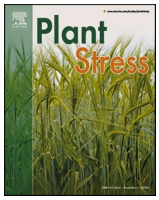
Plant Stress 16 (2025) 100860

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



Functional adaptations of the rhizosphere microbiome for drought-tolerance promotion in common bean

Ana Vitória Reina da Silva [a], Izadora de Cássia Mesquita Cunha [a], Thierry Alexandre Pellegrinetti [a], Eduardo Henrique Marcandalli Boleta [a], Luis Felipe Guandalin Zagatto [a,b], Solange dos Santos Silva Zagatto [a], Caroline Sayuri Nishisaka [a,c,g], Teresa Maria Lorizolla Mafra [a], Camila Maistro Patreze [a,d], Gordon F. Custer [e], Francisco Dini-Andreote [f], Rodrigo Mendes [g], Siu Mui Tsai [a], Lucas William Mendes [a,*]

[a] Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), Piracicaba, SP 13416-000, Brazil
[b] Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen NL-6700 AB, the Netherlands
[c] Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP 13418-900, Brazil
[d] Institute of Biosciences, Federal University of the State of Rio de Janeiro, Rio de Janeiro, RJ 22290-240, Brazil
[e] Department of Natural Sciences, The University of Maryland Eastern Shore, Princess Anne, MD, USA
[f] Department of Plant Science & Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA
[g] Laboratory of Environmental Microbiology, Embrapa Environment, Jaguariuna 18020-000, Brazil

ARTICLE INFO

Keywords:
Abiotic stress
Metagenomics
Plant-microbe interaction
Water stress

ABSTRACT

Drought stress threatens global food security, highlighting the need for resilient crops. Harnessing rhizosphere microorganisms can improve plant performance in harsh conditions. Here, we investigated the rhizosphere microbiomes of drought-tolerant (BAT477, SEA5) and susceptible (IAC Milênio, IAC-Carioca 80SH) common bean cultivars (*Phaseolus vulgaris* L.) under contrasting water regimes in mesocosm experiments to assess microbiome functional modulation under drought. Analysis of plant growth, physiological responses, nutrient dynamics, and rhizosphere microbial functional diversity revealed that drought-tolerant cultivars exhibited greater water management, minimal growth reductions, and enrichment of beneficial microbial functions, including genes linked to drought tolerance. Notably, drought stress triggered differential abundance in 1864 microbial genes, highlighting a robust functional shift. Specifically, drought-tolerant cultivars showed an enrichment of genes related to osmotic response, photosynthetic efficiency (82–87 % reduction in photosynthesis in susceptible cultivars), oxidative stress mitigation, and osmoprotectant production, whereas susceptible cultivars relied more on genes associated with DNA repair and antioxidant defense, indicating a reactive rather than proactive stress response. Additionally, the rhizosphere microbiomes of drought-tolerant cultivars were enriched in functions related to biofilm formation, dormancy survival, and oxidative stress resistance. These cultivars also maintained higher photosynthetic activity and transpiration rates with more stable stomatal conductance. Upon rehydration, they partially restored physiological functions (e.g., 48–57 % recovery in photosynthesis), further demonstrating microbiome-conferred resilience. These findings underscore the potential of plant-microbiome interactions in adapting to water stress, suggesting that microbiome selection could be a promising strategy for developing drought-resilient crops and advancing sustainable agricultural practices.

1. Introduction

Climate change is causing drastic shifts in precipitation patterns, sunlight duration, and soil and air humidity. These changes exacerbate agricultural water stress, degrade soil quality, reduce microbial biodiversity, and lower food production, critical concerns given projected population growth (Martineau et al. 2017; Seleiman et al. 2021). Drought poses one of the major threats to global agricultural production,

* Corresponding author.
E-mail address: lwmendes@cena.usp.br (L.W. Mendes).

https://doi.org/10.1016/j.stress.2025.100860
Received 17 December 2024; Received in revised form 14 April 2025; Accepted 17 April 2025
Available online 17 April 2025

as water stress harms key agricultural regions, damages economies, and disrupts societies (Lesk et al. 2016). Water scarcity impairs crop development by reducing leaf area (Hernández et al., 2004) and decreasing the photosynthetic rate (Chaves et al. 2009). It also disrupts the balance between reactive oxygen species production and the antioxidant defense system (Monakhova and Cherniad'ev, 2002), ultimately reducing biomass production.

One potential strategy to mitigate the effects of abiotic stress is to harness functions that promote plant tolerance in microbial communities associated with plants. Drought-tolerant microorganisms also can enhance plant growth and development under water-deficient conditions (Poudel et al. 2021). These microorganisms may form more rigid cell walls, enter a dormant state, accumulate osmolytes, and produce exopolysaccharides, thereby reducing the negative impacts of water scarcity (Kumar and Verma, 2018). The production of osmolytes, such as proline and trehalose, is a strategy that helps maintain cellular integrity under water stress conditions by balancing the osmotic potential between the cell interior and the external environment (Feng et al. 2022). Additionally, these microorganisms can activate genes related to water stress response, contributing to plant adaptation and survival in arid environments (Lai et al. 2018; Chai et al. 2020). In recent years, researchers have focused extensively on breeding crops for drought tolerance, while emerging studies highlight the crucial role of soil microorganisms in enhancing plant resilience to water stress (Poudel et al. 2021). While some studies have elucidated how different plant cultivars can select for particular microbiome assemblies to address biotic stressors (Mendes et al. 2018; Lazcano et al. 2021; Tie et al. 2023), research into the role of microbial communities in alleviating abiotic stress is still in its early stages (de Vries et al. 2020). To enhance crop tolerance, researchers must unravel the microbial contributions to abiotic stress resilience. Integrating plant breeding with microbiome research is a powerful strategy for enhancing agricultural sustainability, as it leverages both plant resilience and microbial interactions to improve crop productivity, stress tolerance, and soil health in the face of climate change.

Molecular exchanges from plant roots, including enzymes, alkaloids, glycosides, and exometabolites, influence the selection of the rhizosphere microbiome (Mendes et al. 2011; Philippot et al. 2013). These root exudates regulate microbial density and interactions and serve as an energy source for microbial communities (Venturi and Keel, 2016). The rhizosphere microbiome plays a significant role in plant development and enhances resistance to drought stress (Briñez et al. 2017). Plants adapt to water deficits through various mechanisms, including biofilm formation, osmolyte production, and morphological changes (Francisco et al. 2019). Activation of molecular defense mechanisms involves genes related to mechanical and osmotic adjustments (Agarwal et al. 2017). Gene regulation is integral to various plant development processes, influencing phenotypic plasticity and adaptive responses to environmental conditions, thereby enhancing long-term plant adaptation to ecological challenges (Gallucci et al. 2017).

Advances in DNA sequencing improve the study of microbiome diversity, enabling gene abundance analysis, metabolic pathway identification, and functional predictions (Mendes et al. 2017; Upadhyay et al. 2022). In genetic breeding programs, such as those targeting common beans, the goal is to identify drought-tolerant genotypes. This is particularly important because common beans are sensitive to water deficits and hold substantial nutritional and socio-economic value (Pereira et al. 2011). Common beans (*Phaseolus vulgaris* L.), a staple food in many regions, are highly vulnerable to water stress, which can severely impact their growth and productivity (Assefa et al., 2019). By assessing the rhizosphere microbiome of drought-tolerant common beans, researchers can gain valuable insights into how beneficial microorganisms interact with plant roots under water-limited conditions. These microbes may improve drought tolerance through mechanisms such as enhanced water uptake, increased nutrient availability, or strengthened stress responses. Understanding these interactions is crucial for developing sustainable agricultural practices and enhancing crop resilience. Characterizing the rhizosphere microbiome in drought-tolerant varieties could significantly advance crop management strategies and help address climate change challenges.

Here, we investigated the extent to which drought-tolerant common bean cultivars rely on the rhizosphere microbiome to withstand water stress. We hypothesized that, although drought tolerance primarily involves the physical and physiological mechanisms of the plant, tolerant cultivars may also select a specific microbiome that contributes to stress tolerance through the beneficial functions of the associated microbes. This selection pattern could emerge as the plant's rhizosphere favors certain beneficial microorganisms under drought conditions, potentially selecting specific taxa and functions that help alleviate drought stress. To test the hypothesis, we analyzed the rhizosphere microbiome of four common bean cultivars with different levels of drought tolerance and evaluated their functional profiles through metagenomic sequencing. The plants were grown under both regular watering and water stress conditions, allowing us to investigate how the microbiome's functional profile influences the plants' biometric, physiological, nutritional, and genetic parameters. This study emphasizes the importance of plant-microbiome interactions in adapting to water stress and suggests that selecting specific microbial genes could be an effective strategy for developing more drought-resilient cultivars

2. Material and methods

2.1. Mesocosm experiment design and bioassay

Soil samples from a depth of 0–20 cm were collected from the experimental area of the Luiz de Queiroz College of Agriculture, University of Sao Paulo, in Piracicaba, Brazil. The soil, classified as a medium-textured Red-Yellow Latosol, was analyzed for its physical and chemical properties, sieved through a 4 mm mesh, and its pH was adjusted with lime. Basal fertilization was applied according to common bean cultivation recommendations, using a 04–14–08 NPK formulation following the directives in Boletim 200 of Campinas Agronomic Institute (IAC) (Aguiar et al. 2014).

During the pre-experimental installation phase, soil samples were collected non-destructively using a volumetric ring to determine macroporosity, microporosity, and total porosity (m^3/m^3) with a tension table. The samples were then analyzed in the soil physics laboratory to measure soil water content (θ), residual water content (θr), and saturated water content (θs), based on the *van Genuchten* model for the soil water retention curve. Using the soil water retention potential curve and a TEROS-12 soil moisture sensor (Meter Group®; precision: 0.001 m^3/m^3), the daily water volume required per pot (in milliliters) was calculated to maintain specific soil moisture levels.

Mesocosms were set up in polyethylene pots (30 cm height x 20 cm diameter), each filled with approximately 6 kg of dry soil, and the experiments were conducted in a greenhouse under controlled conditions. The experimental design followed a randomized complete block layout with five replicates per treatment. Four common bean genotypes (*Phaseolus vulgaris* L.) with varying drought tolerance were used: the susceptible cultivars IAC-Carioca 80SH (Blair et al. 2012) and IAC Milenio (Carbonell et al. 2014), sourced from the IAC, the moderately tolerant cultivar BAT477 (Recchia et al., 2018), and the tolerant SEA5 (Briñez et al. 2017), obtained from the seed bank of the Cell and Molecular Biology Laboratory. SEA5 and BAT477 are well-established common bean cultivars widely used in drought tolerance studies due to their ability to maintain productivity under water-deficit conditions (Asfaw and Blair, 2012; Polania et al., 2017; Briñez et al., 2017). In contrast, the IAC Milênio and IAC-Carioca 80SH cultivars are recognized for their higher susceptibility to drought stress, exhibiting greater reductions in growth and yield under limited water availability (Recchia et al., 2018).

