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PHYSIOLOGICAL RESPONSES OF ARABICA COFFEE SEEDLINGS TREATED WITH POTASSIUM, BORON, AND *TRICHODERMA* UNDER DROUGHT STRESS

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ABSTRACT

Arabica coffee productivity recently has faced risk of climate change-induced drought and rising temperatures. This study evaluated the physiological responses of Arabica coffee seedlings treated with potassium (K), boron (B), and the biological agent *Trichoderma* spp. under drought stress. A screenhouse experiment was conducted by using a Randomized Block Design with eight treatment combinations and four replications. Drought was induced by withholding irrigation for 18 days, followed by a 15-day recovery period. Parameters measured included the chlorophyll index, stomatal density, and net assimilation rate (NAR). Findings indicated that the application of nutrient and biological inputs resulted in similar physiological values across all treatments. The leaf chlorophyll index remained stable between 43.47 and 48.83 SPAD units, while stomatal density was recorded within the range of 129.94 to 219.11 stomata mm $^{-2}$. Furthermore, the net assimilation rate varied from 0.08913 to 0.41975 g m $^{-2}$ week $^{-1}$. While providing similar overall outcomes, the effects of drought stress tended to be counterbalanced by the treatments. Several combinations produced higher physiological values compared to the control, indicating their potential as alternative agronomic strategies to maintain seedling resilience under drought. This study establishes a baseline for understanding the interactive effects of multi-input strategies on early-stage coffee resilience.

Key words: Abiotic, Bioagent, Coffee, Foliar, Nursery.

INTRODUCTION

Arabica coffee (*Coffea arabica* L.) is highly vulnerable to global climate change. Increased temperatures, shifts in rainy and dry seasons, and prolonged drought periods cause a decline in Arabica coffee production by up to 80% on non-irrigated lands (Syakir & Surmaini, 2017; Silva *et al*., 2022). The nursery phase is one of the critical periods in Arabica coffee cultivation because the plant roots are still shallow, making them extremely sensitive to drought stress (Randriani & Dani, 2018; Sulistiani *et al*., 2023; Andreazi *et al*., 2025). Soil water capacity below 74.6% can adversely affect coffee seedlings and risk causing plant mortality under severe drought conditions (Dominghetti *et al*., 2016). Water deficit during the nursery stage triggers an imbalance between water absorption by roots and evaporation through leaves, prompting the plant to close its stomata to reduce water loss (Lopes & Almeida, 2017). Furthermore, water shortage causes a decrease in cell turgor pressure and inhibits nutrient transport (Yang *et al*., 2021; Aman *et al*., 2024). These physiological disturbances directly reduce the quality of ready-to-plant seedlings.

To overcome these challenges, agronomic input engineering strategies are highly necessary to enhance the physiological resistance of Arabica coffee seedlings against drought stress. Providing nutrition through the application of potassium (K) and boron (B) is a potential mitigation strategy. Potassium is an essential macronutrient that plays a crucial role in regulating turgor pressure, the mechanism of stomatal opening, and the activation of stress-responsive enzymes (Hasanuzzaman *et al*., 2018; Wulanjari *et al*., 2022). Applying K fertilizer has been proven to increase water use efficiency in coffee plants (Ramírez-Builes & Küsters, 2021). Meanwhile, boron is an essential micronutrient that functions to maintain cell wall strength, stimulate root growth, and assist carbohydrate transport (Rosolem *et al*., 2020; Souza *et al*., 2022). B application can reduce the negative impact of water shortage on coffee growth (Ramírez-Builes *et al*., 2024). Alongside inorganic nutrients, the utilization of the biological agent *Trichoderma* spp. is an alternative option. *Trichoderma* spp. acts as a growth promoter that stimulates roots through the production of Indole Acetic Acid (IAA) and improves the efficiency of water and nutrient absorption during dry conditions (Sinaga *et al*., 2023; Akbari *et al*., 2024). This root symbiosis is capable of improving plant responses, such as increasing chlorophyll levels and photosynthetic rates when facing stress (Harman *et al*., 2021; Akbari *et al*., 2024).

