Original scientific paper
DOI: /10.5513/JCEA01/27.3.5126
Journal of Central European Agriculture, 2026, 27(3), p.717-727

Short-term physiological responses of tomato plants to *Trichoderma brevicompactum under* different watering regimes

Kratkoročni fiziološki odgovori biljaka paradajza na *Trichoderma brevicompactum* u različitim režimima zalivanja

Igor VUKELIĆ[1], Gordana RACIĆ[2], Mirjana BOJOVIĆ[3], Vedrana PROROK[4], Svetlana ROLJEVIĆ NIKOLIĆ[2], Ljiljana PROKIĆ[5], Dejana PANKOVIĆ[6] (✉)

[1] Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia
[2] Research and Development Institute Tamiš, Novoseljenski put 33, Pančevo, Serbia
[3] Faculty of Ecological Agriculture, Educons University, Vojvode Putnika 87, 21208 Sremska Kamenica, Serbia
[4] University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[5] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia
[6] Julius Kuehn Institute, Institute for Resistance Research and Stress Tolerance, Erwin Baur Strasse 27, 06484 Quedlinburg, Germany

✉ Corresponding author: dejana.pankovic@julius-kuehn.de

Received: December 5, 2025; accepted: February 4, 2026

ABSTRACT

Climate change and increasing water scarcity present critical challenges for sustainable crop production, particularly affecting tomato (*Solanum lycopersicum* L.), a globally important horticultural crop. Beneficial soil fungi, such as *Trichoderma* spp. are widely known as plant growth promoters, particularly under abiotic stress such as drought. However, short-term physiological responses to specific strains remain unclear, as *Trichoderma*-plant interactions are strain-genotype dependent. This study presents the results on the influence of *T. brevicompactum* on the physiological and biochemical parameters of tomato, under optimal watering and cessation of watering conditions, aiming to evaluate its potential to mitigate drought induced stress. The results demonstrated that *T. brevicompactum* induced a rapid reduction in stomatal conductance in well-watered plants, accompanied by changes in abscisic acid (ABA) dynamics and water status. Cessation of watering caused the expected increase in ABA levels in both leaves and roots, while in combination with *T. brevicompactum* this accumulation was reduced. Multivariate PCA analysis confirmed that ABA, stomatal conductance, leaf water potential and epidermal flavonols are the most sensitive early indicators of stress and fungal modulation of plant responses. These findings emphasize the potential of *T. brevicompactum* as a biostimulant to mitigate drought effects and support eco-sustainable tomato production, while highlighting the need for further studies under field conditions and at the molecular level to elucidate the underlying regulatory networks.

Keywords: symbiotic endophyte, *Solanum lycopersicum* L., water deficit, ABA, stomatal conductance, chlorophyll

SAŽETAK

Klimatske promene i sve izraženije smanjene količine padavina predstavljaju ključne izazove za održivu poljoprivrednu proizvodnju, posebno utičući na paradajz (*Solanum lycopersicum* L.), jednu od najznačajnijih hortikulturnih kultura u svetu. Gljive roda *Trichoderma* poznate su kao promotori rasta biljaka, naročito u uslovima abiotičkog stresa suše. Ipak, rane fiziološke reakcije biljaka na interakciju sa gljivama roda *Trichoderma* ostaju nedovoljno razjašnjene, budući da ta interakcija zavisi od kombinacije soja gljive i genotipa biljke. Ovo istraživanje prikazuje rezultate o uticaju *T. brevicompactum* na fiziološke i biohemijske parametre paradajza, pod uslovima optimalnog zalivanja i prestanka

snabdevanja vodom, sa ciljem procene potencijala gljiva u ublažavanju stresa izazvanog sušom. Rezultati su pokazali da *T. brevicompactum* izaziva brzo smanjenje provodljivosti stoma kod dobro zalivenih biljaka, praćeno promenama u dinamici apscisinske kiseline (ABA) i vodnom statusu biljke. Prestanak zalivanja doveo je do očekivanog porasta nivoa ABA u listovima i korenu, dok je u kombinaciji sa *T. brevicompactum* ovo nakupljanje bilo smanjeno. Analiza glavnih komponenti (PCA) potvrdila je da su ABA, provodljivost stoma, vodni potencijal listova i epidermalni flavonoli najosetljiviji rani indikatori stresa i modulacije izazvane gljivom. Dobijeni rezultati naglašavaju potencijal *T. brevicompactum* kao biostimulatora u ublažavanju efekata suše i unapređenju ekološki održive proizvodnje paradajza. U cilju razjašnjavanja regulatornih mehanizama koji stoje u osnovi ovih procesa buduća istraživanja je potrebno sprovesti u poljskim uslovima sa fokusom istraživanja na molekularnom nivou.

Ključne reči: simbiotske endofite, *Solanum lycopersicum* L., vodni deficit, ABA, provodljivost stoma, hlorofil

INTRODUCTION

Climate change, together with intensive agricultural practices, has contributed to biodiversity loss, redistribution of pests and diseases, and accumulation of agrochemical pollutants in air, water, and soil, all of which negatively impact agroecosystems and human health (DeClerck et al., 2023). Frequent policy shifts in agriculture further promote the reduction of synthetic chemicals while promoting the use of beneficial soil microorganisms as sustainable alternatives.

Tomato (*Solanum lycopersicum* L.) is one of the most economically significant vegetable crops worldwide. It has become an essential crop in open and covered production systems of the Mediterranean region (Tüzel et al., 2025). It ranks globally as a major crop after sweet potato (Quinet et al., 2019). Globally, tomato cultivation covers approximately 5 million hectares, yielding around 180 million tons annually, with a total production value exceeding 90 billion USD (FAO, 2022). With its wide cultivation range, short developmental cycle, nutritional value, and relatively small genome suitable for genetic manipulation and transformation, the tomato also serves as a valuable model species for biotic and abiotic stress research.