The pots were arranged in a randomized complete block design, with 5 replicates of each treatment. Four common bean genotypes, a control

treatment without plants (Bulk soil), two water regimes (well-watered and drought stress), and three sampling stages (before stress, after stress, and after rehydration), were used in factorial design of 125 samples [(4 genotypes x 2 water regimes x 3 sampling stages x 5 replicates) + 5 bulk soil]. Sample collections were predominantly destructive, except for those from the bulk soil treatment. Three seeds of each genotype were transplanted into their respective pots, which were maintained at 28/19 °C (day/night) with a 12-hour photoperiod and regularly irrigated with 200 mL of water to maintain 80 % WHC. After seedling emergence, thinning was performed, leaving one plant per pot. Irrigation was monitored daily using a tensiometer and precision scale, maintaining humidity at 80 % of water holding capacity (WHC).

2.2. Experiment sampling and measurements of plant parameters

Irrigation was maintained at a constant level of 85 % WHC until the plants reached the pre-flowering developmental stage (R5, approximately 50 days), a critical phase when reliance on stored soil moisture increases, and the impact of terminal drought becomes more pronounced, particularly affecting subsequent flowering and pod filling (Rosales-Serna et al. 2004; Recchia et al. 2018). Then, irrigation was reduced over 96 hours to 40 % of WHC to simulate a severe drought event (Recchia et al. 2018). Following this period, irrigation was restored to 80 % WHC for another 96 hours to allow plant recovery. Irrigation levels were closely monitored using a tensiometer and precision scale. Soil and rhizosphere samples, along with plant measurements, were collected at three key stages: before stress (50 days post-sowing, when the plant reached stage R5), after 96 hours of drought stress, and after 96 hours of rehydration. Parameters such as plant height and gas exchange were recorded. Plant height was measured using a millimeter tape, while gas exchange parameters, including photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), intercellular CO_2 (μmol CO_2 mol air^{-1}), and transpiration (μmol H_2O m^{-2} s^{-1}), were measured using an Infra-Red Gas Analyzer (IRGA), model LICOR XT 6400. Third trefoil leaves were collected, flash-frozen, and stored at -80 °C for gene expression analysis.

Rhizospheric soil was collected by carefully removing the plants from their pots and gently shaking the roots to retain the adhering soil, which was then stored in microtubes at -20 °C. For chemical characterization, 400 g of soil was stored at 7 °C and analyzed according to the method described by Camargo et al. (2009). Plant roots and shoots were separated, weighed for fresh mass, dried at 65 °C for 72 hours, and then weighed for dry mass. For foliar nutrient analysis, common bean leaves were air-dried, ground, and stored in plastic bags. The concentrations of phosphorus (P), potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), boron (B), cupper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were determined using ICP-MS, while total nitrogen was measured through sulfuric acid digestion followed by Kjeldahl distillation (Malavolta et al. 1997).

2.3. Leaf RNA extraction and gene expression profiling

Common bean trefoils were ground in liquid nitrogen using a mortar and pestle, aliquoted into 1.5 mL microtubes, flash-frozen, and stored at -80 °C. Total RNA was extracted using the SV Total RNA Isolation System (Promega). RNA quality was assessed with a spectrophotometer NanoDrop 2000c (ThermoFisher Scientific), ensuring A260/280 ratios between 1.7 and 2.2, and integrity was confirmed by 1.5 % agarose gel electrophoresis. For cDNA synthesis, 1 μg of RNA was reverse transcribed using the SuperScript III First-Strand Synthesis SuperMix kit (Invitrogen®) with Oligo (dT)20 primers, and the resulting cDNA was stored at -20 °C. To evaluate relative gene expression, the first-strand cDNA product was diluted 5:45 in nuclease-free water. Primers were selected based on relevant literature for gene expression studies in common beans (Supplementary Table 1). The reference gene, Actin (*ACT*), was chosen along with target genes including Aquaporin (*AQUA*), Dehydration-Responsive Element-Binding (*DREB*), and Delta-Pyrroline-5-Carboxylate Synthetase (*P5CS*).

Relative gene expression was evaluated using qPCR on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). The reaction mixture included 2.8 μl of diluted cDNA (1:50), 5 μl of SYBR Green Master Mix PowerUp, 1 μl of primers, and 0.2 μl of BSA. Each plate contained the reference gene *ACT* along with the target genes. The *ACT* gene is commonly used as a reference gene because of its stable expression under both biotic and abiotic stress conditions (Borges et al., 2011). The amplification conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. A melting curve analysis was conducted at 95 °C for 15 s, 60 °C for 1 min, and data collection at each 0.7 °C increase between 60 °C and 95 °C. The StepOne™ Software version 2.2.2 captured the results, ensuring that technical replicates had Ct value differences of less than 0.5. The specificity of the reactions was confirmed through melting curve analysis.

2.4. Soil DNA extraction, metagenomic sequencing, and data processing

Total DNA from rhizosphere and bulk soil samples was extracted using the DNeasy PowerSoil® Kit (QIAGEN Mobio) according to the manufacturer's protocol. DNA concentrations were quantified with a NanoDrop 2000c spectrophotometer, and quality was assessed by 1 % agarose gel electrophoresis stained with GelRed™. For functional characterization of the soil and rhizosphere microbiome, DNA samples underwent shotgun metagenomic sequencing at Novogene using the Illumina NovaSeq 150PE platform. A total of 125 metagenomic libraries were prepared with the NEBNext Ultra DNA Library Prep for Illumina system, generating fragments of approximately 300 bp and yielding 6 Gb per sample, equating to roughly 30 million sequences.

Following sample sequencing, the raw reads were quality-controlled using FastQC v0.11.9 and MultiQC v1.12 (Ewels et al. 2016). In this step, sequences with quality (phred) lower than 30 and length lower than 50 pb were removed with Trimmomatic v.0.39 (Bolger et al., 2014). Sequences of all samples were processed in the Diting pipeline (Xue et al. 2021) for metabolism inference and functional gene annotation. During this phase, annotations were conducted against the KEGG Orthology platform, employing the Kofam koala software (Aramaki et al. 2020). The resultant functional gene abundance table was then subjected to various ecological diversity analyses. The sequences are publicly available at NCBI SRA under the identification PRJNA1198935.

2.5. Data analysis

Diversity and composition of functional gene abundance were assessed using the Microeco package in R (Liu et al. 2021). Ecological indices, including functional richness and Shannon diversity, were calculated with the vegan package in R (Oksanen et al. 2007). Beta-diversity analysis was employed to identify differences in functional genes across samples, complemented by Principal Coordinate Analysis (PCoA) using the Bray-Curtis distance metric. Statistical differences were evaluated using the Permutational Analysis of Variance (PERMANOVA) with a significance threshold of $p < 0.05$. A Redundancy Analysis (RDA) ordination plot was generated using the Microeco and ggplot2 packages, with environmental variables selected through '*envfit*' in the vegan package. The differential abundance of functions within each treatment was determined using a two-sided Welch's test with Benjamini-Hochberg FDR correction in STAMP software (Parks et al. 2014), and a heatmap visualizing distinctive features was generated using the pheatmap package in R.

The data from biometric analyses, soil chemistry, and plant physiological parameters were assessed for normality using the Shapiro-Wilk test. Data variation and significance were analyzed using the ExpDes.pt package, which conducts Analysis of Variance (ANOVA) followed by post-hoc analysis using the Tukey test. Regarding gene expression, raw

amplification data were exported for analysis with the LinRegPCR program (Ruijter et al. 2009, 2013, 2014), which enabled baseline corrections and the setting of the window of linearity for each gene-sample combination. The program then generated linear regression data to determine the Ct values for each sample—representing the number of cycles required for the fluorescent signal to surpass the detection threshold—and to calculate the Efficiency (E) values for each gene. Gene expression ratios (2-log) were assessed using the Pair Wise Fixed Reallocation Randomization test for statistical analysis in the Relative Expression Software Tool (REST) (Pfaffl et al. 2002). Expression values were normalized to the reference gene Actin and compared across various treatments involving different common bean cultivars.

3. Results

3.1. Effect of the drought stress on soil chemistry properties

The soil chemical analysis showed that several parameters varied significantly across treatments (Supplementary Table 2). In the bulk soil, drought conditions increased levels of P, K, Ca, Mg, S, Zn, Mn, B, the sum of bases, cation exchange capacity (CEC), and base saturation (V %), while decreasing Cu levels. In the rhizosphere, the pH averaged 4.4 for all cultivars but increased during drought for the SEA5 cultivar. The BAT477, SEA5, and Milenio cultivars reduced Al levels in the rhizosphere, and all genotypes decreased aluminum saturation. Additionally, S concentration increased in the rhizosphere of the BAT477 cultivar.

3.2. Effect of the drought stress on plant parameters

The plant biometric analysis revealed slight variations among treatments (Supplementary Figures 1 and 2). Plant height did not differ significantly among cultivars under drought conditions ($P > 0.05$) (Supplementary Figure 2a). Significant trends were observed in both fresh and dry mass of shoots and roots under drought conditions. Shoot biomass varied significantly among cultivars ($P < 0.05$), with BAT477 showing the greatest decrease during drought, and Milenio experiencing the largest decline during rehydration. Root biomass also differed, with SEA5 exhibiting the lowest fresh root mass during drought (52 % decrease), and Milenio the greatest loss during rehydration (33 % decrease). For dry biomass, BAT477 was most affected by drought (56 % decrease), while Milenio suffered the most during rehydration (41 % decrease). SEA5 consistently had lower biomass overall, while the 80SH cultivar retained a higher proportion of biomass, demonstrating resilience (Supplementary Figure 2b).

Leaf gas exchange analysis showed that all measured parameters decreased significantly during drought (Supplementary Figure 3). Photosynthesis rates dropped by 82 % in BAT477 and 87 % in 80SH during the drought. After recovery, Milenio showed a 48 % decrease, while BAT477 had a 57 % reduction. Stomatal conductance was least affected in SEA5 and most affected in BAT477 throughout both drought and rehydration. Drought decreased Intracellular CO_2 content by 14 % in Milenio and 48 % in BAT477 but increased in all cultivars during rehydration, with 80SH showing a 13 % rise. Leaf transpiration decreased by 89 % in SEA5 and 93 % in BAT477 during drought, with post-rehydration reductions of 41 % in 80SH and 71 % in BAT477.

