid in approximately 475 mL of water. The pH of the buffer was adjusted to 10.0 using 12 M and 1 M NaOH, and the final volume was also adjusted to 500 ml with ddH₂O. The phosphate buffer was prepared by dissolving 3.54 g KH₂PO₄ and 5.66 g Na₂HPO₄ in 800 mL of distilled water, adjusting the pH to 7.0 with 50% (w/v) NaOH, and making the final volume up to 1 L. The HPLC column was SHARPSIL C18 (4.6 mm × 25 cm, 5 µm), and a UV-Vis detector was used. Gradient elution profile: 0-15 minutes was kept at 89% of buffer A; 15-17 minutes, linear gradient change to 50% of buffer A; 17-20 minutes, kept at 50% of buffer A; 20-22 minutes, linear gradient change to 89% of buffer A; 22-30 minutes, kept at 89% of buffer A. 0.8 ml/minute was set as the flow rate, and spectrofluorometric detection was at an absorbance wavelength of 228 nm.

Data Analysis

The data were analysed using the General Linear Model (GLM) procedure to assess the main effects of water status, temperature, light conditions, and treatment duration on GABA accumulation and physiological responses in tea shoots. Mean comparisons among treatment levels were conducted using the Least Significant Difference (LSD) test at $P < 0.05$. Correlation analysis was used to determine relationships between parameters. Pearson correlation analysis was performed using pooled data from all treatment combinations.

RESULT

Effect of Water Status on GABA Accumulation and Physiological Responses

The various water status levels affected GABA, proline, water content, and stomatal conductance ($p < 0.05$) (Figure 1). The water treatment factor significantly influenced GABA accumulation. Soaking treatment resulted in the highest GABA concentration, at 0.09 mg/g DW, which was significantly higher than the control (0.05 mg/g DW). Meanwhile, the

withering treatment showed an intermediate GABA concentration of 0.07 mg/g DW, but it was not significantly different from the soaking treatment and the control (Figure 1a).

A different pattern from GABA was found in proline accumulation. The withering treatment resulted in the highest proline concentration, at 10.72 $\mu\text{mol/g DW}$, although it was not significantly different from that of the soaking treatment (9.02 $\mu\text{mol/g DW}$). Meanwhile, the control treatment accumulated the least proline, at 5.28 $\mu\text{mol/g DW}$, which was significantly lower than the soaking and withering treatments (Figure b).

The soaking treatment maintains the highest water content at 73.95%, with no significant difference from the control at 71.77%. Conversely, the withering treatment significantly reduces the water content to 65.23%, indicating water loss (Figure 1c). Similarly, stomatal conductance decreases significantly in each water-status treatment. The tea shoot in the control condition shows the highest stomatal conductance, approximately 29.03 $\text{mmol/m}^2/\text{s}$, which then decreases in the soaking treatment to 19.47 $\text{mmol/m}^2/\text{s}$, and reaches the lowest value in the withering treatment at 3.51 $\text{mmol/m}^2/\text{s}$ (Figure 1d).