Regarding the state of the art, research evaluating the benefits of K, B, and *Trichoderma* spp. separately has been widely studied in mitigating drought stress. However, there is still a gap in previous findings, as information about the effect of simultaneously combining these three components on the physiological responses of Arabica coffee seedlings under drought stress is still very limited. Previous studies generally focused on providing a single type of element or nutrient combination in other plantation crops or mature coffee plants. There is still a lack of studies that specifically combine the application of K, B, and *Trichoderma* spp. to overcome the physiological problems of Arabica coffee seedlings during the nursery phase under drought periods. Therefore, the novelty of this research lies in evaluating the interactive effects of these multi-input strategies, establishing a new baseline for understanding early-stage coffee resilience against abiotic stress. This research aims to analyze the effect of applying K, B, and *Trichoderma* spp. on the physiological responses of Arabica coffee seedlings under drought stress and to evaluate the most effective treatment combination for increasing their tolerance to drought stress.

MATERIALS AND METHODS

Place and time of study

The experiment took place in a screenhouse at the Bale Tatanen, Faculty of Agriculture, Padjadjaran University, Jatinangor, West Java, at an altitude of ±800 m above sea level. The research was conducted over a period of four months, from February to May 2026.

Materials and equipment

The primary plant material used in this study was six-month-old Arabica coffee seedlings. The cultivation medium used was topsoil. The treatment materials included urea fertilizer, boron (B) fertilizer, potassium (K) fertilizer, and the biological agent *Trichoderma* spp. in a solid

granular formulation. The equipment used to measure the targeted physiological parameters included a Chlorophyll Meter SPAD-502Plus (Minnolta inc., JPN), an Olympus CX23 microscope (Evident Europe GmbH, DEU), clear nail polish and tape for preparing stomatal specimens, an SC-1 Portable Leaf Area Meter (Scitek Global Co., Ltd., CHN), an oven, and an analytical balance.

Experiment design

A Randomized Block Design (RBD) was used for this study. The experiment evaluated eight treatment combinations under drought stress conditions. The treatments were: (A) Control (without K, B, and *Trichoderma* spp.); (B) K fertilizer (1.38 g L^{-1}); (C) B fertilizer (384 mg L^{-1}); (D) *Trichoderma* spp. (20 g $plant^{-1}$); (E) K + B; (F) K + *Trichoderma* spp.; (G) B + *Trichoderma* spp.; and (H) K + B + *Trichoderma* spp. Each treatment was replicated four times. The study utilized a total of 64 seedlings, consisting of 32 main plants for non-destructive observation and 32 additional plants for destructive sampling.

Research procedures

The application of *Trichoderma* spp. was conducted by mixing 20 g $plant^{-1}$ of the biological agent into the topsoil medium one week before transplanting the coffee seedlings, allowing sufficient time for root colonization (Sinaga *et al*., 2023). The foliar application of K and B fertilizers was carried out five times at a seven-day interval, applied in the morning between 07.00 and 10.00 WIB (Erwiyono *et al*., 2006). The spraying volume was calibrated to 15 mL $plant^{-1}$. Following the treatment period, simulated drought stress was induced at the seventh week by completely withholding irrigation for 18 days, which was followed by a 15-day recovery phase where water was restored to 100% field capacity (Reis *et al*., 2022). For plant maintenance, 2 g $plant^{-1}$ of urea was applied every two weeks. Pests and weeds were controlled mechanically without the use of synthetic chemicals to avoid bias in physiological responses.