Significant differences across all measured elements emerged from the nutritional content analysis of common bean shoots (Supplementary Table 3). P levels varied among samples during drought, while N and Mn showed differences during rehydration. Tolerant cultivars increased their nutritional content under water deficit conditions. BAT477, for instance, increased its K content by 24 %, and SEA5 showed substantial increases in Fe, Cu, and Zn by 147 %, 223 %, and 50 %, respectively. Additionally, tolerant cultivars exhibited higher S and Ca content even before stress, with SEA5 maintaining the highest levels throughout and after the drought period.

The relative expression analysis of the *P5CS, AQUA*, and *DREB* genes revealed distinct patterns under water stress (Fig. 1). Overall, the resistant cultivars exhibited higher expression levels of drought-responsive genes, with the tolerant cultivar BAT477 showing a significant increase in the expression of *P5CS* and *AQUA* genes. The tolerant cultivar SEA5 displayed a tendency toward increased expression of *P5CS* and *AQUA* genes, although this was not statistically significant ($P > 0.05$). Interestingly, SEA5 showed significant downregulation of the *DREB* gene, a pattern also observed in the susceptible cultivar 80SH.

3.3. Effect of drought stress on rhizosphere microbial functional structure

Drought stress increased the richness of observed functions in Bulk soil and 80SH samples, while it decreased in the drought-tolerant cultivars BAT477 and SEA5 ($P < 0.05$) (Fig. 2a). Regarding functional diversity, the drought-tolerant cultivars SEA5 and BAT477 also showed decreased functional diversity under water stress. During rehydration, however, only Bulk soil exhibited a significant reduction in functional diversity (Fig. 2b).

The RDA revealed distinct differences between bulk soil and rhizosphere samples. Bulk soil correlated with Mn, S, Ca, K, Zn, B, and P, while rhizosphere samples were linked to Al and Fe (Fig. 3a and Supplementary Table 4). Comparing rhizosphere functional profiles under drought stress across cultivars revealed negative correlations with photosynthesis and transpiration. In contrast, control conditions demonstrated positive correlations with stomatal conductance, intracellular CO_2, and sulfur (Fig. 3b and Supplementary Table 4). Examining the three experimental stages, the Pre-Drought functional composition was consistent across cultivars (Fig. 3c and Supplementary Table 4). However, during the Drought stage, each cultivar displayed distinct functional structures, characterized by increased soil Fe, P, and Mn, as well as elevated plant Fe and Zn (Fig. 3d and Supplementary Table 4). During the rehydration stage, no discernible patterns linked to return after the drought stress or cultivar differentiation were observed (Fig. 3e and Supplementary Table 4).

3.4. Effect of drought stress on microbial functional gene composition

Differential abundance analysis of functional genes revealed significant differences among cultivars (Fig. 4). Few genes (191) differed in abundance among all cultivars during the pre-drought stage, showing minimal differentiation in functional gene patterns. However, during drought stress, a total of 1864 genes exhibited differential abundance across all cultivars, indicating a widespread microbial functional response to the treatment. Interestingly, the tolerant cultivars exhibited clear differentiation, particularly in cluster 7 which shows an increased abundance of specific functional genes. Specifically, the tolerant cultivars exhibited a higher abundance of several crucial genes that can aid plants in surviving drought stress (Fig. 4). These genes were associated with nitrate reductase and nitrite oxidoreductase (*narH, nary*, and *nxrB*), the trehalose and maltose transport system (*thuE, thuF, lpqY*, and *sugA*), the redox-sensing transcriptional repressor (*rex*), membrane protein (*K07058*), two-component system (*prrA* and *dtaR*), and the osmoprotectant transport system (*opuC*).

The pair-wise comparison for each cultivar during the drought and rehydration stages showed that drought-tolerant cultivars increased microbial genes related to biofilm formation (*tag*TUV), controlling gene activation (*rsb*W), long-term survival in dormant states (*tgs*), and oxidative stress resistance (*fqr*) ($P < 0.05$) (Fig. 5a). SEA5 also showed an increased abundance of genes linked to lipid production (*dag*K), whereas in the BAT477, there was an increase in membrane proteins, transporters necessary for cell division (*ded*A), and enzymes essential for the plant photorespiratory cycle (*glx*K). Susceptible cultivars increased genes related to transporter enzymes that catalyze DNA ends, including replication/repair (*lig*D) and ribosome biogenesis (*rimJ*). The Milenio exhibited a higher abundance of genes involved in catalyzing the final step of fatty acid oxidation in microbial metabolisms in extreme

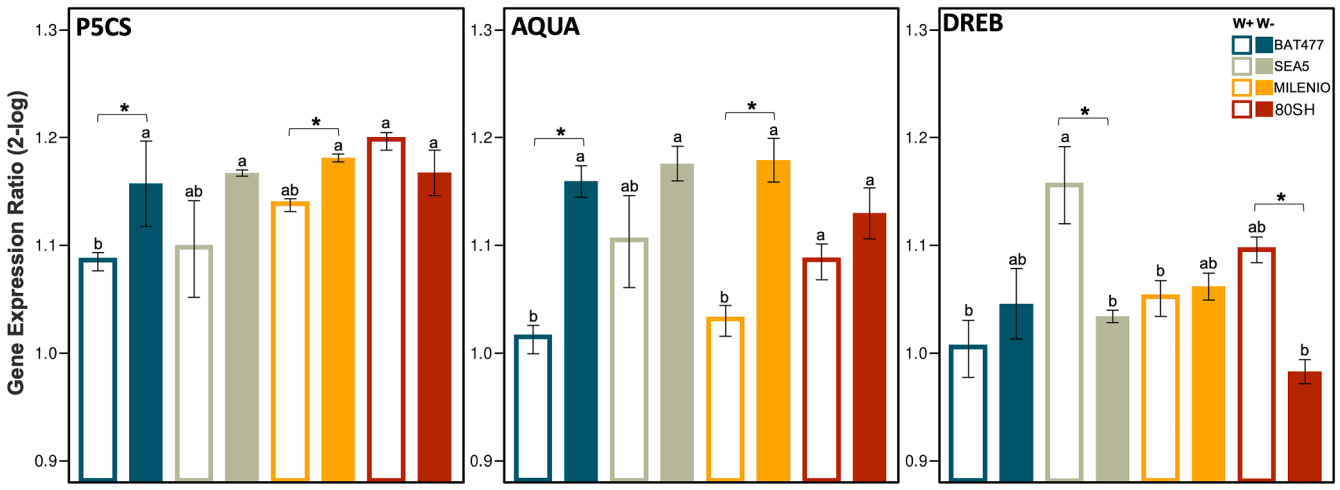


Fig. 1. Gene expression analysis of drought-related genes in common bean leaves. The figure depicts the relative expression levels of drought-responsive genes in common bean plants subjected to drought stress. The genes analyzed include Delta-Pyrroline-5-Carboxylate Synthetase (P5CS), Aquaporin (AQUA), and Dehydration-Responsive Element-Binding (DREB). Expression levels were normalized using the housekeeping gene Actin (ACT) as an internal control. Statistically significant differences among treatments are indicated by different lowercase letters above the bars, while differences within each treatment (comparing control with drought-stress conditions) are denoted by asterisks. Statistical significance was determined using analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$). The genotypes evaluated include drought-tolerant beans (BAT477 and SEA5) and drought-susceptible beans (Milenio and 80SH).

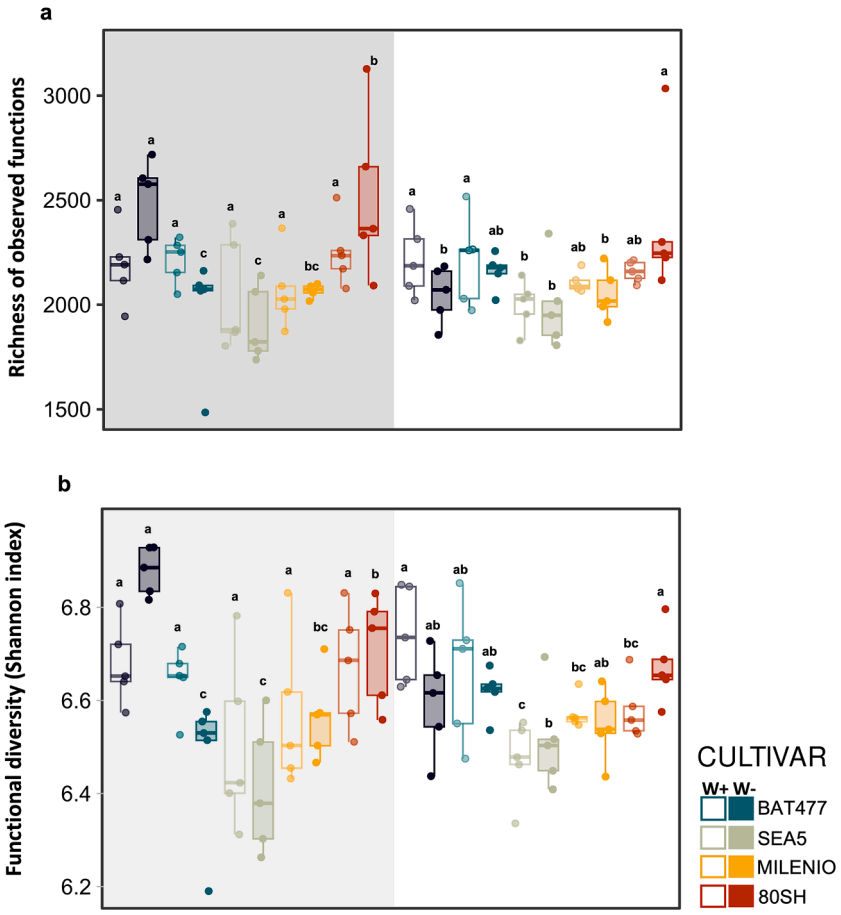


Fig. 2. Functional diversity measurements of the common bean rhizosphere microbiome. Alpha-diversity measurements of **(a)** richness of observed functions, and **(b)** Shannon indexes of the four bean cultivars with distinct tolerance to drought (Tolerant: BAT477 and SEA5; Susceptible: Milenio and 80SH) at two evaluation time stages: Drought (Gray), and Rehydration (White). And two types of irrigation, with 80 % of WHC (W+) and with 40 % of WHC (W-). Different lowercase letters above the bars indicate statistically significant differences between treatments, based on analysis of variance followed by Tukey's test ($p < 0.05$).