Drought is one of the major abiotic stresses, exerting adverse effects on plant development, productivity, and ultimately crop yield (Muhammad et al., 2024). By 2050, drought conditions are expected to adversely affect plant development and lead to decreased crop yields across more than half of the world's cultivable land (Lin et al., 2020). Stress caused by drought triggers a series of biochemical and physiological responses, among which stomatal closure is one of the most important mechanisms, controlling the loss of transpiration and the intake of carbon dioxide (Nguyen et al., 2023). The stomatal closure during drought is primarily regulated by the phytohormone ABA. Higher levels of ABA are the signals for the control of transpiration, photosynthesis, stomatal reactions, and expression of adaptive response genes (Vishwakarma et al., 2017). Generally, tomato is highly susceptible to drought stress, which severely affects its yield. In this context, the cosmopolitan soil-inhabiting fungi *Trichoderma* spp. have demonstrated notable potential in improving tomato tolerance to drought (Woo et al., 2025). Over the past few years, *Trichoderma* has become one of the most promising microorganisms known for its diverse functions as a plant biostimulant, bioprotectant, biodegrader, biofertilizer and bioremediator (Nawaz et al., 2025; Racić et al., 2023; Vukelić et al., 2020). *Trichoderma* spp., by synthesizing ABA precursors such as ABA aldehyde and by reprogramming and activating molecular mechanisms which are involved in ABA biosynthesis, signal transduction, and cross-talk with other hormones, are able to manage the level of ABA and its involvement in plant stress responses (Guzmán-Guzmán et al., 2019). Moreover, beyond its role in ABA regulation, *Trichoderma* spp. influences additional endogenous phytohormones, such as auxin, salicylic acid, jasmonic acid and ethylene (Racić et al., 2018). *Trichoderma* spp. also play a significant role in enhancing plant tolerance to drought via several other mechanisms. One of the most efficient strategies is the stimulation of

root growth, allowing the plant to better exploit available water. Root interaction with fungal hyphae also permits higher uptake of capillary moisture strongly held in fine soil pores. Early research also shows that *Trichoderma* spp. can induce the synthesis of plant metabolites associated with drought resistance (Zhang et al., 2025) and enhance the antioxidant defensive system (Gulzar et al., 2023). One more important reaction of plants to drought is the production of reactive oxygen species (ROS). Plants interacting with *Trichoderma* spp. exhibited higher activity of chitinases, lipoxygenases, glucanases, peroxidases and hydroperoxidases (Zhang et al., 2022). These enzymes are involved in phenolics and phytoalexin production and result in ROS detoxification, thereby promoting plant resistance against water deficit stress (Contreras-Cornejo et al., 2016).

This study aimed to identify physiological and biochemical markers of tomato responses to *T. brevicompactum* under different watering regimes. For this purpose, we investigated key physiological parameters such as stomatal conductance, chlorophyll content, epidermal flavonols and leaf water potential, together with biochemical indicators including abscisic acid concentration in leaves and roots. The results provide insight into the role of *T. brevicompactum* in enhancing tomato adaptation to water deficit and highlight its potential as a biostimulant for sustainable tomato production under climate variability.

MATERIALS AND METHODS

Plant material and growth conditions

Tomato plants (*Solanum lycopersicum* Mill. cv. Ailsa Craig) were germinated in pots containing commercial substrate (Klasman Potgrond H.). Seedlings were grown under controlled conditions in a phytotron chamber (250 µmol photons m^{-2} s^{-1}, photoperiod 14 h, day/night temperature 23 °C/17 °C, relative humidity 60%). Young plants were optimally watered daily and maintained at 75% soil water content (37% Volumetric Water Content) until the 4th leaf development phase, after which two factors were applied: cessation of watering and addition of *T. brevicompactum*. The following treatments were applied: optimally watered plants (C), optimally watered plants treated with *T. brevicompactum* (C_T), cessation of watering (D), and cessation of watering with the addition of *T. brevicompactum* (D_T) (Figure 1). Each treatment consisted of 16 plants.

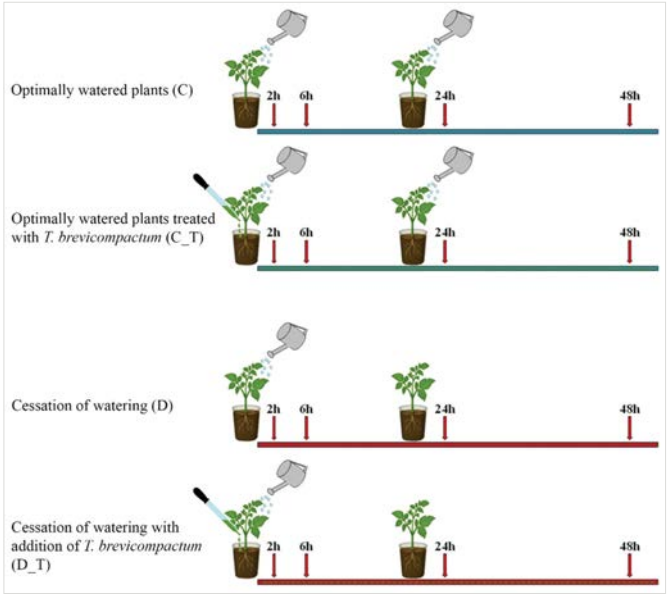


Figure 1. Schematic overview of the experimental design showing the two watering regimes (optimal watering and water cessation), with or without *T. brevicompactum*, and the measurement time points at 2 h, 6 h, 24 h and 48 h after treatment application

Fungal strain, preparation and application

T. brevicompactum SZMC 22661 was isolated from the A horizon (5–30 cm) of agricultural soil, and species identification was based on ITS and TEF1-α sequences (Racić et al., 2017). Before preparing the suspension, the fungal isolate was incubated for four days on potato dextrose agar (PDA) medium in the dark at 25 °C. Colonies of pure cultures were scraped off the Petri dish and suspended in 100 mL of water. The reagent bottles containing the fungal spore suspension were placed in a shaker (TalBoys, Kingwood, Houston, TX, USA) and mixed for 2 h at a speed of 50 rpm. The fungal spores were counted using a hemocytometer. The density of the suspension was set to 8 × 10^6 CFU mL^{-1} and applied to the root zone of the plants. The volume of the inoculum used to treat the plants was 5 mL.

Determination of Volumetric Water Content

The Volumetric Water Content (VWC) was measured at a depth of 6 cm using a Theta probe (Delta-T, Cambridge, UK) (Racić et al., 2017). VWC was monitored in the time interval from 2 h to 48 h (2 h, 6 h, 24 h, 48 h) after the application of *T. brevicompactum*. The Volumetric Water Content was measured in all pots during the experiment and expressed as the mean value for each treatment ± SD.

In vivo measurement of chlorophyll content, epidermal flavonols and nitrogen balance index

Indices of chlorophyll (Chl), epidermal flavonols (Flav), and the nitrogen balance index (NBI) were measured non-destructively using a Dualex sensor (Force-A, Orsay, France) (Racić et al., 2018). Measurements were performed on uniform and fully developed young tomato leaves 2, 6, 24, and 48 h after inoculation with *T. brevicompactum*. The obtained results are expressed as the mean values of four biological and three technical replicates for each treatment ± SD.

Measurement of stomatal conductance

Stomatal conductance (Gs) was measured in fully developed young leaves using a porometer (AP4, Delta-T, Cambridge, UK). Stomatal conductance was measured at 2, 6, 24, and 48 h after the application of *T. brevicompactum*. Measurements were performed on three uniform, fully developed young leaves per plant. The obtained results are expressed as the mean value of three biological and three technical replicates for each treatment ± SD.