Analysis method

The evaluation was focused on three main physiological parameters:

1. Leaf Chlorophyll Index: Measured non-destructively using a SPAD-502Plus meter clamped onto the third fully expanded leaf from the shoot apex. Data were recorded periodically every three days during both the drought stress and recovery phases.
2. Stomatal Density: Evaluated microscopically using the replica method (Haryanti, 2010). Clear nail polish was applied to the abaxial surface of the third leaf, allowed to dry, and then peeled off using clear tape and placed on a glass slide. The specimens were observed under an Olympus CX23 microscope at 40x magnification, and the stomatal density was calculated as the number of stomata divided by the 1 mm^2 field of view area.
3. Net Assimilation Rate (NAR): Measured destructively using data on leaf area (LA) and total dry weight (W) collected at designated intervals (T_1 and T_2). Leaf area was measured using a Portable Leaf Area Meter, while total dry weight was obtained by

drying the plant organs in an oven at 70°C for 24 hours (Chekol *et al*., 2023). The NAR was mathematically formulated using the equation established by Gardner *et al*. (1991):

$$NAR = \frac{(W_2 - W_1)}{(T_2 - T_1)} \times \frac{(ln\, L_{A2} - L_{A1})}{(L_{A2} - L_{A1})}$$

Statistical Analysis

Data obtained from the observations were analyzed using Analysis of Variance (ANOVA) in accordance with the RBD format at a 5% significance level. If the analysis showed a significant effect of the treatments, a further evaluation using Tukey's Test at a 5% significance level was performed to determine the best treatment combination among the variables observed.

RESULTS AND DISCUSSION

Leaf chlorophyll index

The application of K, B, and *Trichoderma* spp., whether applied individually or in combination, resulted in comparable leaf chlorophyll indices across all treatments during the 18 days of drought stress (Table 1). The index values remained stable between 43.47 and 48.83 SPAD units, indicating that the moderate severity of the drought period had not reached the critical threshold capable of degrading the photosynthetic pigments. Similar findings demonstrated that the chlorophyll index in coffee plants typically remains stable during the early phases of water deficit, and a severe reduction generally occurs only when the stress period is extended beyond 30 to 60 days (Chutteang *et al*., 2023; Chekol *et al*., 2024).

The uniform responses across treatments were also attributed to the specific nature and working mechanisms of the applied inputs. The biological agent *Trichoderma* spp. requires a prolonged incubation period and does not directly govern pigment synthesis during the early vegetative phase, which is predominantly controlled by inherent genetic factors and plant age (Syaharani *et al*., 2022; Aji *et al*., 2025). Furthermore, nutrients like K and B primarily function as osmotic regulators and structural components of the cell wall, rather than as direct biochemical precursors for the chlorophyll porphyrin ring (Wulanjari *et al*., 2022). Consequently, these multi-input strategies did not trigger an excessive increase in SPAD values during the stress period. However, it is worth noting that the combined application of K, B, and *Trichoderma* spp. (Treatment H) maintained a slightly higher index (45.73 SPAD units) at the peak of the stress (18 DAS) compared to the control (43.75 SPAD units), showing a slight positive trend in protecting the photosynthetic apparatus.

During the 15-day recovery phase, the chlorophyll levels slowly increased and continually maintained uniform values. This recovery capability demonstrates a highly efficient intrinsic mechanism of Arabica coffee seedlings to prevent cellular water loss and protect the photosynthetic apparatus from permanent damage after experiencing severe water shortages (Menezes-Silva *et al*., 2017; Chekol *et al*., 2024). The uniform recovery across all treatments confirms that the combination of nutrition and biological agents acted more as a root osmotic

buffer to regulate water absorption efficiency rather than directly modifying the leaf pigment concentration (Chutteang *et al*., 2023).

Stomatal density

Based on the observations, the stomatal density of Arabica coffee leaves exhibited uniform values across all treatments during both the drought stress and recovery phases (Table 2). The average density varied within the range of 129.94 to 203.82 stomata mm^{-2} during the peak of the stress phase, and between 119.75 and 219.11 stomata mm^{-2} during the 15-day recovery period. According to Karubuy *et al*. (2018), stomatal densities below 300 stomata mm^{-2} are generally categorized as low for leaf anatomical traits. The consistent density values across all applied inputs demonstrate that the fundamental structural anatomy of the coffee leaf epidermis was retained and remained unaltered by the multi-input agronomic strategies or the severe lack of water. Nevertheless, at the peak of the drought stress (18 DAS), treatments utilizing *Trichoderma* spp. (D), K + B (E), and K + *Trichoderma* spp. (F) recorded visibly higher stomatal densities (203.82, 198.73, and 183.44 stomata mm^{-2}, respectively) compared to the control (147.77 stomata mm^{-2}). This suggests a tendency of these treatments to better preserve the fundamental structural anatomy of the leaves under extreme stress compared to untreated plants.