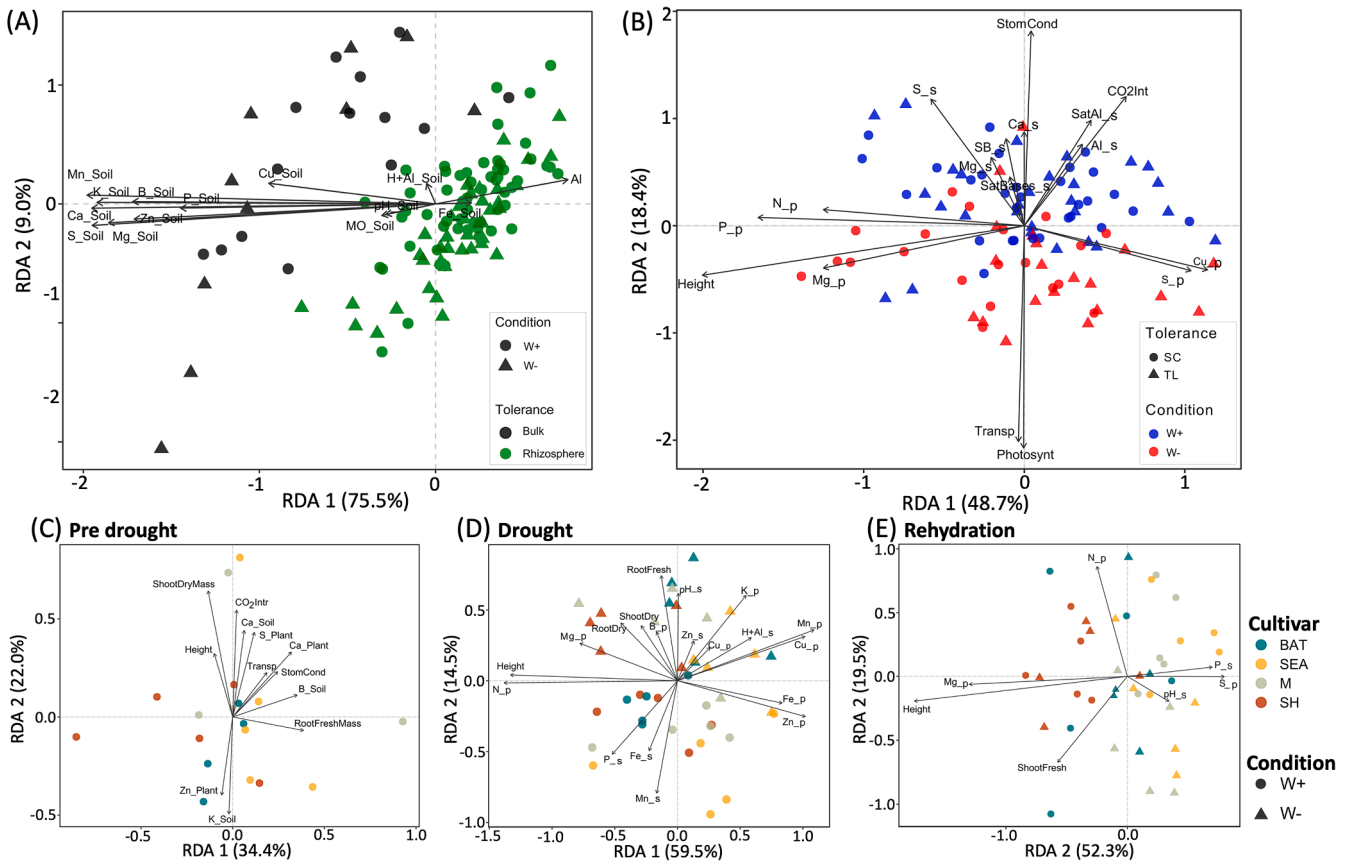


Fig. 3. Functional structure of the common bean rhizosphere microbiome. Redundancy analysis (RDA) of the four bean cultivars with distinct tolerance to drought (Tolerant: BAT477 and SEA5; Susceptible: Milenio and 80SH). **(a)** The RDA comparing bulk soil and rhizosphere samples. **(b)** RDA comparing the rhizosphere samples between control (W+) and drought-stressed treatment (W-). RDA comparing the rhizosphere samples at each experimental stage at three evaluation time stages between the susceptible (SC) and tolerant (TL) plants. **(c)** Pre drought (before water stress), **(d)** Drought (at the end of the 96-hour water stress period), and **(e)** Rehydration (96 hours after water rehydration).

environments (*ACAT*), oxidative damage prevention (*G6PD*), key electron donors in defense against oxidants (*hem*L), antioxidant effects (*TST*), and DNA repair involving removal of oxidized purines from damaged DNA (*mut*M). The 80SH showed an increased abundance of genes to carbohydrate uptake (*ABC.MS.P*) and oxidative stress (*kat*E). Some genes showed increased abundance due to drought stress in both tolerant and susceptible cultivars. For example, the gene *pls*C, responsible for an integral membrane protein, increased in SEA5 and 80SH plants. Additionally, the gene *dag*K, involved in lipid production, was overrepresented in SEA5 and Milenio cultivars.

During rehydration (Fig. 5b), the previously stressed plants showed increases in specific genes. BAT477 increased genes for DNA replication, recombination, and repair (*ssb*), integral membrane enzyme (*lgt*), osmotic stress adaptation (*msc*L), and assistance in lateral membrane protein transport (*yid*C). SEA5 showed an increase in a two-component system (*K02483*), fatty acid metabolism (*paa*F), and phosphorylation catalysis (*isp*E). In the susceptible plants, Milenio increased thiamine synthesis (*apb*E) and sporulation, biofilm, and cell wall biosynthesis (*prk*C). In 80SH, an increase in the catalysis of decarboxylation and phosphorylation (*E4.1.1.32*) and the translocation and biogenesis of membrane proteins (*sec*D) were observed.

3.5. Correlation among differential genes and biometric and chemical variables

Among the differential genes, 21 showed strong correlations with biometric and chemical variables (Fig. 6). Genes like *hpx*Q, *GGCT*, *tgs*, *tet*R, *fqr*, *lit*R, *nar*A, *gpg*P, and *IAH1*, involved in stress response, metabolism, and microbial regulation, were negatively correlated with photosynthesis and stomatal conductance, suggesting their role in stress adaptation mechanisms. Some of these genes also showed negative correlations with root dry mass and intracellular CO_2, while being positively correlated with K, Mn, Cu, and S. Genes like *tau*B (sulfonate transport), *nad*M (NAD biosynthesis), *prn*B-D (secondary metabolism and antifungal production), *pyr*E (pyrimidine synthesis), *dxr* (isoprenoid biosynthesis), *dnd*D (DNA modification), *gli* (gliotoxin biosynthesis), *abg*R (transcriptional regulation), *wsp*R (biofilm formation via cyclic-di-GMP signaling), and *tkt*A-B (pentose phosphate pathway) were positively correlated with photosynthesis and stomatal conductance, highlighting their potential roles in supporting metabolic and regulatory processes under favorable conditions. Genes such as *wsp*R and *tkt*A-B were also positively correlated with root dry mass and intracellular CO_2, while *prn*B-D showed correlations with these variables, as well as Fe, shoot dry mass, and height. Among these cited functions, some variables exhibited negative correlations, such as K (*abg*R, *prn*B-D), Mn (*dxr*, *pyr*E), Cu (*prn*D, *tau*B), Ca (*dnd*D, *prn*B), and S (*dnd*D, *prn*B-D).

4. Discussion

Our results revealed that the levels of most soil nutrients increased during the dry period, likely due to the concentration effect. This effect could be attributed to the reduced soil moisture that leads to a higher concentration of dissolved nutrients in the soil solution, increasing the concentration but not the absolute amounts (Naylor and Derr, 2018). The bulk and rhizosphere soil pH remained relatively stable but increased significantly under drought conditions, especially in the SEA5

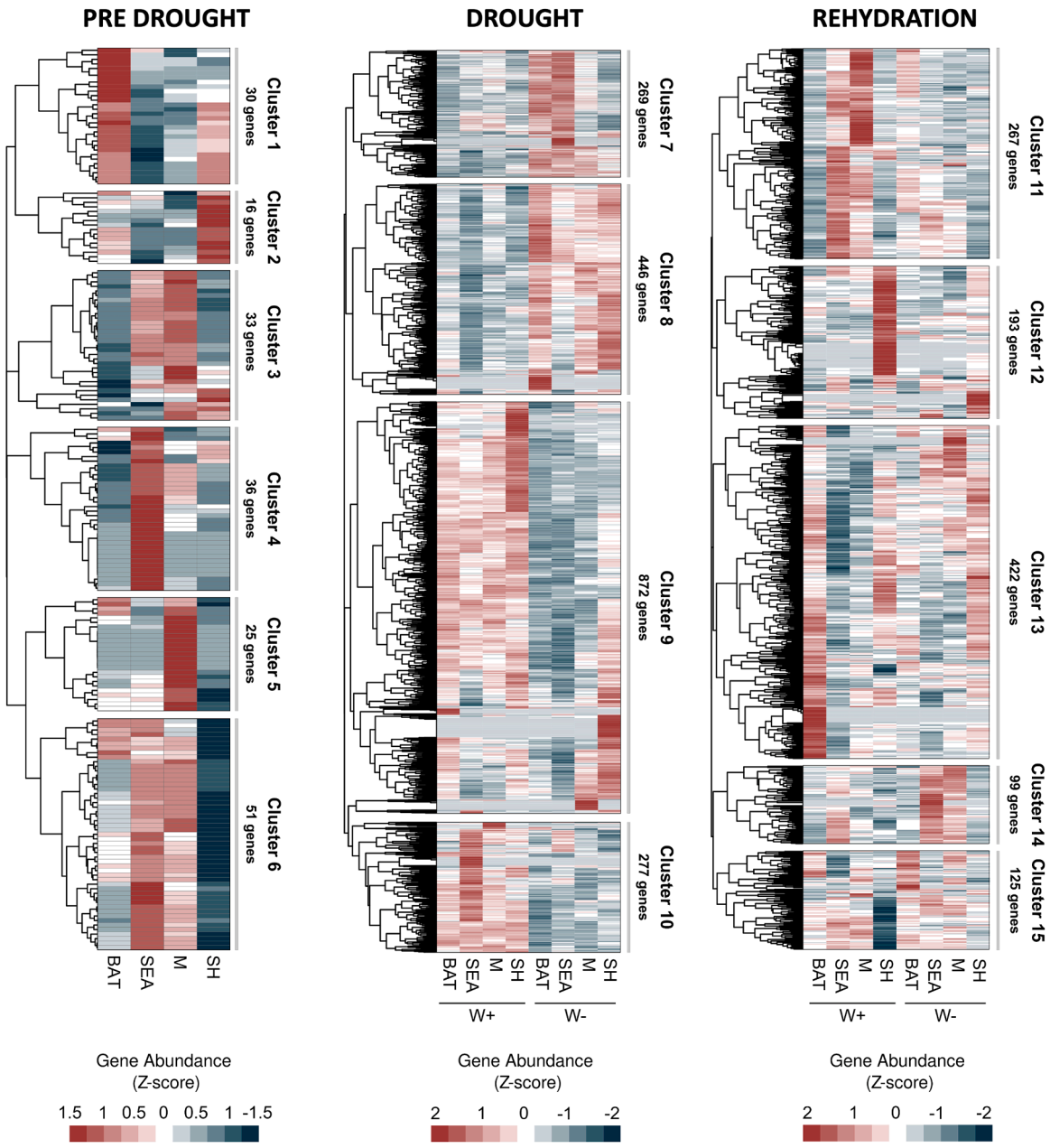


Fig. 4. Functional profile of the common bean rhizosphere microbiome. Heatmap of the relative functional abundance and prevalence of the microbiome according to different treatments, control (W+) and drought-stressed treatment (W-) based on metagenomic gene sequencing. The color key relates the heatmap colors to the standard score (z-score), representing the deviation from the row mean in units of standard deviation above or below the mean. In each heatmap hierarchical groupment represents differential functions among treatments (clusters). Tolerant beans: BAT477 (BAT) and SEA5 (SEA); Susceptible beans: Milenio (M) and 80SH (SH).

cultivar. This increase in pH benefits the microbial community, as rhizosphere acidification can hinder root growth, reduce microbial diversity, and limit nutrient availability (Silva et al. 2006). The elevated pH in SEA5 during drought likely promotes a more favorable environment for beneficial microbes, supporting nutrient cycling and plant-microbe interactions (Guo et al. 2022).