Measurement of leaf water potential

Leaf water potential (ψH_2O) was determined using a chamber under nitrogen gas pressure (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) as described by Scholander et al. (1965). The ψH_2O of leaves was measured at 2, 6, 24, and 48 h after *T. brevicompactum* inoculation. Measurements were performed on three uniform, fully developed young leaves per plant. The obtained results are expressed as the mean values of three biological replicates for each treatment ± SD.

Determination of abscisic acid concentration in leaves and roots

The abscisic acid (ABA) content in the leaves and roots was determined using an indirect enzyme-linked immunosorbent assay (ELISA) (Asch, 2000; Quarrie et al., 1988). Briefly, the assay was performed using the MAC 252 monoclonal antibody for ABA (John Innes Centre, Colney, Norwich, UK). After the enzymatic reaction, the absorbance was measured at 405 nm using a microplate reader (Sunrise, Tecan, Switzerland). ABA concentration was monitored at 2, 6, 24, and 48 h after *T. brevicompactum* inoculation. At each time interval, for the determination of ABA, samples were collected from three plants for one biological replicate from each treatment. The obtained results are expressed as the mean value of three biological and three technical replicates for each treatment ± SD.

Statistical Analysis

To determine statistically significant differences ($P < 0.05$), one-way analysis of variance (ANOVA) with Tukey's Post Hoc test was used. The results were subjected to the Shapiro-Wilk test, which determined that they had a normal distribution. The analysis was performed using GraphPad Prism (version 6.01; GraphPad Software, San Diego, USA). Pearson correlations and heat maps were generated using Microsoft Excel 2024 (Microsoft Corporation, Redmond, USA), while principal component analysis (PCA) was performed using Python (Python Software Foundation, Wilmington, USA) in Google Colab (Google LLC).

RESULTS

The experiment included four treatments differing in watering regime and fungal inoculation. Under optimal watering, the volumetric water content (VWC) was maintained at approximately 37% (C), whereas after 48 h of water cessation (D) it decreased to about 12%. During the first 6 h following *T. brevicompactum* application, no significant changes in VWC were observed among treatments. After 24 h, VWC in C_T was 9% higher than in C. After 48 h, statistically significant differences were

recorded between all treatments. VWC was 24% higher in C than in D. In addition, *T. brevicompactum* treatment resulted in a 3% higher VWC in C_T compared to C, and an 8.5% higher VWC in D_T compared to D (Figure 2A). The results indicated that after 48 h from the cessation of watering, the plants were subjected to soil drought conditions, and that the presence of *T. brevicompactum* contributed to maintaining higher soil moisture.

ψH_2O did not differ significantly 2 h after inoculation with *T. brevicompactum*. After 6 h, ψH_2O decreased by 61% in both C_T and D_T compared to the respective controls (C and D). At 24 h, ψH_2O in C was approximately twice as high as in D. After 48 h, there were no significant differences between the treatments (Figure 2B).

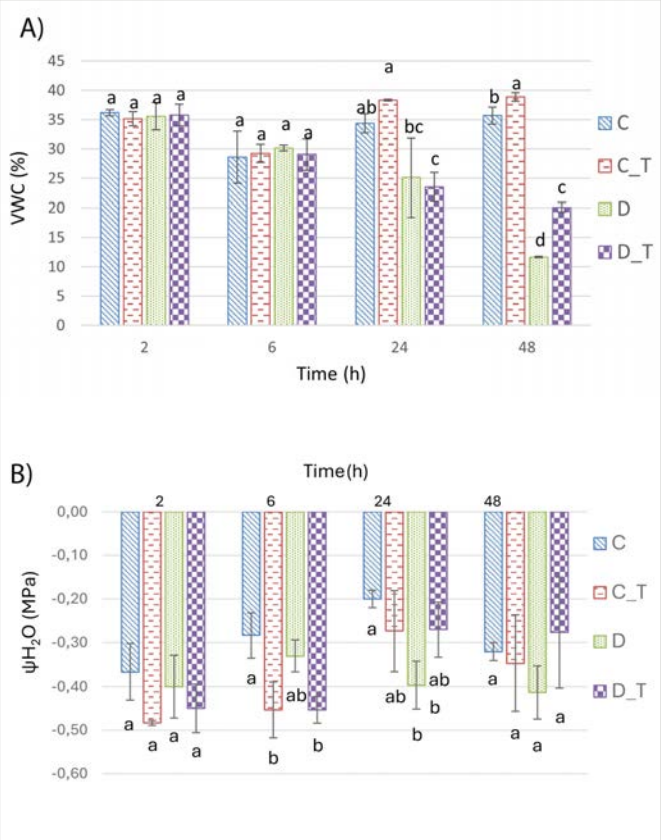


Figure 2. Water status parameters: volumetric water content (VWC) (A) and leaf water potential (ψH_2O) (B) in tomato plants subjected to four treatments: optimally watered (C), optimally watered with *T. brevicompactum* (C_T), cessation of watering (D), and cessation of watering with *T. brevicompactum* (D_T). Values marked with the same letter, measured within the same time period, are not significantly different according to Tukey's test ($P < 0.05$)

The Chl content did not change significantly between the treatments compared to the C (Figure 3A). In contrast to Chl content, a significant increase in Flav was observed as early as 2 h in C_T, showing a 47% increase compared to the C. After 6 h, D treatment resulted in an 11% reduction in Flav in comparison to C. Maximum Flav levels across all treatments were recorded at 24 h, although *T. brevicompactum* exhibited an inhibitory effect under both watering regimes. Specifically, Flav content decreased by 42% in C_T and by 48% in D_T compared to their respective controls. After 48 h, Flav content in D was significantly reduced by 17% relative to C, while inoculation with *T. brevicompactum* exerted a stimulatory effect under both watering regimes, resulting in a significant 28% increase in C_T and an 11% increase in D_T compared to their respective controls (Figure 3B). Regarding NBI, as early as 2 h after *T. brevicompactum* inoculation, C_T and D_T plants showed 32% and 17% inhibition compared to their respective controls (Figure 3C).

Regarding Gs, a significant reduction was detected as early as 2 h in C_T (41%) and D_T (44%) treatments compared to the respective controls. After 6 h, the inhibition was 55% in C_T and 38% in D_T relative to their respective controls. At 24 h, Gs values in C, C_T and D_T were comparable, while a pronounced decrease was recorded in D. This indicates that inoculation with *T. brevicompactum* mitigated the negative effect of drought, resulting in about 70% higher Gs in D_T compared to D. After 48 h, significant differences were evident across all treatments, with Gs reduced relative to C by 21% in C_T, 61% in D_T, and 84% in D (Figure 3D).