Rather than permanently modifying the structural quantity of the stomata to cope with the severe water deficit, the primary defense mechanism deployed by the seedlings was functional regulation. When the plant roots detect a substantial water deficit in the growing medium, internal chemical signals trigger a rapid loss of turgor pressure in the guard cells, leading to immediate stomatal closure without degrading the stomata quantity itself (Bertolino *et al*., 2019; Mostofa *et al*., 2022; Chekol *et al*., 2023). This functional adjustment acts as an essential barrier that effectively restricts the leaf hydraulic conductance, allowing the plant to rapidly suppress daily water loss via transpiration and prevent fatal dehydration (Chekol *et al*., 2023).

Modifying the physical quantity of stomata is a long-term anatomical adaptation that requires a high biological cost and prolonged cellular differentiation. Therefore, relying on rapid stomatal closure is a much more efficient strategy for early-stage seedlings (Tounekti *et al*., 2018). Consequently, the simultaneous application of K, B, and *Trichoderma* spp. did not alter the physical stomatal traits, as the seedlings systematically prioritized functional stomatal closure over structural adaptation to navigate the heavy 18-day drought period safely.

Net Assimilation Rate (NAR)

Based on the destructive evaluations, the net assimilation rate (NAR) produced comparable outcomes for all applied inputs under continuous water scarcity, with overall values ranging from 0.08913 to 0.41975 g m^{-2} $week^{-1}$ (Table 3). NAR is a crucial physiological parameter that indicates the plant's capability to accumulate dry matter from carbon assimilation per unit leaf area over a specific time. The uniform values suggest that the application of K, B, and *Trichoderma* spp., either individually or in combination, resulted in a similar photosynthate accumulation rate during the severe drought stress. Drought during the seedling stage acts as

a major environmental constraint that uniformly reduces relative growth and assimilation rates (Dias *et al*., 2007; Worku & Astatkie, 2010).

When coffee seedlings experience a severe lack of water, the drop in leaf water potential immediately triggers stomatal closure as a primary defense mechanism to minimize transpirational water loss (Chekol *et al*., 2023). However, this essential functional closure severely restricts the diffusion of carbon dioxide (CO_2) into the leaf mesophyll, which subsequently halts carbon assimilation and limits biomass production (Andreazi *et al*., 2025; Dao *et al*., 2025). Consequently, early-stage coffee seedlings uniformly experience slowed or halted growth under water-restricted environments, regardless of the agronomic inputs provided (Dias *et al*., 2007). Beyond the restricted CO_2 supply, the decline in NAR during advanced drought is also heavily influenced by biochemical damage to chloroplasts, reduction in photosynthetic pigments, and the inactivation of crucial metabolic enzymes (Menezes-Silva *et al*., 2017).

Furthermore, under severe stress conditions, plants naturally shift their assimilate allocation priority. Instead of supporting active canopy development, the limited photoassimilates are redirected toward osmotic adjustment and cellular defense mechanisms to maintain turgor pressure (DaMatta & Ramalho, 2006). However, despite the overall similarities, specific treatments showed a tendency to counterbalance the severe water deficit. For instance, the application of K + *Trichoderma* spp. (Treatment F) and B fertilizer (Treatment C) produced notably higher NAR values (0.41975 and 0.33858 g m^{-2} $week^{-1}$, respectively) compared to the control (0.19163 g m^{-2} $week^{-1}$). This trend indicates that the synergistic and individual applications of these agronomic inputs provided a physiological buffer that supported carbon assimilation slightly better than untreated seedlings, demonstrating their potential as an alternative strategy during prolonged drought (Akbari *et al*., 2024).