No significant differences in plant growth were observed under water stress; however, all cultivars exhibited a tendency for reduced plant height. Different studies have reported reductions in plant height and dry matter production of common beans cultivated under water stress (Gonçalves et al., 2015; Kaur et al., 2021). The drought-tolerant cultivar SEA5 demonstrated resilience by maintaining a consistent architecture, with minimal reduction in height under stress. Water deficit can significantly influence plant development, leading to reduced biomass production due to decreased photosynthesis, which diminishes leaf development and expansion (Pantin et al. 2011). SEA5 maintained a consistently lower biomass across all conditions, aligning with a study that found this genotype performed well under water stress, despite an observed biomass increase in their findings (Polania et al. 2016). This suggests that the SEA5 cultivar maintains a competitive water balance, allowing for more efficient water use in terms of plant physiological responses to water deficit stress (Ribeiro et al. 2019). This phenomenon can be seen as a survival strategy, as the reduction in shoot growth rate implies a decrease in structures with high transpiration capacity, consequently reducing the plant's water demand (Chaves et al. 2009). The drought-tolerant cultivar BAT477 exhibited the highest expression of *P5CS*, consistent with previous studies indicating that drought-tolerant cultivars generally express higher levels of P5CS

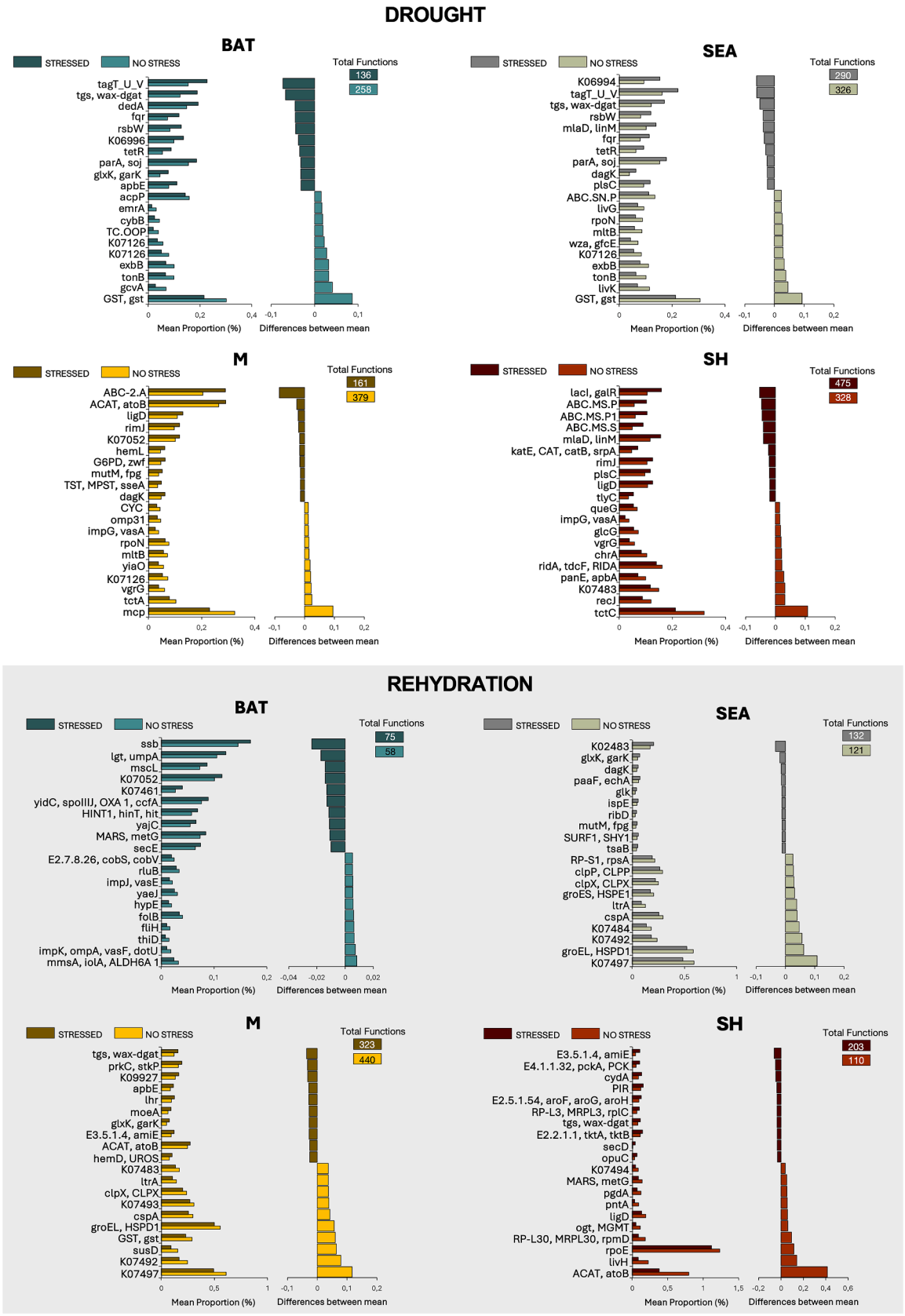


Fig. 5. Functional composition of the common bean rhizosphere microbiome. Differential abundance between treatments using the two-sided Welch's *t*-test with STAMP ($P < 0.05$). **(a)** Drought differential functional among cultivar illustrating the impact of drought conditions on the system. **(b)** Rehydration differential abundance of genes showing differences among cultivars. Statistical differential abundance were assessed using Welch's *t*-test followed by Benjamini-Hochberg FDR correction, with significance determined at $p < 0.05$. Tolerant beans: BAT477 (BAT) and SEA5 (SEA); Susceptible beans: Milenio (M) and 80SH (SH).

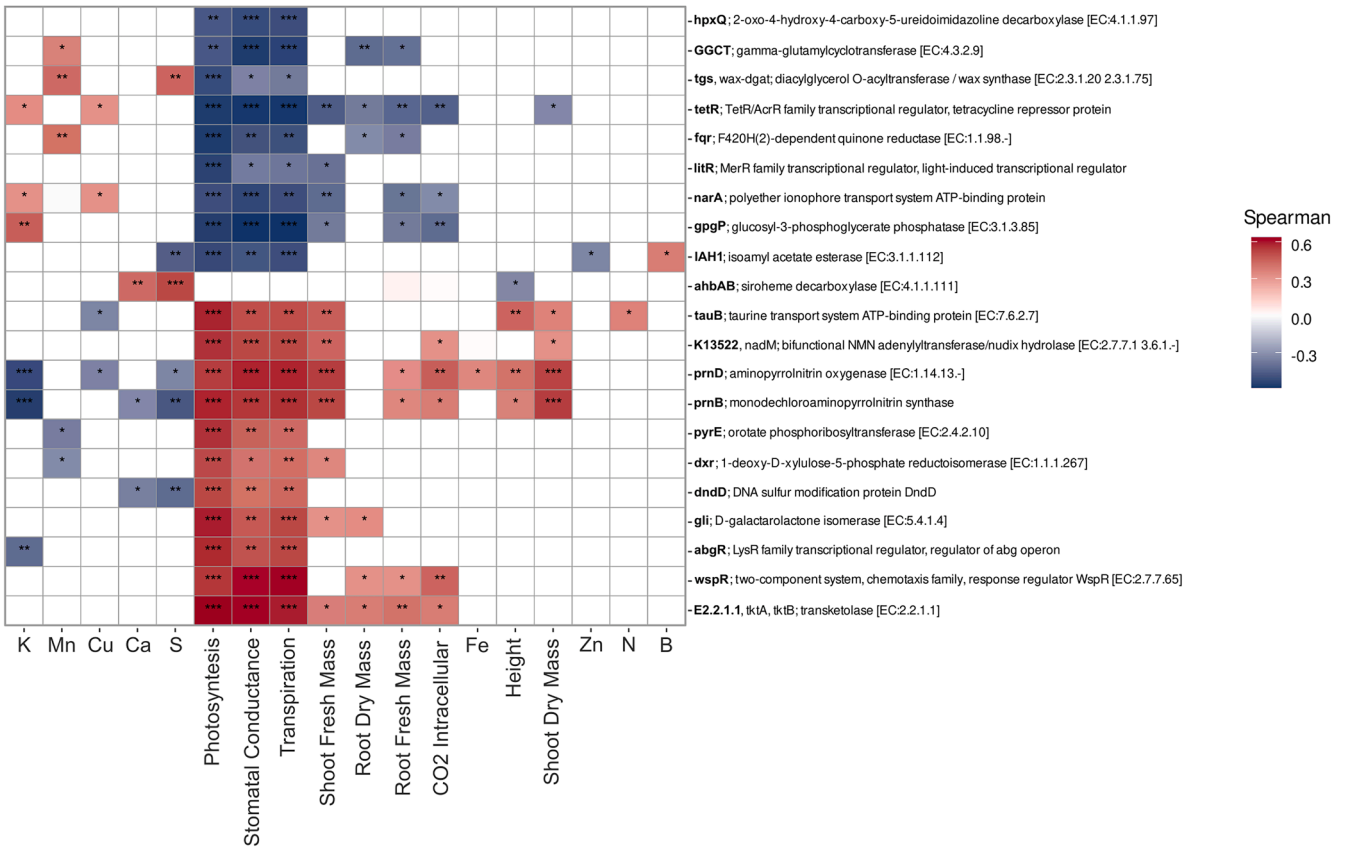


Fig. 6. Correlation between plant parameters and functional microbiome. Spearman correlation analysis between differential genes obtained from metagenomic sequencing and plant biometric, physiological parameters, and soil chemical properties. The intensity and shade of the colors represent the strength and direction (positive or negative) of the correlations: blue tones indicate negative correlations, while red tones represent positive correlations. More intense colors correspond to stronger correlations, whereas colors closer to white indicate no significant correlation. (K) Potassium, (Mn) Manganese, (Cu) Copper, (Ca) Calcium, (S) Sulfur, (Fe) Iron, (Zn) Zinc, (N) Nitrogen and (B) Boron.

compared to drought-sensitive ones (Lanna et al. 2016). The lesser reduction in aboveground growth for BAT477 under water deficit compared to the sensitive genotype may also be attributed to the remobilization of photoassimilates for deep root growth, potentially exploring regions with higher moisture content. Similar findings indicate that genotypes more tolerant to water deficiency tend to expand their roots into deeper layers of the soil profile (Polania et al. 2017).