The dynamics of ABA content in leaves (ABA_L) and roots (ABA_R) during the experimental period followed an identical trend across all treatments, with maximum values reached 6 h after treatment application.

At 2 h after inoculation, *T. brevicompactum* had no significant effect on ABA_L in C_T, whereas in D_T it caused a 39% reduction compared to the D. Treatment D resulted in a two-fold increase in ABA_L relative to C. After 6 h, ABA_L was stimulated in C_T by 47% compared to C.

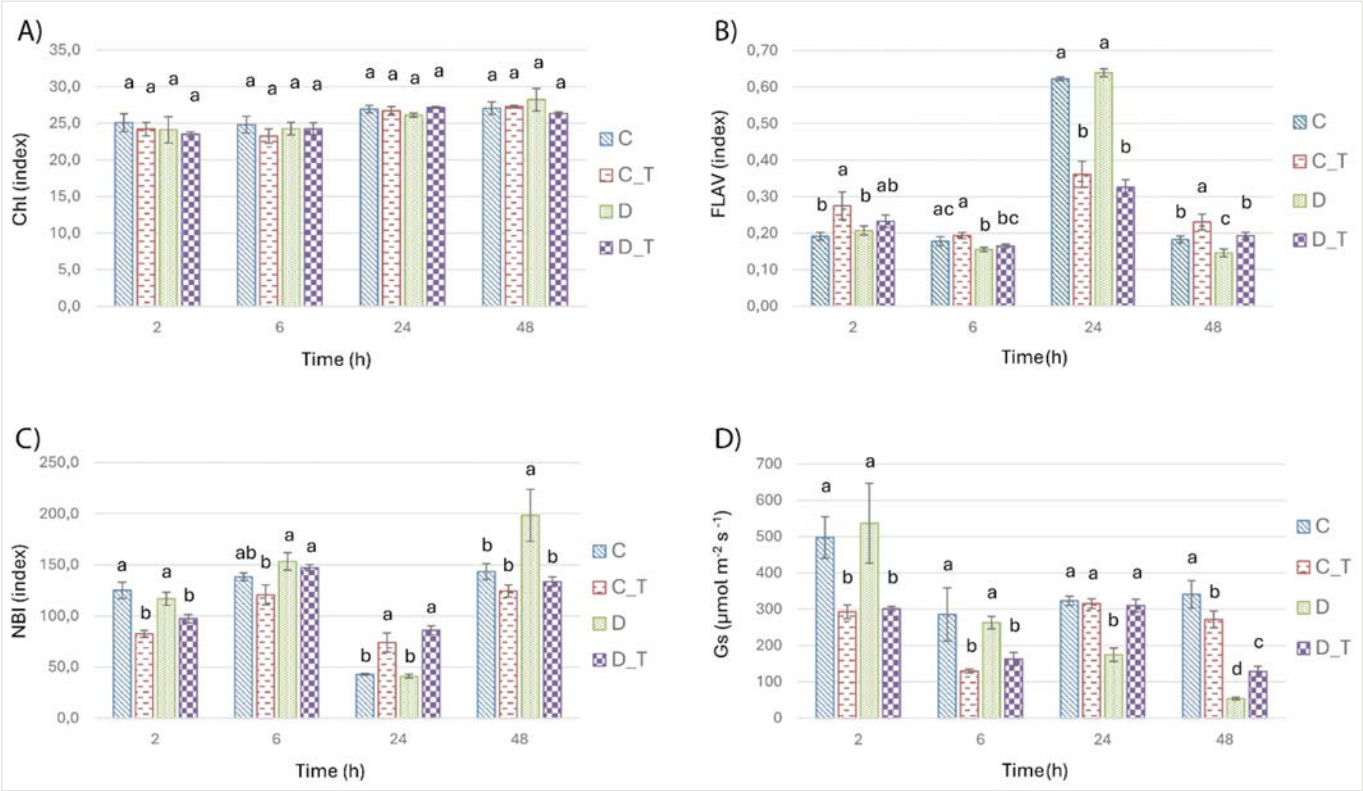


Figure 3. Photosynthetic and metabolic traits: chlorophyll content (Chl) (A), epidermal flavonols content (Flav) (B), nitrogen balance index (NBI) (C), and stomatal conductance (Gs) (D) subjected to four treatments: optimally watered (C), optimally watered with *T. brevicompactum* (C_T), cessation of watering (D), and cessation of watering with *T. brevicompactum* (D_T). Values marked with the same letter, measured within the same time period, are not significantly different according to Tukey's test ($P < 0.05$)

In the same period, D plants exhibited an 85% higher ABA_L content than C. At 24 h, no significant effects of *T. brevicompactum* were detected, while treatment D induced a two-fold increase in ABA_L relative to C. After 48 h, cessation of watering again resulted in a two-fold increase in ABA_L relative to C (Figure 4A).

The ABA_R content differed significantly among all treatments 2 h after *T. brevicompactum* inoculation. Treatment D induced a 400% increase in ABA_R levels compared to C. In C_T, *T. brevicompactum* led to a 74% increase in ABA_R relative to C, whereas in D_T, it had the opposite effect, resulting in a 20% reduction compared to D. After 6 h, ABA_R levels in C_T increased by 50%. During the same period, plants under treatment D showed a 200% increase in ABA_R compared to C. Although the effects of *T. brevicompactum* were not statistically significant after 24 h, ABA_R levels in D remained elevated by 72% compared to C. After 48 h, plants in D exhibited a 400% higher ABA_R content relative to C, while D_T showed a 20% lower ABA_R level compared to D (Figure 4B).

Pearson correlation analysis shows a very close dependence between ABA_L and ABA_R ($r = 0.95$; $P < 0.001$), confirming the coordinated regulation of ABA accumulation in leaves and roots. VWC was positively correlated with Gs ($r = 0.61$; $p < 0.01$), while Chl was moderately correlated with ψH_2O ($r = 0.41$; $P < 0.05$). In contrast, Flav showed a strong negative correlation with NBI ($r = -0.87$; $P < 0.001$). Other relationships among the measured parameters were less correlated (Figure 5).

Principal component analysis (PCA) revealed separation of treatments and traits along the first two principal components (PC). These two components explained 59.1% of the total variance, with PC1 accounting for 32.7% and PC2 for 26.4%. (Figure 6). PC1 separated the cessation of watering treatments (D and D_T) from controls (C and C_T), being positively associated with ABA_L and ABA_R, while it is negatively associated with Gs and VWC.