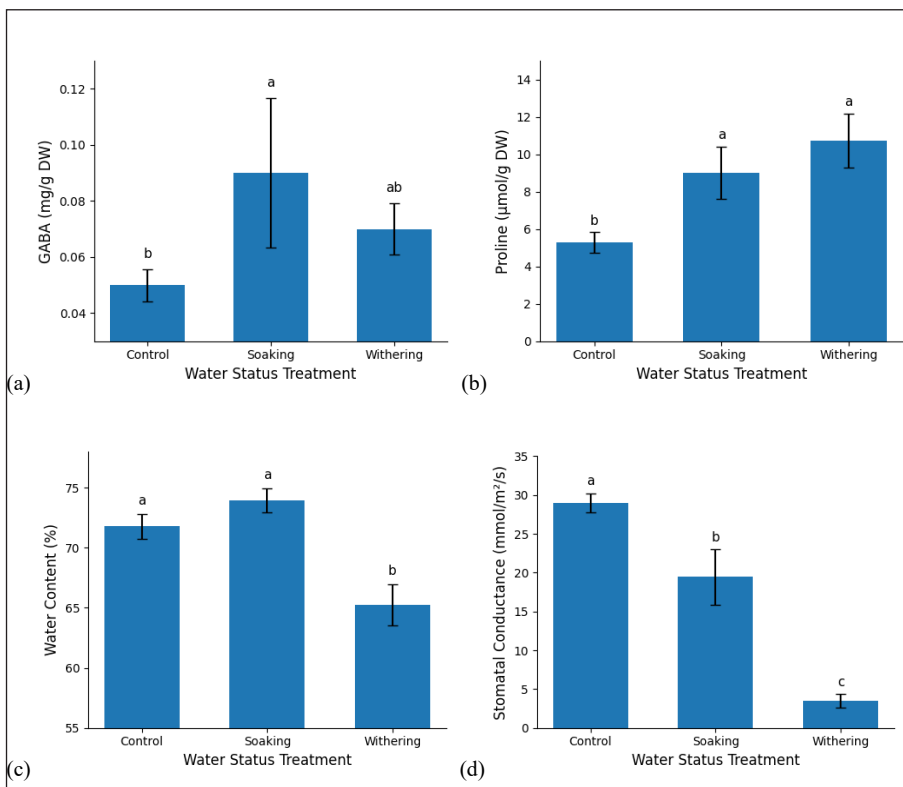


Figure 1. The GABA (a), proline (b), water content (c), and stomatal conductance (d) change in water status treatment. Statistical analysis using least significant difference (LSD). Bars with different letters indicate significant differences ($P < 0.05$)

Effect of temperature on GABA accumulation and physiological responses

Treatment of abiotic stress caused by temperature differences results in the highest GABA accumulation at 37.5 °C. The GABA concentration accumulated higher when placing tea shoots at 37.5 °C (0.11 mg/g DW), which is significantly different from the control treatment (0.05 mg/g DW) and at 25 °C (0.06 mg/g DW). This indicates that the lowest GABA accumulation occurs in the control treatment and that it gradually increases with rising storage temperature (Figure 2a).

The highest proline accumulation was observed when the storage temperature of the tea shoots was increased to 37.5 °C (12.65 μ mol/g DW), which was significantly higher than the control treatment and the 25 °C treatment. The 25 °C temperature treatment also tended to increase proline accumulation to 7 μ mol/g DW compared with the control (5 μ mol/g DW), although the difference was not significant (Figure 2b).

Water content of tea shoots decreased significantly at 37.5 °C, reaching a minimum of 67%, which differed significantly from the control and 25 °C treatments.

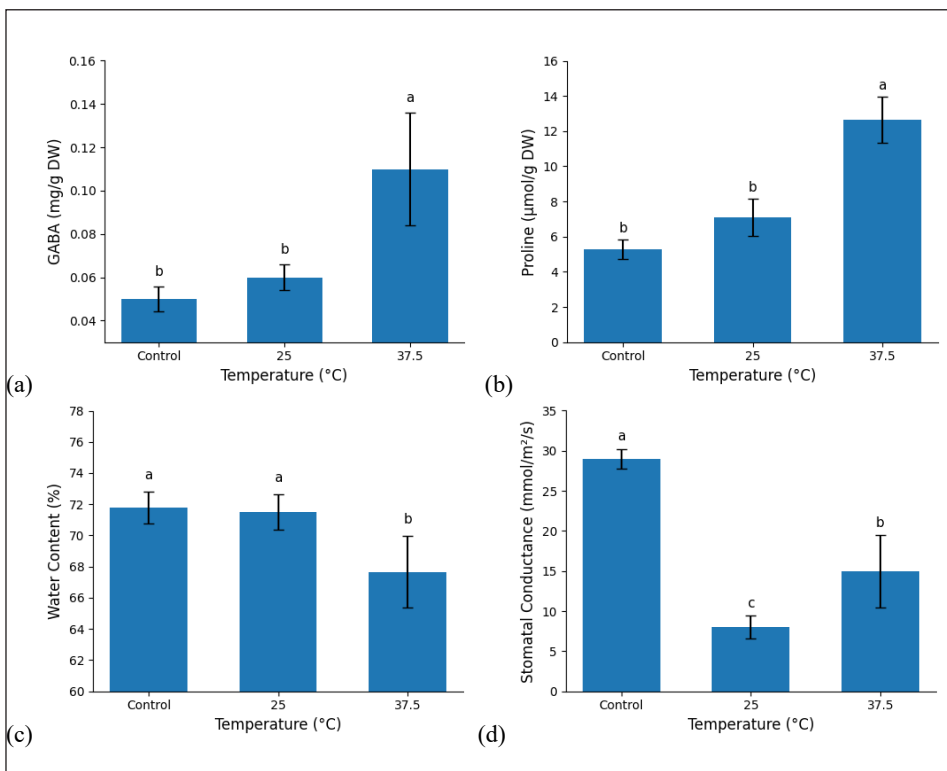


Figure 2. The GABA (a), proline (b), water content (c), and stomatal conductance (d) change with temperature treatment. Statistical analysis using least significant difference (LSD). Bars with different letters indicate significant differences ($P < 0.05$) ($n = 3$)

Meanwhile, at 25 °C, there was no significant difference in the water content of tea shoots compared with the control treatment. In the control treatment, the water content of the tea shoots was 71% and decreased slightly to 70% when the storage temperature was increased to 25 °C (Figure 2c). However, for stomatal conductance, the highest value is observed in the control treatment (29.04 mmol/m²/s), then decreases significantly and reaches its lowest value at 25 °C (8.03 mmol/m²/s). Furthermore, as the temperature increases to 37.5 °C, stomatal conductance rises to 14.95 mmol/m²/s and differs significantly from the 25 °C treatment (Figure 2d).