Water stress significantly impacted all measured plant physiological processes, leading to reduction across all treatments, with the effect being particularly pronounced in the susceptible cultivars. The BAT477 genotype exhibited a significant reduction in photosynthetic and transpiration rates, with a 50 % decrease in carbon dioxide (CO_2) compared to the 48 % reduction for transpiration rate (Lanna et al. 2016). In the current study, BAT477 has effective control over stomatal opening and closure. After the rehydration, stressed plants began to recover gas exchange physiological processes, compared to stressed plants during the drought stage, particularly in intracellular CO_2 processes, which showed increased rates in all cultivars. Similar results were found in other common beans studies, where photosynthetic rate and CO_2 assimilation recovered two days after recovery; though severe water deficit prevented full recovery (Oliveira et al. 2002; Miyashita et al. 2005).

Plant growth relies on a steady nutrient supply for key metabolic functions, and water stress often disrupts nutrient uptake, leading to deficiencies (Arjenaki et al. 2012). However, our results showed that the drought-tolerant cultivar SEA5 maintained a higher transpiration rate and increased calcium levels. Recent studies suggest that nutrient accumulation, such as calcium and potassium during drought in plants like *Cunninghamia lanceolata*, may aid in stress adaptation by supporting osmotic adjustment and CO_2 fixation (Li et al. 2023). In the present study, potassium, calcium, and magnesium levels also elevated post-drought, likely enhancing stress tolerance. Thus, the higher transpiration rates in SEA5 helped maintain calcium absorption and, consequently, enabled the plant to thrive during drought (Mauad et al. 2015).

The drought-tolerant BAT477 cultivar exhibited significantly higher expression of *P5CS* and *AQUA* genes under drought conditions. The upregulation of *P5CS* aligns with previous studies demonstrating elevated expression of this gene in drought-tolerant cultivars compared to drought-sensitive ones (Maghsoudi et al., 2018). This increased expression likely contributes to proline accumulation, which plays a critical role in regulating osmotic potential and protecting cellular structures during stress (Yang et al., 2021; Feng et al., 2022). Similar patterns of *P5CS* overexpression have been reported in other plant species, such as *Hordeum vulgare* L. and *Triticum aestivum* L. (Bandurska et al., 2017), underscoring its conserved role in drought tolerance across diverse taxa. Aquaporin genes, such as *AQUA*, regulate the movement of water and solutes across cell membranes and are thought to play a pivotal role in plant responses to water stress. Their activity may influence stomatal closure and water conservation during drought (Putpeerawit et al., 2017). The upregulation of *AQUA* in *BAT477* suggests that this cultivar may enhance drought tolerance by optimizing water use efficiency through the timely regulation of specific aquaporin genes (Zupin et al., 2017). This is supported by its ability to maintain relatively stable physiological processes, including controlled stomatal regulation, efficient water use, and the capacity to recover gas exchange processes following rehydration, as observed in the experiment. In contrast to *P5CS* and *AQUA*, the expression of *DREB* genes in BAT477 showed only a slight, non-significant increase under drought conditions.

DREB genes are known to be crucial mediators of stress responses in plants, participating in signal transduction pathways that protect against dehydration and regulate processes such as growth, anthocyanin biosynthesis, and hormonal balance (Lai et al., 2018; Chai et al., 2020). Interestingly, the *DREB* gene was downregulated in the drought-tolerant SEA5 cultivar, suggesting that this cultivar may rely on alternative mechanisms for drought adaptation or exhibit a less effective stress response compared to BAT477. The SEA5 cultivar showed minimal reduction in plant height and maintained higher transpiration rates, suggesting that it may rely less on *DREB*-mediated pathways. Instead, SEA5 appears to prioritize nutrient uptake, particularly calcium, to support osmotic adjustment and CO_2 fixation, which may explain its resilience despite lower biomass production. The differential expression of drought-related genes between BAT477 and SEA5 highlights the complexity of drought adaptation mechanisms in common bean cultivars. While BAT477 employs a robust genetic response involving proline synthesis and aquaporin regulation, SEA5 may utilize alternative strategies, such as nutrient accumulation and reduced shoot growth, to mitigate water stress. The differential expression of drought-related genes between the two cultivars underscores the complexity of drought adaptation mechanisms and highlights the importance of cultivar-specific approaches in breeding for drought tolerance.

Prior to drought exposure, the microbial functional diversity, as assessed by the richness and Shannon diversity index, showed no difference among cultivars. However, differences were observed under drought conditions. No significant changes were observed within the same genotype under drought versus control conditions, but drought-tolerant cultivars BAT477 and SEA5 showed a marked decline in microbial richness and diversity compared to more susceptible cultivars. This suggests that during drought stress, the rhizosphere microbiome undergoes changes in the community structure with an impact on functional gene composition (Gao et al. 2024). Furthermore, it can be inferred that tolerant plants selectively recruit specific microbiota, potentially harboring beneficial traits that enhance drought resilience. The dynamics of bacterial communities in the rhizosphere are influenced by both the host plant and environmental stressors, suggesting that plants can selectively enrich beneficial microbial populations that confer drought resilience (Andreo-Jimenez et al. 2019). Previous research has shown that drought stress can alter the diversity and function of root-associated microbiomes, reducing bacterial diversity and increasing the abundance of drought-resistant bacteria in plant rhizospheres (Kavamura et al. 2018; Fuentes et al. 2020; Liu et al. 2021).

During drought, the tolerant cultivars BAT477 and SEA5 increased the abundance of specific genes, such as *nar*H, *nar*Y, and *nxr*B, which regulate nitrate reductase and nitrite oxidoreductase activity. These genes help plants regulate nitrogen levels, a critical nutrient for development (Ye et al. 2022). Nitrogen addition improves water use efficiency, increases plant dry mass, mitigates drought's negative effects on photosynthesis, and prevents carbon deprivation (Gessler et al. 2017). It also aids osmotic adjustment, enhances antioxidant enzyme activity, and promotes drought tolerance by boosting root number, photosynthetic characteristics, and stomatal conductance (Yang et al. 2014). There was an increase in *rex* genes associated with redox-sensing transcriptional regulation, which plays a key role in plant defense signaling (Chae et al. 2023). Water stress induces higher production of ROS in chloroplasts, peroxisomes, and mitochondria. This increase is controlled by an antioxidant system that modulates ROS levels and maintains the cell's redox status. Elevated ROS act as alarm signals, triggering acclimation and defense responses via pathways involving ABA, calcium fluxes, and sugar sensing (Cruz, 2008).

An increased abundance of genes associated with trehalose and maltose transport systems, including *thu*E, *thu*F, *lpq*Y, and *sug*A, was observed in the drought-tolerant cultivars. Trehalose, a non-reducing sugar present in plants and microorganisms and used as a storage carbohydrate in fungal spores, plays a crucial role in enhancing drought tolerance (Schwarz and Van Dikck, 2016). It helps maintain cell membranes, regulate stomata, support photosynthesis, enhance nutrient uptake, and osmolyte accumulation, activate stress proteins, and detoxify ROS, thereby supporting the antioxidant system (Moller et al. 2007). Foliar applications of maltose and trehalose have been shown to induce drought tolerance in plants, enhancing growth and mitigating drought-induced damage (Ibrahim and Abdellatif, 2016). The genotypes BAT477 and SEA5 also showed an increase in the *opu*C gene, associated with the osmoprotectant transport system, which aids in the uptake and synthesis of various osmoprotectants (Du et al. 2011). These include amino acids (proline and glutamate), carbohydrates (trehalose), sugar alcohols (inositol and mannitol), quaternary ammonium compounds (glycine betaine), and tertiary sulfonium compounds (dimethylsulfoniopropionate) (Ejaz et al. 2020). These osmoprotectant compounds enable plants and microorganisms to adjust their osmotic potential, regulate water flow, and prevent dehydration or cell rupture under stress conditions (Hoffmann and Bremer, 2017).

The tolerant cultivars exhibited a higher abundance of genes associated with biofilm production, stress response, and energy storage. Genes such as *wsp*R and *tag*TUV, which are involved in biofilm production, help microorganisms protect against adverse conditions and retain water and nutrients around the roots (Carvalho et al. 2019; Brito, 2020). The *rsb*W gene regulates stress response activation through signaling cascades involving protein interactions, phosphorylation, and dephosphorylation, thereby facilitating stress adaptation (Cheng and Case, 2023). The *GGCT* gene is involved in glutathione metabolism, which is essential for maintaining redox balance and protecting cells from oxidative stress. By regulating growth and signaling under stress conditions, it enhances plant resilience and overall health (Dorion et al. 2021; Zhang et al. 2024). The rhizosphere microbiome of the drought-tolerant cultivar SEA5 exhibited a higher abundance of the *dag*K gene, which is involved in lipid biosynthesis essential for carbon storage and energy production (Taiz and Zeiger, 2004). Similarly, the rhizosphere microbiome of the BAT477 cultivar exhibited a higher abundance of the *ded*A gene. This gene is linked to cell membrane maintenance, essential for preventing dehydration, sustaining energy production, enabling nutrient uptake, and supporting signaling (Padhi and Chatterjee, 2023).

Conversely, susceptible cultivars showed an increased abundance of genes related to genome integrity (*lig*D), DNA repair, and antioxidant effects (*TST*), suggesting that these plants prioritize maintaining genetic stability and mitigating oxidative damage. Under water stress, oxidative stress in plants leads to the production of free radicals, damaging cellular integrity and reducing productivity (Sankar et al., 2007). In response, plants produce antioxidant enzymes (APX, GPX, CAT, and SOD) to detoxify ROS and protect vital processes (Caverzan et al. 2016). Drought-tolerant plants significantly increase these enzyme activities to maintain redox balance, with ROS also serving as signals for regulating various stress responses, such as stomatal opening and closing, root gravitropism, cell wall strengthening, and cell cycle control (Oliveira, 2015). The Milenio cultivar showed an increased abundance of the *ACAT* gene, which is crucial for the final step of fatty acid oxidation in extreme microbial environments. This gene supports molecular adaptations that influence the organism's physiology, metabolism, and cell signaling (Martínez-Espinosa, 2020). Interestingly, the 80SH cultivar showed a higher abundance of the *ABC.MS.P* gene, associated with carbohydrate absorption, suggesting a mechanism for energy storage. During drought, plants accumulate sugars to maintain water balance and cell turgor (Maia et al. 2007). Elevated carbohydrates, resulting from reduced growth and starch breakdown, are linked to abscisic acid, which enhances survival by increasing root-to-shoot ratio and inducing stomatal closure (Lisar et al. 2012).