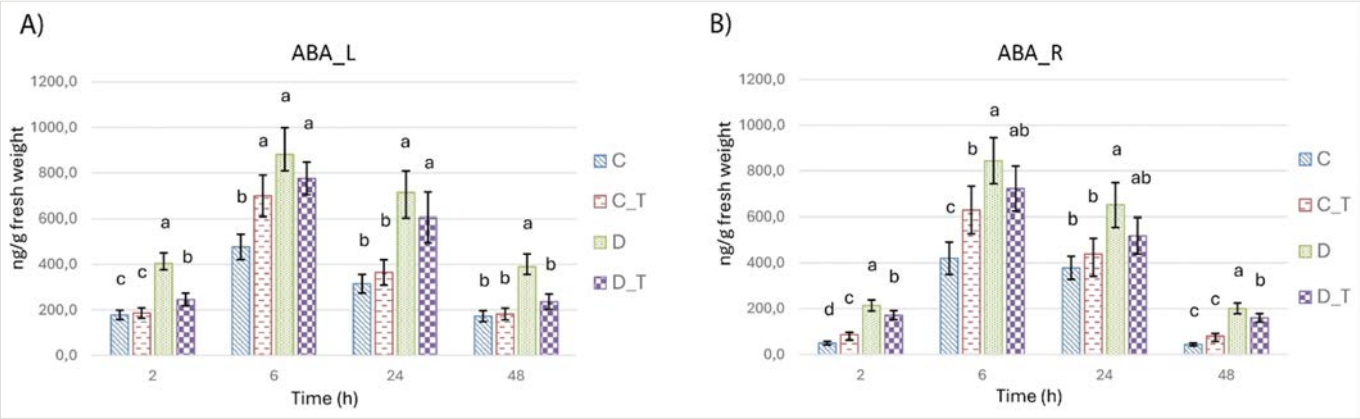


Figure 4. Hormonal responses: abscisic acid concentration in leaves (A) and roots (B) in tomato plants subjected to four treatments: optimally watered (C), optimally watered with *T. brevicompactum* (C_T), cessation of watering (D), and cessation of watering with *T. brevicompactum* (D_T). Values marked with the same letter, measured within the same time period, are not significantly different according to Tukey's test ($P < 0.05$)



Figure 5. Heatmap of Pearson correlation coefficient (r) among measured physiological and biochemical parameters: stomatal conductance (Gs), leaf water potential (ψH_2O), volumetric water content (VWC), chlorophyll content (Chl), epidermal flavonols content (Flav), nitrogen balance index (NBI), abscisic acid concentration in leaves (ABA_L) and roots (ABA_R)

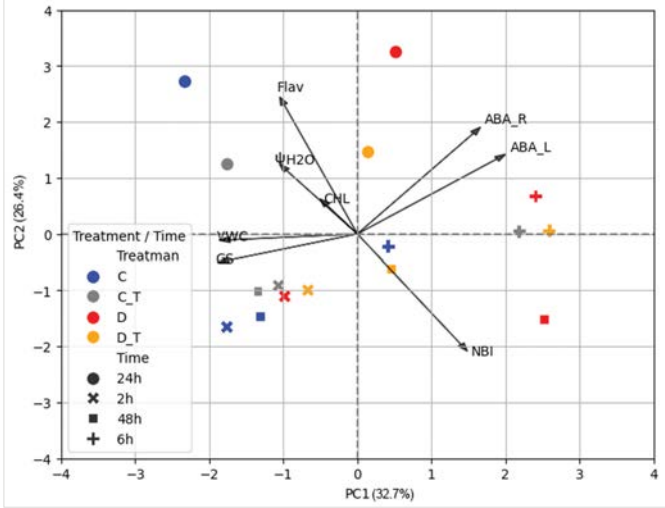


Figure 6. Principal component analysis (PCA) of physiological and biochemical traits under four treatments: optimally watered (C), optimally watered with *T. brevicompactum* (C_T), cessation of watering (D), and cessation of watering with *T. brevicompactum* (D_T)

PC2 was mainly influenced by Flav and ψH_2O, contributing to the separation of *Trichoderma*-treated plants (C_T and D_T) from their respective controls. The analysis identified ABA and Gs as the main markers of drought stress, with *T. brevicompactum* modulating some of these responses in the early phase.

DISCUSSION

The beneficial effects of certain strains of the genus *Trichoderma* in agriculture are well known. These strains can promote plant growth, modulate local and systemic defense responses against abiotic and biotic stresses and influence future stress responses through the activation of transcriptional memory (Woo et al., 2023). In this work, the physiological and biochemical responses of tomato plants (*Solanum lycopersicum* L. cv. Ailsa Craig) to *T. brevicompactum* were investigated under short-term water deficit (Figure 7).

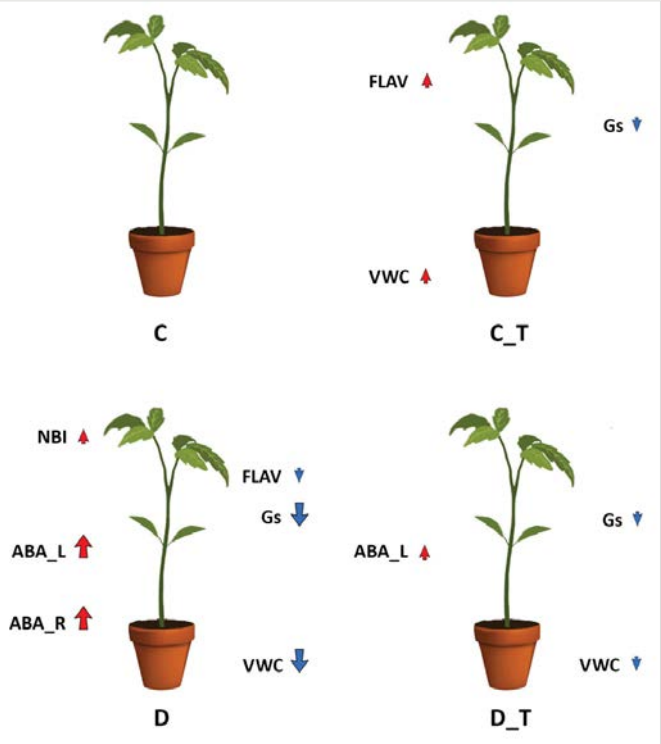


Figure 7. Schematic representation of the early response of tomato plants to *T. brevicompactum* under different treatments after 48h: optimal watering (C), optimal watering in the presence of *T. brevicompactum* (C_T), water withholding (D), and water withholding in the presence of *T. brevicompactum* (D_T). The following parameters were measured: chlorophyll content (CHL), epidermal flavonols content (FLAV), stomatal conductance (Gs), abscisic acid content in the leaf (ABA_L), leaf water potential (ψH_2O), abscisic acid content in the root (ABA_R) and volumetric water content (VWC). The intensity of stimulation is indicated by the size of the red arrows, while the intensity of inhibition is indicated by the size of the blue arrows, relative to the control conditio.