Effect of Light Availability on GABA Accumulation and Physiological Responses

Light availability treatment had a significant effect on changes in GABA accumulation in tea shoots (Figure 3a). Tea shoots that received dark treatment after harvesting appeared to accumulate the highest GABA, at 0.09 mg/g DW, which was significantly higher than the control treatment (0.05 mg/g DW) but not significantly different from the light treatment (0.07 mg/g DW). Conversely, light and dark treatments showed a significantly greater increase in proline accumulation than the control treatment (Figure 3b). The proline concentration accumulated in the light treatment was 9.90 µmol/g DW, which was not significantly different from that in the dark treatment (9.84 µmol/g DW). The initial or control proline concentration was 5.28 µmol/g DW.

Under light and dark conditions, water content in tea shoot tissue decreased from 71.77% in the control treatment to 70.48% in the light treatment. This decrease appears very small and is not statistically significant. Unlike the dark treatment, the water content decrease was very low (68.69%) and significantly different from the control treatment, but not significantly different from the light treatment (Figure 3c).

Among all the physiological parameters observed, stomatal conductance showed the most significant response to the light availability treatment (Figure 3d). The highest stomatal conductance was observed in the control condition (29.03 mmol m⁻² s⁻¹), which was significantly higher than in the light and dark treatments. The light treatment resulted in a stomatal conductance of 14.34 mmol m⁻² s⁻¹, while the lowest value was found in the dark treatment, at 8.64 mmol m⁻² s⁻¹.

Effect of Treatment Duration on GABA Accumulation and Physiological Responses

The treatment duration of abiotic stress showed a significant effect on GABA accumulation in tea shoots. The control treatment (0 h) showed the lowest GABA accumulation, at 0.05 mg/g DW. As storage time increased to 2 hours, GABA accumulation increased significantly compared to the control treatment and reached the highest level, at 0.10 mg/g DW. However, the difference was not statistically significant compared with

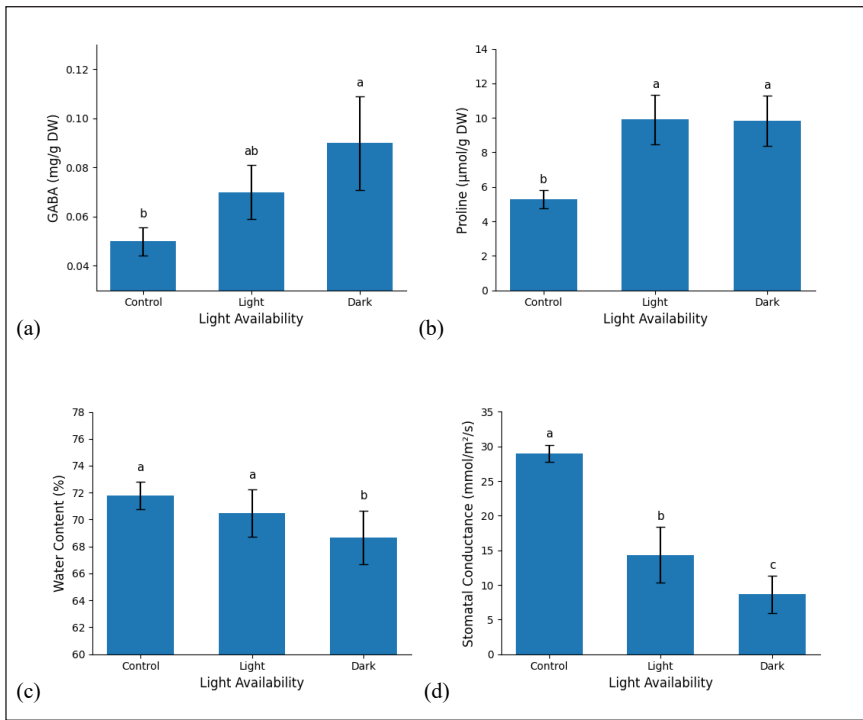


Figure 3. The GABA (a), proline (b), water content (c), and stomatal conductance (d) change in light availability treatment. Statistical analysis using least significant difference (LSD). Bars with different letters indicate significant differences ($P < 0.05$) ($n = 3$)

tea shoots treated for 4 hours (0.07 mg/g DW) and 8 hours (0.08 mg/g DW). This trend indicates that GABA content gradually declined after peaking at 2 hours (Figure 4a).