During the rehydration phase, the tolerant cultivar BAT477 showed a higher abundance of the *msc*L and *ssb* genes. The *msc*L gene helps adapt to osmotic stress by managing cell volume and solute concentration (Larcher, 2000), while the *ssb* gene encodes proteins that control DNA accessibility (Zhou et al. 2011). The SEA5 cultivar had higher levels of

the K02483 gene, linked to a bacterial two-component system helping sense environmental changes (Guerra and Lourenço, 2018), as well as the *isp*E gene, which plays a key role in phosphorylation during photosynthesis. Additionally, the *kat*E gene offers protection against oxidative stress by scavenging harmful hydrogen peroxide, minimizing damage, and signaling stress conditions (Tondo et al. 2020). Susceptible cultivars like Milenio showed a higher abundance of the *prk*C gene, crucial for cell wall biosynthesis, providing resistance and protection against stress. This gene is also linked to sporulation, allowing microorganisms to form resilient spores under stress (Amabis and Martho, 2004). Additionally, there was an increase in the *apb*E gene, associated with thiamine, which enhances stress tolerance in various plant species such as beans, wheat, and *Arabidopsis*. Thiamine improves germination, growth, and stress responses, and is linked to antioxidant action, pathogen defense, and better water absorption by seeds (Vendruscolo et al. 2020; Ezzat, 2021; Paraizo et al. 2021).

Metagenomic analysis of the rhizosphere microbiome in drought-tolerant and susceptible common bean cultivars revealed microbial mechanisms that may support plant adaptation to drought. While drought-tolerant cultivars showed an enrichment of genes related to osmotic response, photosynthetic efficiency, oxidative stress mitigation, and osmoprotectant production, susceptible cultivars relied more on genes associated with DNA repair and antioxidant defense, indicating a reactive rather than proactive stress response. These findings suggest that drought-tolerant cultivars selectively recruit and enrich microbial populations with beneficial traits, such as enhanced nitrogen cycling, osmoprotectant synthesis, and biofilm formation, which collectively contribute to drought resilience. Harnessing these microbial mechanisms through microbiome engineering or bioinoculant development could offer promising strategies to enhance drought tolerance in common bean and other crops, particularly in water-scarce regions. Future research should focus on validating the functional roles of these microbial genes and their applications in sustainable agriculture.

While our study provides valuable insights into the physiological, molecular, and microbial mechanisms associated with drought tolerance in common bean cultivars, several limitations must be acknowledged. First, the experiment was conducted under controlled drought conditions, which may not fully capture the complexity and variability of field environments, including fluctuating soil moisture, temperature extremes, and biotic interactions. Future field-based studies are needed to validate whether the responses observed here, particularly in SEA5 and BAT477, translate into agronomic advantages under real-world conditions. Second, although we identified key genes and microbial functions associated with drought tolerance, our approach was limited to functional inference based on gene abundance. Metatranscriptomic or metaproteomic analyses would provide a more dynamic view of gene expression and microbial activity under stress conditions, helping to distinguish between the presence and the actual functionality of microbial genes. Additionally, the study focused on a relatively short-term drought-recovery cycle. Long-term monitoring of plant performance and rhizosphere dynamics across multiple drought events could help determine whether the observed physiological and microbiome responses are stable or transient adaptations. This is particularly important for understanding plant memory and resilience to recurring stress events. Our analysis also revealed contrasting drought-adaptive strategies between SEA5 and BAT477, ranging from transcriptional regulation of stress-related genes to nutrient accumulation and shifts in microbial functional potential. However, the underlying regulatory networks that coordinate these plant-microbe interactions remain largely unexplored. Future work should aim to integrate multi-omics data (e.g., genomics, transcriptomics, metabolomics, and microbiomics) with plant phenotyping to unravel the complex signaling cascades governing drought responses at the host-microbe interface. Lastly, although we observed changes in microbial diversity and function in the rhizosphere, the causal relationships between microbiome composition and plant performance under drought remain to be experimentally validated. Synthetic community experiments or microbiome transplant assays could provide causal evidence for microbiome-mediated drought resilience.

5. Conclusion

This study highlights the resilience of drought-tolerant common bean cultivars, specifically SEA5 and BAT477, under water stress conditions, both in terms of physiological responses and microbial dynamics. While drought led to reduced plant growth and physiological processes in all cultivars, the tolerant varieties demonstrated more efficient water use, better root development, and enhanced osmotic regulation, contributing to their resilience. Notably, these cultivars exhibited unique microbial profiles, with increased abundances of genes related to stress adaptation, nutrient cycling, and osmoprotection. The observed microbial shifts suggest that drought-tolerant plants recruit specific beneficial microorganisms to optimize stress responses, thereby enhancing their overall drought resilience. The presence of specific genes, such as *nar*H, *nar*Y, *nxr*B, *rex*, trehalose transport system genes, and *tag*TUV, highlights the complex interactions between plant and microbial communities in mitigating drought stress. In contrast, susceptible cultivars like Milenio and 80SH exhibited distinct gene profiles, emphasizing the importance of microbial functional diversity in plant stress responses. This underscores the importance of understanding plant-microbe interactions in the context of drought stress, as they play a crucial role in improving plant performance under water-deficit conditions. Future studies should identify key microbial taxa and genes enhancing drought resilience in crops, guiding breeding programs to improve water-use efficiency and stress tolerance for climate-resilient agriculture.

CRediT authorship contribution statement

Ana Vitória Reina da Silva: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Izadora de Cássia Mesquita Cunha:** Writing – review & editing, Methodology, Investigation. **Thierry Alexandre Pellegrinetti:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation. **Eduardo Henrique Marcandalli Boleta:** Writing – review & editing, Investigation, Formal analysis. **Luis Felipe Guandalin Zagatto:** Investigation, Formal analysis. **Solange dos Santos Silva Zagatto:** Methodology, Investigation, Formal analysis. **Caroline Sayuri Nishisaka:** Writing – review & editing, Formal analysis. **Teresa Maria Lorizolla Mafra:** Methodology, Investigation. **Camila Maistro Patreze:** Methodology, Investigation, Formal analysis. **Gordon F. Custer:** Formal analysis. **Francisco Dini-Andreote:** Supervision, Formal analysis. **Rodrigo Mendes:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Siu Mui Tsai:** Supervision, Funding acquisition, Conceptualization. **Lucas William Mendes:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

Acknowledgments

This study was supported by grants from Fundação de Amparo à pesquisa do Estado de São Paulo (FAPESP 2019/16043–7, 2020/12890–4, 2021/08804–8, 2022/00552–2, 2022/08729–9, 2023/00429–9), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 402050/2023–1), and PRPI-USP (22.1.08498.01.0).

LWM also thank CNPq for the Productivity Research Grant (CNPq 307670/2021–0).

Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2025.100860.

Data availability

The sequences are publicly available at NCBI SRA under the identification PRJNA1198935

References

Agarwal, P.K., Gupta, K., Lopato, S., Agarwal, P., 2017. Dehydration responsive element binding transcription factors and their applications for the engineering of stress tolerance. J. Exp. Bot. 68 (9), 2135–2148. https://doi.org/10.1093/jxb/erx118.

Aguiar, A.T.E., Gonçalves, C., Paterniani, M.E.A.G.Z., Tucci, M.L.S., Castro, C.E.F., 2014. Instruções Agrícolas Para As Principais Culturas Econômicas, 7. Instituto Agronômico de Campinas, Campinas, SP, p. 452.

Amabis, J.M., Martho, G.R., 2004. Biologia moderna first ed. Moderna.

Andreo-Jimenez, B., Vandenkoornhuyse, P., Lê Van, A., Heutinck, A., Duhamel, M., Kadam, N., Jagadish, K., Ruyter-Spira, C., Bouwmeester, H., 2019. Plant host and drought shape the root associated fungal microbiota in rice. PeerJ. 7, 7463. https://doi.org/10.7717/peerj.7463.

Aramaki, T., Blanc-Mathieu, R., Endo, H., Ohkubo, K., Kanehisa, M., Goto, S., Ogata, H., 2020. KOFAMKOALA: KEGG ortholog assignment based on profile HMM and adaptive score threshold. Bioinformatics. 36 (7), 2251–2252. https://doi.org/10.1093/bioinformatics/btz859.

Arjenaki, F.G., Jabbai, R., Morshedi, A., 2012. Evaluation of drought stress on relative water content, chlorophyll content and mineral elements of wheat (*Triticum aestivum L.*) varieties. Int. J. Agric. Crop. Sci. 4, 726–729. https://doi.org/10.3390/agronomy11091792.

Assefa, T., Mahama, A.A., Brown, A.V., Cannon, E.K.S., Rubyogo, J.C., Rao, I.M., Blair, M.W., Cannon, S.B., 2019. A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (*Phaseolus vulgaris* L.). Mol. Breed. 39, 20. https://doi.org/10.1007/s11032-018-0920-0.

Asfaw, A., Blair, M.W., 2012. Quantitative trait loci for rooting pattern traits of common beans grown under drought stress versus non-stress conditions. Mol. Breed. 30 (2), 681–695. https://doi.org/10.1007/s11032-011-9654-y.

Bandurska, H., Niedziela, J., Pietrowska-Borek, M., Nuc, K., Chadzinikolau, T., Radzikowska, D., 2017. Regulation of proline biosynthesis and resistance to drought stress in two barley (*Hordeum vulgare L.*) genotypes of different origin. Plant Physiol. Biochem. 118, 427–437. https://doi.org/10.1016/j.plaphy.2017.07.006.

Blair, M.W., Galeano, C.H., Tovar, E., 2012. Development of a mesoamerican intra-gene pool genetic map for quantitative trait loci detection in a drought tolerant × susceptible common bean (*Phaseolus vulgaris L.*) cross. Mol. Breed. 29, 71–88. https://doi.org/10.1007/s11032-010-9527-9.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Borges, A., Tsai, S.M., Caldas, D.G.G., 2011. Validation of reference genes for RT-qPCR normalization in common bean during biotic and abiotic stresses. Plant Cell Rep. 31, 827–838. https://doi.org/10.1007/s00299-011-1204-x.