The observed significant decrease in VWC and a decreasing trend in ψH_2O under drought conditions confirmed that tomato plants were exposed to a mild to moderate soil water deficit after 48 h of water cessation. VWC was higher in treatments with *T. brevicompactum* under both optimal watering and drought conditions. This may suggest that fungal inoculation either reduced water uptake or improved water use efficiency by modulating root hydraulic properties and plant transpiration rates. Previous studies have shown that *Trichoderma* colonization can alter root architecture and increase aquaporin expression, thereby improving water transport efficiency and maintaining better hydration under drought conditions (Battaglia et al., 2024; Rawal et al., 2022). Interestingly, in our research, plants treated with *T. brevicompactum* had lower ψH_2O values during the early phase (2-6 h) compared to their respective controls. This transient decline likely reflects the initial stage of stomatal and hydraulic adaptation rather than a loss of leaf hydration. To our knowledge, no data are available in the literature on ψH_2O in such short intervals (2, 6, 24 and 48 hours) after *Trichoderma* inoculation. Most published research has focused on long-term responses. Khoshmanzar et al. (2020) showed that *Trichoderma* isolates, in the long term, increase ψH_2O in tomato plants under water deficit conditions. Vieira et al. (2017) demonstrated that improvements in ψH_2O became evident only after several days of water deficit in transgenic *Nicotiana tabacum* plants overexpressing an aquaglyceroporin gene from *Trichoderma harzianum*. However, other studies have reported no effect of *Trichoderma* on ψH_2O depending on the combination of the fungal strain and water deficit (Mesquita et al., 2025). Taken together, our results suggest that *T. brevicompactum* induces an early, transient modulation of ψH_2O that precedes the longer-term improvements in plant water status reported in previous studies. This early modulation may reflect a controlled decrease in ψH_2O to trigger stomatal regulation and osmotic adjustment, contributing to plant acclimation to the onset of drought.

ABA plays a central role in plant adaptation to drought stress. In our study, its accumulation in both leaves and roots exhibited a rapid increase under drought conditions as early as 2 h after treatment, while *T. brevicompactum* contributed to reducing this accumulation in plants exposed to water cessation. ABA levels showed a transient increase, reaching a maximum after 6 h, whereas after 48 h *T. brevicompactum* reduced ABA levels in plants subjected to drought. Plants treated with *T. brevicompactum* showed an attenuated ABA response, suggesting that the fungus mitigated stress-induced ABA accumulation. This modulation likely reflects an adaptive regulation of hormonal signalling that allows plants to maintain stomatal responsiveness without triggering a full drought

stress response. The inhibitory effect of *T. brevicompactum* on ABA accumulation in drought-stressed plants aligns with previous findings showing that *Trichoderma* spp. induce ethylene production in tomato (Leonetti et al., 2017), and that ethylene can inhibit ABA biosynthesis (She and Song, 2012). Such hormonal cross-talk can modify the plant's perception of stress, contributing to enhanced tolerance rather than avoidance responses (Guzmán-Guzmán et al., 2019).

Moreover, *Trichoderma*-induced changes in ABA may contribute to stress adaptation by mediating signal perception, transduction, and protection of photosynthetic pigments, thereby reducing water loss under drought (Akbari et al., 2024). In our previous study, *T. brevicompactum* treatment induced the upregulation of the ABA biosynthesis-related gene NCED1 in tomato roots under drought, while ABA accumulation was lower than in non-inoculated plants (Racić et al., 2018). This indicates that the *T. brevicompactum* modulates ABA metabolism through regulatory feedback or hormonal cross-talk mechanisms, rather than directly suppressing its synthesis.

Stomatal conductance exhibited a rapid reduction after 2 h in both *Trichoderma*-treated groups, reflecting a fast physiological adjustment, indicating that plants recognized the fungal signal. This transient reduction in Gs may represent a mechanism regulated by ABA and other stress-related signals. Similar findings were reported by Contreras-Cornejo et al. (2015), who demonstrated that *Trichoderma* species induce stomatal limitation. However, by 24–48 h, stomatal conductance in plants treated with *T. brevicompactum* under drought increased, indicating that the fungus enabled faster stomatal reopening and improved regulation of gas exchange. This adjustment appears to stabilize as part of a controlled homeostatic response, contributing to improved water-use efficiency. Comparable patterns were reported by Akbari et al. (2024), who observed similar stomatal dynamics in Arabidopsis exposed to *Trichoderma* under water deficit. It should be noted that Gs is not only regulated by ABA. Other chemical signals, including salicylic acid, ethylene, proline, polyamines, H_2S, Ca^{2+} ions, and nitric oxide, also participate in regulating stomatal aperture (Agurla et al., 2018). The observed recovery of Gs coincided with lower ABA levels and higher ψH_2O, suggesting that fungal inoculation mitigated drought-induced stomatal dysfunction and preserved photosynthetic capacity.

Flavonols act as antioxidants, UV protectants, and signalling molecules, playing key roles in stress perception and redox regulation (Laoué et al., 2024). In this study, the content of flavonols increased already 2 h after the application of *T. brevicompactum*, indicating a rapid activation of secondary metabolism. The temporary decline in flavonol content observed after 24 h may correspond to the metabolic reprogramming required for successful root colonization. Brotman et al. (2013) reported that genes involved in secondary metabolite synthesis are transiently downregulated 24 h after *Trichoderma* inoculation, facilitating symbiotic establishment. This observation may explain the decrease in flavonols at 24 h, which was followed by a significant increase after 48 h in plants treated with *T. brevicompactum*. The inverse trend observed between the nitrogen balance index (NBI) and flavonols was expected, as chlorophyll content remained stable and NBI reflects the Chl/Flav ratio. These findings are consistent with previously reported correlations between chlorophyll and flavonol indices measured by Dualex sensor and their corresponding biochemical quantifications, confirming the reliability of these non-destructive indicators for evaluating pigment dynamics (Cerovic et al., 2012).

Principal component analysis (PCA) revealed that ABA_L, ABA_R, Gs, ψH_2O and Flav were the primary contributors to treatment separation, suggesting that *T. brevicompactum* modulated both hormonal and physiological networks. These findings agree with earlier metabolomic analyses showing that *Trichoderma* can reprogram host metabolism and water relations within 48 h, promoting faster recovery from transient stress (Wen et al., 2025; Senizza et al., 2025).

Overall, our results indicate that *T. brevicompactum* acts as an early modulator of drought responses by regulating ABA signaling, promoting balanced control of stomatal conductance, and enhancing secondary metabolite accumulation. These coordinated adjustments confer an adaptive advantage to tomato plants under short-term water deficit. The dual modulation of hormonal and metabolic pathways underscores the strategic potential of *T. brevicompactum* as a biostimulant for enhancing drought resilience and supporting sustainable tomato cultivation under variable climate conditions.