The same trend was observed in proline accumulation resulting from treatments with different durations of abiotic stress (Figure 4b). In the control conditions, proline concentration was the lowest among treatments with increased durations, at 5.28 $\mu\text{mol/g DW}$. Proline accumulation reached its peak at 2 hours, at 9.46 $\mu\text{mol/g DW}$, which was not significantly different from the treatments at 4 hours (8.70 $\mu\text{mol/g DW}$) and 8 hours (11.46 $\mu\text{mol/g DW}$). However, generally, the pattern of accumulation change indicates that proline accumulation increased during the 2-hour treatment, then decreased at 4 hours, and gradually increased significantly at 8 hours.

The variation in treatment duration also significantly affects changes in water content in tea shoot tissue (Figure 4c). Control treatment showed a tissue water content of 71.77%, which then gradually decreased during the 2-hour treatment (71.72%), although this decrease was not significant. As the duration of the treatment increased, the water content continued to decrease significantly at 4 hours (69.43%) and 8 hours (68.05%) compared to the control condition.

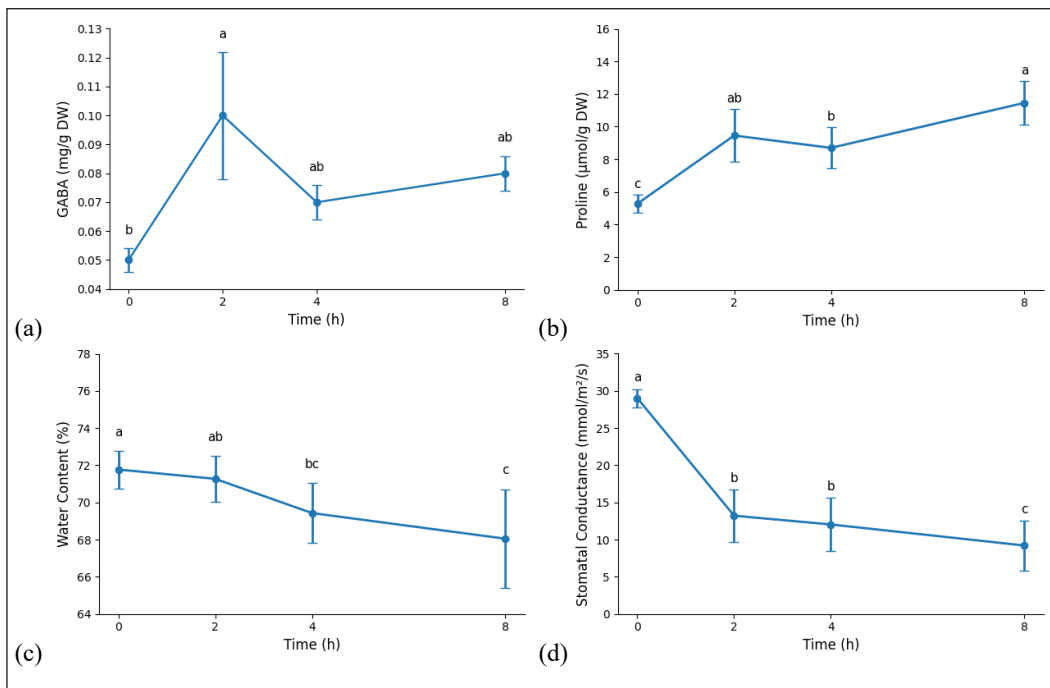


Figure 4. The GABA (a), proline (b), water content (c), and stomatal conductance (d) change in different treatment durations. Statistical analysis using least significant difference (LSD). Dots with different letters indicate significant differences ($P < 0.05$) ($n = 3$)

Unlike water content, the initial shoot condition (control treatment) had the highest stomatal conductance ($29.03 \text{ mmol/m}^2/\text{s}$), which then decreased significantly with increasing treatment duration. However, stomatal conductance at the 2-hour treatment ($13.22 \text{ mmol/m}^2/\text{s}$) was not significantly different from that at the 4-hour treatment ($12.04 \text{ mmol/m}^2/\text{s}$), but it was significantly different from that at the 8-hour treatment ($9.21 \text{ mmol/m}^2/\text{s}$) (Figure 4d).

Pearson Correlation among GABA and Physiological Parameters

Pearson correlation analysis indicates varying relationships between GABA accumulation and physiological parameters of tea shoots during post-harvest stress treatment (Table 1). GABA shows a highly significant positive correlation with proline, but there is no significant correlation with other physiological responses, namely water content and stomatal conductance. Conversely, proline shows a significant negative correlation with water content and stomatal conductance. Meanwhile, for the water content and stomatal conductance parameters, a highly significant positive correlation was also observed.