CONCLUSION

1. The application of K, B, and *Trichoderma* spp. resulted in comparable overall physiological responses, specifically the leaf chlorophyll index, stomatal density, and net assimilation rate, in Arabica coffee seedlings under 18 days of drought stress.
2. Despite the uniform outcomes, several treatment combinations demonstrated a tendency to counterbalance the effects of the severe water deficit. These multi-input strategies produced higher physiological values compared to the control, indicating their potential as alternative agronomic strategies to maintain early-stage coffee resilience under drought.

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Table 1. Leaf chlorophyll index of Arabica coffee seedlings under drought stress and recovery phases

Treatment	0 DAS	3 DAS	6 DAS	9 DAS	12 DAS	15 DAS	18 DAS	3 DAR	6 DAR	9 DAR	12 DAR	15 DAR
A	44.78	44.25	45.80	45.15	44.65	43.75	43.75	44.21	45.01	45.71	48.72	48.39

B	43.68	45.25	44.93	44.43	43.93	44.48	43.55	44.75	45.49	46.19	47.76	48.29
C	46.05	46.88	47.63	46.73	46.23	44.45	43.53	45.38	46.18	46.88	47.55	48.79
D	43.63	45.25	43.70	45.70	45.20	44.80	43.47	44.43	44.73	45.43	49.00	48.60
E	47.28	45.45	41.79	44.69	44.22	45.61	44.78	44.54	47.20	47.90	49.07	49.02
F	45.83	43.23	45.63	46.58	46.08	46.15	45.28	45.20	46.00	46.70	48.70	49.48
G	46.45	44.78	45.73	46.03	45.53	45.03	44.18	46.43	47.23	47.93	49.00	49.83
H	45.45	45.30	47.55	47.70	46.63	48.83	45.73	45.00	45.80	46.50	49.53	49.25

Explanation: A = Control; B = K fertilizer; C = B fertilizer; D = *Trichoderma* spp.; E = K + B fertilizers; F = K fertilizer + *Trichoderma* spp.; G = B fertilizer + *Trichoderma* spp.; H = K + B fertilizers + *Trichoderma* spp. DAS = Days after stress; DAR = Days after recovery. Values without notation letters in the same column indicate no significant difference according to Tukey's Test at a 5% significance level.

Table 2. Stomatal density of Arabica coffee leaves under drought stress and recovery phases

Treatment	0 DAS	9 DAS	18 DAS	15 DAR
A	146.50	141.40	147.77	119.75
B	129.94	138.86	138.86	219.11
C	150.32	142.68	174.52	187.26
D	147.77	153.22	203.82	170.70
E	137.58	160.51	198.73	201.28
F	152.87	156.69	183.44	184.72
G	142.68	175.80	169.43	185.99
H	157.97	161.79	174.53	184.72

Explanation: A = Control; B = K fertilizer; C = B fertilizer; D = *Trichoderma* spp.; E = K + B fertilizers; F = K fertilizer + *Trichoderma* spp.; G = B fertilizer + *Trichoderma* spp.; H = K + B fertilizers + *Trichoderma* spp. DAS = Days after stress; DAR = Days after recovery. Values without notation letters in the same column indicate no significant difference according to Tukey's Test at a 5% significance level.

Table 3. Net assimilation rate of Arabica coffee seedlings under drought stress

Treatment	Net Assimilation Rate (NAR)
A	0.19163
B	0.26298
C	0.33858
D	0.15505
E	0.08913

F	0.41975
G	0.36330
H	0.29458

Explanation: A = Control; B = K fertilizer; C = B fertilizer; D = *Trichoderma* spp.; E = K + B fertilizers; F = K fertilizer + *Trichoderma* spp.; G = B fertilizer + *Trichoderma* spp.; H = K + B fertilizers + *Trichoderma* spp. Values without notation letters in the column indicate no significant difference according to Tukey's Test at a 5% significance level.