CONCLUSION

In summary, our research highlights how *T. brevicompactum* enhances the resistance of tomato plants to short-term water deficit by modulating physiological and hormonal responses. Among the early responses, the most sensitive parameters were epidermal flavonol content, stomatal conductance, leaf water potential and ABA concentration in roots. The observed coordination between flavonol accumulation and ABA regulation indicates their joint involvement in stress signalling. Plants inoculated with *T. brevicompactum* under drought showed improved adaptive responses, including lower ABA levels in roots and more stable stomatal conductance, suggesting enhanced physiological resilience and sustained gas exchange. Although our study elucidates early responses to the combined effects of drought and *T. brevicompactum*, further research is needed to clarify the underlying molecular mechanisms and validate these findings under field conditions. A deeper understanding of the regulatory networks linking *Trichoderma*-induced hormonal and metabolic changes will be essential for optimizing its use as a biostimulant to mitigate drought effects and promote sustainable tomato cultivation under variable climate conditions.

ACKNOWLEDGMENTS

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant numbers: 451-03-66/2024-03/200032 and 451-03-136/2025-03/200054.

REFERENCES

Agurla, S., Gahir, S., Munemasa, S., Murata, Y., Raghavendra, A.S. (2018) Mechanism of stomatal closure in plants exposed to drought and cold stress. Survival Strategies in Extreme Cold and Desiccation, 215–232.

Akbari, S.I., Prismantoro, D., Permadi, N., Rossiana, N., Miranti, M., Mispan, M.S., Doni, F. (2024) Bioprospecting the roles of *Trichoderma* in alleviating plants' drought tolerance: principles, mechanisms of action, and prospects. Microbiological Research, 283, 127665. DOI: https://doi.org/10.1016/j.micres.2024.127665

Asch, F. (2000) Laboratory manual on determination of abscisic acid by indirect enzyme linked immuno sorbent assay (ELISA). Royal Veterinary and Agricultural University, Department of Agricultural Sciences, Laboratory for Agrohydrology and Bioclimatology, Copenhagen.

Battaglia, M.E., Martinez, S.I., Covacevich, F., Consolo, V.F. (2024) *Trichoderma harzianum* enhances root biomass production and promotes lateral root growth of soybean and common bean under drought stress. Annals of Applied Biology, 185 (1), 36–48. DOI: https://doi.org/10.1111/aab.12909

Brotman, Y., Landau, U., Cuadros-Inostroza, Á., Takayuki, T., Fernie, A.R., Chet, I., Viterbo, A., Willmitzer, L. (2013) *Trichoderma*–plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. PLoS Pathogens, 9 (3), e1003221. DOI: https://doi.org/10.1371/journal.ppat.1003221

Cerovic, Z. G., Masdoumier, G., Ghozlen, N. B., Latouche, G. (2012) A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiologia Plantarum, 146 (3), 251-260. DOI: https://doi.org/10.1111/j.1399-3054.2012.01639.x

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Del-Val, E.K., Larsen, J. (2016) Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. FEMS Microbiology Ecology, 92 (4), fiw036. DOI: https://doi.org/10.1093/femsec/fiw036

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Vergara, A.G., López-Bucio, J. (2015) *Trichoderma* modulates stomatal aperture and leaf transpiration through an abscisic acid-dependent mechanism in Arabidopsis. Journal of Plant Growth Regulation, 34(2), 425–432. DOI: https://doi.org/10.1007/s00344-014-9453-1

DeClerck, F.A.J., Koziell, I., Benton, T., Garibaldi, L.A., Kremen, C., Maron, M., Del Rio, C.R., Sidhu, A., Wirths, J., Clark, M., Dickens, C., Carmona, N.E., Fremier, A.K., Jones, S.K., Khoury, C.K., Lal, R., Obersteiner, M., Remans, R., Rusch, A., Winowiecki, L. (2023) A whole Earth approach to nature-positive food: biodiversity and agriculture. In: Science and Innovations for Food Systems Transformation. Springer, pp. 1–25. DOI: https://doi.org/10.1007/978-3-031-29244-6_1

FAO. 2022. FAOSTAT. License: CC BY-NC-SA 3.0 IGO. Available at: https://fao.org/faostat [Accessed 31 March 2022].

Gulzar, S., Manzoor, M.A., Liaquat, F., Shah, I.H., Rehman, A., Hameed, M.K., Zhang, Y. (2023) Effects of melatonin and *Trichoderma harzianum* on pak choi yield, chlorophyll contents and antioxidant defense system under clubroot disease. South African Journal of Botany, 158, 292–300. DOI: https://doi.org/10.1016/j.sajb.2023.03.010

Khoshmanzar, E., Aliasgharzad, N., Neyshabouri, M.R., Khoshru, B., Arzanlou, M., Asgari Lajayer, B. (2020) Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water-deficit stress. International Journal of Environmental Science and Technology, 17 (2), 869–878. DOI: https://doi.org/10.1007/s13762-019-02455-4

Laoué, J., Fernandez, C., Ormeño, E. (2022) Plant flavonoids in Mediterranean species: a focus on flavonols as protective metabolites under climate stress. Plants, 11 (2), 172. DOI: https://doi.org/10.3390/plants11020172

Leonetti, P., Zonno, M.C., Molinari, S., Altomare, C. (2017) Induction of SA-signaling pathway and ethylene biosynthesis in *Trichoderma harzianum*-treated tomato plants after infection of the root-knot nematode Meloidogyne incognita. Plant Cell Reports, 36 (4), 621–631. DOI: https://doi.org/10.1007/s00299-016-2083-5

Lin, Y., Watts, D.B., Kloepper, J.W., Feng, Y., Torbert, H.A. (2020) Influence of plant growth-promoting rhizobacteria on corn growth under drought stress. Communications in Soil Science and Plant Analysis, 51 (2), 250–264. DOI: https://doi.org/10.1080/00103624.2019.1705329

Mesquita, N. L. S., Novaes, Q. D., Soares, P. P. S., Bonfim, R. A. A., Silva, E. D., Brito, N. D., Coqueiro, D. S. O. (2025) Role of *Trichoderma* spp. in mitigating water deficiency and promoting Eucalyptus urophylla growth. Brazilian Journal of Biology, 85, e291287. DOI: https://doi.org/10.1590/1519-6984.291287

Muhammad, M., Waheed, A., Wahab, A., Majeed, M., Nazim, M., Liu, Y.H., Li, W.J. (2024) Soil salinity and drought tolerance: an evaluation of plant growth, productivity, microbial diversity, and amelioration strategies. Plant Stress, 11, 100319. DOI: https://doi.org/10.1016/j.plantsci.2023.100319