Table 1

Correlation between GABA, proline, water content, and stomata conductance in tea shoots exposed to several water statuses, temperature, light availability, and treatment duration

Parameter	Water Content	Stomatal Conductance	Proline
GABA	0.146 ns	0.127 ns	0.200**
Water Content		0.468**	-0.266**
Stomatal Conductance			-0.192*

Note. Asterisks * and ** denote statistical significance at $P \leq 0.05$ and $P \leq 0.01$, respectively. ns, not significant. All the numbers are obtained from the Pearson correlation

DISCUSSION

Post-harvest abiotic stress treatment affects GABA accumulation and physiological responses in tea shoots. In general, stress circumstances such as water status, temperature, light availability, and treatment duration increase GABA content relative to controls. These modifications also affect proline accumulation, water content, and stomatal conductance. This study found that harvested tea shoots retain physiological activity and metabolic processes, enabling them to adapt to environmental changes after harvest. Previous research has shown that post-harvest tea shoots can still respond to environmental stress through physiological and metabolic changes (Shao et al., 2021).

Tea shoots accumulate more GABA after soaking as a response to changes in water status and oxygen availability. Reduced oxygen availability in tea shoot tissues during soaking may increase glutamate decarboxylase (GAD), which turns glutamate into GABA via the GABA shunt pathway (Crawford et al., 1994; Liao et al., 2017). In addition to water-status treatment, high temperature (37.5 °C) also promotes GABA accumulation. GABA accumulation in intact plants is associated with membrane protection and mitochondrial activity under heat stress (Nayyar et al., 2014; Priya et al., 2019), which may explain the increased GABA content in this study under high temperatures.

Dark conditions tend to increase GABA accumulation compared with light conditions. In detached tea shoots, the absence of light temporarily halts photosynthesis, while cellular respiration continues, leading to CO₂ accumulation and a decrease in intracellular pH. This acidic condition activates the enzyme GAD, which converts glutamate into GABA via the GABA shunt pathway (Yogeswara et al., 2020). Furthermore, during treatment, GABA content tends to increase with prolonged postharvest stress, suggesting that prolonged stress may intensify metabolic changes in tea shoots.

Proline plays a role in maintaining osmotic stability and protecting plant cells during abiotic stress. The withering and soaking treatments increase proline levels relative to the control because both trigger post-harvest stress: withering induces osmotic stress from water loss (Lv et al., 2011), while soaking induces hypoxia due to low oxygen availability

(Siahaan & Palupi, 2025). Although proline levels during withering tend to be higher than during soaking, the difference is not significant, indicating that both treatments induce a similar level of physiological stress that triggers proline accumulation.

Treatment at 37.5 °C significantly increased proline accumulation compared to the control and 25.5 °C, indicating the role of proline as a reactive oxygen species (ROS) scavenger and protein stabiliser during heat stress in tea shoots (Roychoudhury et al., 2015; Xiong et al., 2012). Under light availability treatment, both light and dark conditions differed significantly from the control, but did not differ significantly from each other. Proline content in dark conditions tended to be lower than in light, which is suspected to be related to the use of carbohydrate reserves during post-harvest respiration, considering that proline synthesis is influenced by carbohydrate availability (Stewart, 1972). Meanwhile, the highest proline levels were observed at an 8-hour treatment duration, although they were not significantly different from those at the 2-hour treatment. Based on previous research, this increase in proline is associated with a sharp spike in Pyrroline-5-carboxylate synthetase gene expression during the first 2 hours of stress (H. Wang et al., 2015).

Water content and stomatal conductance show consistent physiological changes in response to various post-harvest stress treatments on tea shoots. The withering treatment causes a significantly greater decrease in water content than the control or soaking, indicating water loss due to the withering process. Conversely, the soaking treatment maintains a higher water content due to direct contact between the tissue and water (Zeng et al., 2024). In terms of temperature treatment, exposure to 37.5 °C increases water loss compared to the control and 25 °C, although not significantly, whereas dark conditions and longer treatment durations (4 and 8 hours) significantly reduce water content. The decrease in water content during the treatment is suspected to be related to increased post-harvest respiration and transpiration, which leads to greater utilisation of tissue water reserves as the treatment duration lengthens (S. Mei et al., 2022).

The change in water content is in line with the response of stomatal conductance, which tends to decrease under stress conditions. Soaking treatment reduces stomatal conductance compared to the control and experiences a sharper decline during withering. This finding further indicates that as leaves lose water content, stomatal conductance correspondingly decreases. Stomatal conductance depends on the plant's water potential or hydration status (Giménez et al., 2013). In temperature treatments, the lowest stomatal conductance was observed at 25 °C and was significantly different from both the control and 37.5 °C. This decrease is likely due to stomatal closure from water stress after tea shoots are harvested. However, the increase in stomatal conductance again at 37.5 °C may be due to disrupted stomatal regulation under heat stress, leading to passive stomatal leakage even when the tissue is under stress (Marchin et al., 2022). Light treatment results in higher stomatal conductance compared to darkness. Light directly or indirectly influences the biosynthesis

and degradation of Abscisic acid (ABA) (Ye et al., 2021). Degradation of ABA may occur, resulting in continued stomatal opening due to the absence of closure signals. Abscisic acid (ABA) is a plant hormone that responds to abiotic and biotic stresses, significantly affecting stomatal movement and transpiration (Bharath et al., 2021). For the duration treatment, an 8-hour treatment resulted in the largest decrease in stomatal conductance compared with other treatments.

Pearson's correlation indicates that water content is strongly positively correlated with stomatal conductance, meaning that the higher the water content in leaves, the wider the stomatal openings. Conversely, a decrease in water content triggers stomatal closure to prevent dehydration (da S. Lopes et al., 2024). Proline shows a significant negative correlation with water content and stomatal conductance, indicating its role in the plant's natural defence mechanism (osmoregulation) when facing stress (Hayat et al., 2012). Conversely, GABA shows no significant correlation with water content or stomatal conductance but a highly significant positive correlation with proline. This suggests that GABA accumulation is more closely related to metabolic and biochemical responses to post-harvest stress than to direct physiological changes in water status and stomatal activity.

CONCLUSION

This study reveals that post-harvest water status, temperature, light availability, and treatment time affect GABA accumulation and physiological responses in harvested tea shoots. Generally, post-harvest abiotic stress treatments enhance GABA and proline and decrease water content and stomatal conductance compared to the initial condition after picking. Pearson correlation analysis indicates that proline is a physiological parameter affecting GABA accumulation. These findings imply that post-harvest tea shoots still maintain active physiological activity and metabolism, allowing them to respond to environmental changes after harvesting.

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REFERENCE

Aaqil, M., Peng, C., Kamal, A., Nawaz, T., Zhang, F., & Gong, J. (2023). Tea harvesting and processing techniques and its effect on phytochemical profile and final quality of black tea: A review. *Foods*, 12(24), Article 4467. <https://doi.org/10.3390/foods12244467>

- Bharath, P., Gahir, S., & Raghavendra, A. S. (2021). Abscisic acid-induced stomatal closure: An important component of plant defense against abiotic and biotic stress. *Frontiers in Plant Science*, *12*, Article 615114. <https://doi.org/10.3389/fpls.2021.615114>
- Chen, C. T., Chen, L.-M., Lin, C., & Kao, C. H. (2001). Regulation of proline accumulation in detached rice leaves exposed to excess copper. *Plant Science*, *160*(2), 283-290. [https://doi.org/10.1016/S0168-9452\(00\)00393-9](https://doi.org/10.1016/S0168-9452(00)00393-9)
- Crawford, L. A., Bown, A. W., Breikreuz, K. E., & Guinel, F. C. (1994). The synthesis of γ -aminobutyric acid in response to treatments reducing cytosolic pH. *Plant Physiology*, *104*(3), 865–871. <https://doi.org/10.1104/pp.104.3.865>
- da S. Lopes, A., de Andrade Júnior, A. S., Bastos, E. A., de Sousa, C. A. F., Casari, R. A. D. C. N., & de Moura, M. S. B. (2024). Detecção do estado hídrico em híbridos de milho com imagens aéreas obtidas por aeronave remotamente pilotada [Assessment of maize hybrid water status using aerial images from an unmanned aerial vehicle]. *Revista Caatinga*, *37*, Article e11701. <https://doi.org/10.1590/1983-21252024v37i11701rc>
- Duursma, R. A., Blackman, C. J., Lopez, R., Martin-StPaul, N. K., Cochard, H., & Medlyn, B. E. (2018). On the minimum leaf conductance: Its role in models of plant water use, and ecological and environmental controls. *New Phytologist*, *221*(2), 693–705. <https://doi.org/10.1111/nph.15395>
- Faralli, M., Matthews, J., & Lawson, T. (2019). Exploiting natural variation and genetic manipulation of stomatal conductance for crop improvement. *Current Opinion in Plant Biology*, *49*, 1–7. <https://doi.org/10.1016/j.pbi.2019.01.003>
- Giménez, C., Gallardo, M., & Thompson, R. B. (2013). Plant–water relations. In Daniel Hillel (Ed.), *Reference Module in Earth Systems and Environmental Sciences*, 231-238, Elsevier. <https://doi.org/10.1016/B978-0-12-409548-9.05257-X>
- Hayat, S., Hayat, Q., Alyemini, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments: A review. *Plant Signaling & Behavior*, *7*(11), 1456–1466. <https://doi.org/10.4161/psb.21949>
- Kaspal, M., Kanapaddalagamage, M. H., & Ramesh, S. A. (2021). Emerging roles of γ -aminobutyric acid (GABA) gated channels in plant stress tolerance. *Plants*, *10*(10), Article 2178. <https://doi.org/10.3390/plants10102178>
- Khan, N., & Mukhtar, H. (2013). Tea and health: Studies in humans. *Current pharmaceutical design*, *19*(34), 6141–6147. <https://doi.org/10.2174/1381612811319340008>
- Khan, N., & Mukhtar, H. (2019). Tea polyphenols in promotion of human health. *Nutrients*, *11*(1), Article 39. <https://doi.org/10.3390/nu11010039>
- Lawson, T., & Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology*, *164*(4), 1556–1570. <https://doi.org/10.1104/pp.114.237107>
- Liang, Z., Gao, Y., Zhang, M., Pu, K., Zhang, L., Ma, Y., Huo, J., Zhang, J., Li, J., & Xie, J. (2026). Foliar application of exogenous gamma-aminobutyric acid alleviates the detrimental effects of combined low-temperature and low-light stress in pepper (*Capsicum annuum* L.). *Plant Physiology and Biochemistry*, *235*, Article 111338. <https://doi.org/10.1016/j.plaphy.2026.111338>