Nawaz, M., Shabbir, S., Manzoor, N., Xu, H., Wang, Z., Arshad, K.T., Sun, J. (2025) Recent advances in biofertilizer development. In: Agricultural Nutrient Pollution and Climate Change: Challenges and Opportunities. Springer, pp. 271–309. DOI: https://doi.org/10.1007/978-3-031-41899-2_12

Nguyen, T.B.A., Lefoulon, C., Nguyen, T.H., Blatt, M.R., Carroll, W. (2023) Engineering stomata for enhanced carbon capture and water-use efficiency. Trends in Plant Science, 28 (11), 1290–1309. DOI: https://doi.org/10.1016/j.tplants.2023.09.002

Quarrie, S.A., Whitford, P.N., Appleford, N.E.J., Wang, T.L., Cook, S.K., Henson, I.E., Loveys, B.R. (1988) A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. Planta, 173 (3), 330–339. DOI: https://doi.org/10.1007/BF00393323

Quinet, M., Angosto, T., Yuste-Lisbona, F.J., Blanchard-Gros, R., Bigot, S., Martinez, J.P., Lutts, S. (2019) Tomato fruit development and metabolism. Frontiers in Plant Science, 10, 1554. DOI: https://doi.org/10.3389/fpls.2019.01554

Racić, G., Körmöczi, P., Kredics, L., Raičević, V., Mutavdžić, B., Vrvić, M.M., Panković, D. (2017) Effect of the edaphic factors and metal content in soil on the diversity of *Trichoderma* spp. Environmental Science and Pollution Research, 24 (4), 3375–3386.

Racić, G., Vukelić, I., Kordić, B., Radić, D., Lazović, M., Nešić, L., Panković, D. (2023) Screening of native *Trichoderma* species for nickel and copper bioremediation potential determined by FTIR and XRF. Microorganisms, 11(3), 815. DOI: https://doi.org/10.3390/microorganisms11030815

Racić, G., Vukelić, I., Prokić, L., Ćurčić, N., Zorić, M., Jovanović, L., Panković, D. (2018) The influence of *Trichoderma* brevicompactum treatment and drought on physiological parameters, abscisic acid content, and signalling pathway marker gene expression in leaves and roots of tomato. Annals of Applied Biology, 173 (3), 213–221. DOI: https://doi.org/10.1111/aab.12448

Rawal, R., Scheerens, J.C., Fenstemaker, S.M., Francis, D.M., Miller, S.A., Benitez, M.S. (2022) Novel *Trichoderma* isolates alleviate water deficit stress in susceptible tomato genotypes. Frontiers in Plant Science, 13, 869090. DOI: https://doi.org/10.3389/fpls.2022.869090

Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A., Hammel, H.T. (1965) Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. Science, 148, 339–346. DOI: https://doi.org/10.1126/science.148.3668.339

Senizza, B., Araniti, F., Lewin, S., Wende, S., Kolb, S., Lucini, L. (2023) *Trichoderma* spp.-mediated mitigation of heat, drought, and their combination on the Arabidopsis thaliana holobiont: a metabolomics and metabarcoding approach. Frontiers in Plant Science, 14, 1190304. DOI: https://doi.org/10.3389/fpls.2023.1190304

She, X., Song, X. (2012) Ethylene inhibits abscisic acid-induced stomatal closure in Vicia faba via reducing nitric oxide levels in guard cells. New Zealand Journal of Botany, 50 (2), 203–216. DOI: https://doi.org/10.1080/0028825X.2012.671577

Shi, L., Guo, M., Ye, N., Liu, Y., Liu, R., Xia, Y., Cui, S., Zhang, J. (2015) Reduced ABA accumulation in the root system is caused by ABA exudation in upland rice (*Oryza sativa* L. var. Gaoshan1) and this enhanced drought adaptation. Plant and Cell Physiology, 56 (5), 951–964. DOI: https://doi.org/10.1093/pcp/pcv022

Tüzel, Y., Biyke, H., Harouna, O.S., Durdu, T., Tepecik, M., Oztekin, G.B., Gruda, N.S. (2025) Deficit irrigation response and climate resilience of Mediterranean tomato landraces. Horticulturae, 11 (1), 74. DOI: https://doi.org/10.3390/horticulturae11010074

Vieira, P. M., Santos, M. P., Andrade, C. M., Souza-Neto, O. A., Ulhoa, C. J., Aragão, F. J. L. (2017) Overexpression of an aquaglyceroporin gene from *Trichoderma harzianum* improves water-use efficiency and drought tolerance in *Nicotiana tabacum*. Plant Physiology and Biochemistry, 121, 38-47. DOI: https://doi.org/10.1016/j.plaphy.2017.10.012

Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R.K., Kumar, V., Verma, R., Upadhyay, R.G., Pandey, M., Sharma, S. (2017) Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. Frontiers in Plant Science, 8, 161. DOI: https://doi.org/10.3389/fpls.2017.00161

Vukelić, I., Racić, G., Bojović, M., Ćurčić, N., Mrkajić, D., Jovanović, L., Panković, D. (2020) Effect of *Trichoderma harzianum* on morpho-physiological parameters and metal uptake of tomato plants. Zbornik Matice Srpske za Prirodne Nauke, 139, 61–71. DOI: https://doi.org/10.2298/ZMSPN2039061V

Wen, X., Qi, H., Niu, Q., Tang, R., Yin, S. (2025) *Trichoderma* asperellum 152-42 enhances growth of perennial ryegrass (*Lolium perenne* L.) by modulation of plant hormones and carbon–nitrogen metabolism. Scientia Horticulturae, 345, 114138. DOI: https://doi.org/10.1016/j.scienta.2025.114138

Woo, S.L., Hermosa, R., Lorito, M., Monte, E. (2023) *Trichoderma*: a multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. Nature Reviews Microbiology, 21 (5), 312–326. DOI: https://doi.org/10.1038/s41579-022-00819-5

Zhang, C., Wang, W., Hu, Y., Peng, Z., Ren, S., Xue, M., Liu, Z., Hou, J., Xing, M., Liu, T. (2022). A novel salt-tolerant strain *Trichoderma atroviride* HN082102. 1 isolated from marine habitat alleviates salt stress and diminishes cucumber root rot caused by Fusarium oxysporum. BMC microbiology, 22 (1), 67. DOI: https://doi.org/10.1186/s12866-022-02479-0

Zhang, K., Sun, M., Feng, H., Wei, X., Xie, W., Fu, W., Chen, B. (2025) Synergistic effects of *Rhizophagus irregularis* and *Trichoderma harzianum* co-inoculation on enhancing drought tolerance and secondary metabolite production in licorice (*Glycyrrhiza uralensis*). Journal of Fungi, 11(7), 488. DOI: https://doi.org/10.3390/jof11070488