- Liao, J., Wu, X., Xing, Z., Li, Q., Duan, Y., Fang, W., & Zhu, X. (2017). γ -Aminobutyric acid (GABA) accumulation in tea (*Camellia sinensis* L.) through the GABA shunt and polyamine degradation pathways under anoxia. *Journal of Agricultural and Food Chemistry*, 65(14), 3013–3018. <https://doi.org/10.1021/acs.jafc.7b00304>
- Lin, C.-C., Hsieh, C.-Y., Chen, L.-F., Chen, Y.-C., Ho, T.-H., Chang, S.-C., & Chang, J.-F. (2023). Versatile effects of GABA oolong tea on improvements in diastolic blood pressure, alpha brain waves, and quality of life. *Foods*, 12(22), Article 4101. <https://doi.org/10.3390/foods12224101>
- Lv, W. -T., Lin, B., Zhang, M., & Hua, X. -J. (2011). Proline accumulation is inhibitory to *Arabidopsis* seedlings during heat stress. *Plant Physiology*, 156(4), 1921–1933. <https://doi.org/10.1104/pp.111.175810>
- Malekzadeh, P., Khara, J., & Heydari, R. (2014). Alleviating effects of exogenous gamma-aminobutyric acid on tomato seedling under chilling stress. *Physiology and Molecular Biology of Plants*, 20(1), 133–137. <https://doi.org/10.1007/s12298-013-0203-5>
- Marchin, R. M., Backes, D., Ossola, A., Leishman, M. R., Tjoelker, M. G., & Ellsworth, D. S. (2022). Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable plant species. *Global Change Biology*, 28(3), 1133–1146. <https://doi.org/10.1111/gcb.15976>
- Mei, S., Yu, Z., Chen, J., Zheng, P., Sun, B., Guo, J., & Liu, S. (2022). The physiology of postharvest tea (*Camellia sinensis*) leaves, according to metabolic phenotypes and gene expression analysis. *Molecules*, 27(5), Article 1708. <https://doi.org/10.3390/molecules27051708>
- Mei, X., Chen, Y., Zhang, L., Fu, X., Wei, Q., Grierson, D., Zhou, Y., Huang, Y., Dong, F., & Yang, Z. (2016). Dual mechanisms regulating glutamate decarboxylases and accumulation of gamma-aminobutyric acid in tea (*Camellia sinensis*) leaves exposed to multiple stresses. *Scientific Reports*, 6, Article 23685. <https://doi.org/10.1038/srep23685>
- Nayyar, H., Kaur, R., Kaur, S., & Singh, R. (2014). γ -Aminobutyric acid (GABA) imparts partial protection from heat stress injury to rice seedlings by improving leaf turgor and upregulating osmoprotectants and antioxidants. *Journal of Plant Growth Regulation*, 33(2), 408–419. <https://doi.org/10.1007/s00344-013-9389-6>
- Priya, M., Sharma, L., Kaur, R., Bindumadhava, H., Nair, R. M., Siddique, K. H. M., & Nayyar, H. (2019). GABA (γ -aminobutyric acid), as a thermo-protectant, to improve the reproductive function of heat-stressed mungbean plants. *Scientific Reports*, 9(1), Article 7788. <https://doi.org/10.1038/s41598-019-44163-w>
- Roychoudhury, A., Banerjee, A., & Lahiri, V. (2015). Metabolic and molecular-genetic regulation of proline signalling and its cross-talk with major effectors mediates abiotic stress tolerance in plants. *Turkish Journal of Botany*, 39(6), 887–910. <https://doi.org/10.3906/bot-1503-27>
- Shao, C., Zhang, C., Lv, Z., & Shen, C. (2021). Pre- and post-harvest exposure to stress influence quality-related metabolites in fresh tea leaves (*Camellia sinensis*). *Scientia Horticulturae*, 281, Article 109984. <https://doi.org/10.1016/j.scienta.2021.109984>
- Siahaan, L., & Palupi, D. (2025). Analisis kadar prolin daun teh pascapanen dalam kondisi hipoksia [Analysis of proline levels in post-harvest tea leaves under hypoxic conditions]. *Agropross: National Conference Proceedings of Agriculture*, 7, 266–272. <https://doi.org/10.25047/agropross.2025.835>

- Stewart, C. R. (1972). Effects of proline and carbohydrates on the metabolism of exogenous proline by excised bean leaves in the dark. *Plant Physiology*, *50*(5), 551–555. <https://doi.org/10.1104/pp.50.5.551>
- Tsushida, T., & Murai, T. (1987). Conversion of glutamic acid to γ -aminobutyric acid in tea leaves under anaerobic conditions. *Agricultural and Biological Chemistry*, *51*(11), 2865–2871. <https://doi.org/10.1080/00021369.1987.10868498>
- Wang, H., Tang, X., Wang, H., & Shao, H. B. (2015). Proline accumulation and metabolism-related genes expression profiles in *Kosteletzkya virginica* seedlings under salt stress. *Frontiers in Plant Science*, *6*, Article 792. <https://doi.org/10.3389/fpls.2015.00792>
- Wang, Y., Xiong, F., Nong, S., Liao, J., Xing, A., Shen, Q., Ma, Y., Fang, W., & Zhu, X. (2020). Effects of nitric oxide on the GABA, polyamines, and proline in tea (*Camellia sinensis*) roots under cold stress. *Scientific Reports*, *10*, Article 12240. <https://doi.org/10.1038/s41598-020-69253-y>
- Wu, Q. Y., Ma, S. Z., Zhang, W. W., Yao, K. B., Chen, L., Zhao, F., & Zhuang, Y. Q. (2018). Accumulating pathways of γ -aminobutyric acid during anaerobic and aerobic sequential incubations in fresh tea leaves. *Food Chemistry*, *240*, 1081–1086. <https://doi.org/10.1016/j.foodchem.2017.08.004>
- Xiong, J., Zhang, L., Fu, G., Yang, Y., Zhu, C., & Tao, L. (2012). Drought-induced proline accumulation is uninvolved with increased nitric oxide, which alleviates drought stress by decreasing transpiration in rice. *Journal of Plant Research*, *125*, 155–164. <https://doi.org/10.1007/s12065-011-0417-y>
- Xu, Z., & Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany*, *59*(12), 3317–3325. <https://doi.org/10.1093/jxb/ern185>
- Ye, J.-H., Lv, Y.-Q., Liu, S.-R., Jin, J., Wang, Y.-F., Wei, C.-L., & Zhao, S.-Q. (2021). Effects of light intensity and spectral composition on the transcriptome profiles of leaves in shade grown tea plants (*Camellia sinensis* L.) and regulatory network of flavonoid biosynthesis. *Molecules*, *26*(19), Article 5836. <https://doi.org/10.3390/molecules26195836>
- Yogeswara, I. B. A., Maneerat, S., & Haltrich, D. (2020). Glutamate decarboxylase from lactic acid bacteria—A key enzyme in GABA synthesis. *Microorganisms*, *8*(12), Article 1923. <https://doi.org/10.3390/microorganisms8121923>
- Yong, B., Xie, H., Li, Z., Li, Y. P., Zhang, Y., Nie, G., Zhang, X. Q., Ma, X., Huang, L. K., Yan, Y. H., & Peng, Y. (2017). Exogenous application of GABA improves PEG-induced drought tolerance positively associated with GABA-shunt, polyamines, and proline metabolism in white clover. *Frontiers in Physiology*, *8*, Article 1107. <https://doi.org/10.3389/fphys.2017.01107>
- Zeng, Z., Han, C., Wang, Q., Yuan, H., Zhang, X., & Li, B. (2024). Analysis of drying characteristic, effective moisture diffusivity and energy, exergy and environment performance indicators during thin layer drying of tea in a convective-hot air dryer. *Frontiers in Sustainable Food Systems*, *8*, Article 1371696. <https://doi.org/10.3389/fsufs.2024.1371696>