Briñez, B., Perseguini, J.M.K.C., Rosa, J.S., Bassi, D., Gonçalves, J.G.R., Almeida, C., Paulino, J.F.C., Blair, M.W., Chioratto, A.F., Carbonell, S.A.M., Valdisser, P.A.M.R., Vianello, R.P., Benchimol-Reis, L.L., 2017. Mapping QTLs for drought tolerance in a SEA 5 x AND 277 common bean cross with SSRs and SNP markers. Genet. Mol. Biol. 40 (4), 813–823. https://doi.org/10.1590/1678-4685-GMB-2016-0222.

Brito, L.C.N., Doolittle-Hall, J., Lee, C.T., 2020. The apical root canal system microbial communities determined by next generation sequencing. Sci. Rep. 10, 10932. https://doi.org/10.1038/s41598-020-67828-3.

Camargo, O.A., Moniz, A.C., Jorge, J.A., Valadares, J.M.A.S., 2009. Métodos De Análises química, Mineralógica e Física De Solos Do Instituto Agronômico De Campinas. Governo do Estado de São Paulo, p. 77. Boletim No. 106.

Carbonell, S.A.M., Chiorato, A.F., Bolonhezi, D., Barros, V.L.N.P., Borges, W.L.B., Ticelli, M., Gallo, P.B., Finoto, E.L., Santos, N.C.B., 2014. IAC Milênio" - common bean cultivar with high grain quality. Crop. Breed. Appl. Biotechnol. 14, 273–276.

Carvalho, M.C., Zuolo, M.L., Arruda-Vasconcelos, R., Marinho, A.C.S., Louzada, L.M., Francisco, P.A., Pecorari, V.G.A., Gomes, B.P.F.A., 2019. Effectiveness of XP-Endo Finisher in the reduction of bacterial load in oval-shaped root canals. Braz. Oral Res. 33, 1–8. https://doi.org/10.1590/1807-3107bor-2019.vol33.0021.

Caverzan, A., Casassola, A., Brammer, S.P., 2016. Antioxidant responses of wheat plants under stress. Genet. Mol. Biol. 39 (1), 1–6. https://doi.org/10.1590/1678-4685-GMB-2015-0109.

Chae, H.B., Bae, S.B., Paeng, S.K., Wi, S.D., Phan, K.A.T., Kim, M.G., Kim, W.Y., Yun, D. J., Lee, S.Y., 2023. The physiological role of thiol-based redox sensors in plant defense signaling. New. Phytol. 239 (4), 1203–1211. https://doi.org/10.1111/nph.19018.

Chai, M., Cheng, H., Yan, M., Priyadarshani, S., Zhang, M., He, Q., Huang, Y., Chen, F., Liu, L., Huang, X., Lai, L., Chen, H., Cai, H., Qin, Y., 2020. *Ananas comosus* identification and expression analysis of the DREB transcription factor family in pineapple. PeerJ. 8, 9006.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103 (4), 551–560. https://doi.org/10.1093/aob/mcn125.

Cheng, X., Case, L.B., 2023. Phase separation in chemical and mechanical signal transduction. Curr. Opin. Cell Biol. 85, 102243. https://doi.org/10.1016/j.ceb.2023.102243.

Cruz de Carvalho, M.H., 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal. Behav. 3 (3), 156–165. https://doi.org/10.4161/psb.3.3.5536.

De Vries, F.T., Griffiths, R.I., Knight, C.G., Nicolitch, O., Williams, A., 2020. Harnessing rhizosphere microbiomes for drought-resilient crop production. Science (1979) 368, 270–274. https://doi.org/10.1126/science.aaz5192.

Dorion, S., Ouellet, J.C., Rivoal, J., 2021. Glutathione metabolism in plants under stress: beyond reactive oxygen species detoxification. Metabolites. 11, 641. https://doi.org/10.3390/metabo11090641.

Du, Y., Shi, W.W., He, Y.X., Yang, Y.H., Zhou, C.Z., Chen, Y., 2011. Structures of the substrate-binding protein provide insights into the multiple compatible solute binding specificities of the *Bacillus subtilis* ABC transporter OpuC. Biochem. J. 436 (2), 283–289. https://doi.org/10.1042/BJ20102097.

Ejaz, S., Fahad, S., Anjum, M.A., Nawaz, A., Naz, S., Hussain, S., Ahmad, S., 2020. Role of osmolytes in the mechanisms of antioxidant defense of plants. In: Lichtfouse, E. (Ed.), Role of osmolytes in the mechanisms of antioxidant defense of plants. Sustain. Agric. Rev. Springer 95–117. https://doi.org/10.1007/978-3-030-38881-2_4.

Ewels, P., Magnusson, M., Lundin, S., Käller, M., 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics. 32 (19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354.

Ezzat, S.E., 2021. Effect of thiamine and biotin vitamins on growth and yield of smooth wheat *Triticum aestivum* L. Ann. Rom. Soc. Cell Biol. 25 (5), 5305–5313.

Feng, X., Enquist, B.J., Park, D.S., Boyle, B., Breshears, D.D., Gallagher, R.V., Lien, A., Newman, E.A., Burger, J.R., Maitner, B.S., 2022. A review of the heterogeneous landscape of biodiversity databases: opportunities and challenges for a synthesized biodiversity knowledge base. Glob. Ecol. Biogeogr: J. Macroe. 31, 1242–1260. https://doi.org/10.1111/geb.13497.

Francisco, C.S., Ma, X., Zwyssig, M.M., 2019. Morphological changes in response to environmental stresses in the fungal plant pathogen *Zymoseptoria tritici*. Sci. Rep. 9, 9642. https://doi.org/10.1038/s41598-019-45994-3.

Fuentes, A., Herrera, H., Charles, T.C., Arriagada, C., 2020. Fungal and bacterial microbiome associated with the rhizosphere of native plants from the Atacama Desert. Microorganisms. 8 (2), 209. https://doi.org/10.3390/microorganisms8020209.

Gallusci, P., Dai, Z., Genard, M., Gauffretau, A., Leblanc-Fournier, N., Richard-Molard, C., Vile, D., Brunel-Muguet, S., 2017. Epigenetics for plant improvement: current knowledge and modeling avenues. Trends. Plant Sci. 22 (7), 610–623. https://doi.org/10.1016/j.tplants.2017.04.009.

Gao, H., Huang, Z., Chen, W., Xing, A., Zhao, S., Wan, W., Hu, H., Li, H., 2024. Mild to moderate drought stress reinforces the role of functional microbiome in promoting growth of a dominant forage species (*Neopallasia pectinata*) in desert steppe. Front. Microbiol. 15, 1371208. https://doi.org/10.3389/fmicb.2024.1371208.

Gessler, A., Schaub, M., Mcdowell, N.G., 2017. The role of nutrients in drought-induced tree mortality and recovery. New. Phytol. 214, 513–520.

Gonçalves, J.G.R., Chiorato, A.F., Silva, D.A., Esteves, J.A.F., Bosetti, F., Carbonell, S.A. M., 2015. Combining ability in common bean cultivars under drought stress. Bragantia 74, 149–155. https://doi.org/10.1590/1678-4499.0345.

Guerra, J., Lourenço, L.C., 2018. The 2030 Agenda: trends of transition toward sustainability. In: Delicado, A., Domingos, N., Sousa, L. (Eds.), The 2030 Agenda: trends of transition toward sustainability. Div. Worlds Sustain. Impr. Ciênc. Soc. 54–85. Lisboa.

Guo, Y., Song, B., Li, A., Wu, Q., Huang, H., Li, N., 2022. Higher pH is associated with enhanced co-occurrence network complexity, stability and nutrient cycling functions in the rice rhizosphere microbiome. Environ. Microbiol. 24, 6200–6219. https://doi.org/10.1111/1462-2920.16185.

Hernández, I., Alegre, L., Munné-Bosch, S., 2004. Drought-induced changes in flavonoids and other low molecular weight antioxidants in *Cistus clusii* grown under mediterranean field conditions. Tree Physiol. 24, 1303–1311. https://doi.org/10.1093/treephys/24.11.1303.

Hoffmann, T., Bremer, E., 2017. Guardians in a stressful world: the Opu family of compatible solute transporters from *Bacillus subtilis*. Biol. Chem. 398 (2), 193–214. https://doi.org/10.1515/hsz-2016-0265.

Ibrahim, H.A., Abdellatif, Y.M.R., 2016. Effect of maltose and trehalose on growth, yield and some biochemical components of wheat plant under water stress. Ann. Agric. Sci. 61 (2), 267–274. https://doi.org/10.1016/j.aoas.2016.05.002.

Kaur, H., Kohli, S.K., Khanna, K., Bhardwaj, R., 2021. Scrutinizing the impact of water deficit in plants: transcriptional regulation, signaling, photosynthetic efficacy, and management. Physiol. Plant 172, 935–962. https://doi.org/10.1111/ppl.13389.

Kavamura, V.N., Taketani, R.G., Ferreira, C., Melo, I.S., de Mendes, R., 2018. The role of species turnover in structuring bacterial communities in a local scale in the cactus rhizosphere. Plant Soil. 425, 101–112. https://doi.org/10.1007/s11104-018-3570-4.

Kumar, A., Verma, J.P., 2018. Does plant-microbe interaction confer stress tolerance in plants: a review? Microbiol. Res. 207, 41–52. https://doi.org/10.1016/j.micres.2017.11.004.

Lai, X., Bazin, J., Webb, S., Crespi, M., Zubieta, C., Conn, S.J., 2018. CircRNAs in plants. Adv. Exp. Med. Biol. 1087, 329–343. https://doi.org/10.1007/978-981-13-1426-1_26.

Lanna, A.C., Silva, R.A., Ferraresi, T.M., Mendonça, J.A., Coelho, G.R.C., Moreira, A.S., Valdisser, P.A.M.R., Brondani, C., Vianello, R.P., 2016. Physiological characterization of common bean (*Phaseolus vulgaris L.*) genotypes, water-stress induced with contrasting response towards drought. Aust. J. Crop. Sci. 10, 1–6. https://doi.org/10.1007/s11356-018-3012-0.

Larcher, W., 2000. Temperature stress and survival ability of Mediterranean sclerophyllous plants. Plant Biosyst. 134, 279–295. https://doi.org/10.1080/11263500012331350455.

Lazcano, C., Boyd, E., Holmes, G., Hewavitharana, S., Pasulka, A., Ivors, K., 2021. The rhizosphere microbiome plays a role in the resistance to soil-borne pathogens and nutrient uptake of strawberry cultivars under field conditions. Sci. Rep. 11, 3188. https://doi.org/10.1038/s41598-021-82768-2.

Lesk, C., Rowhani, P., Ramankutty, N., 2016. Influence of extreme weather disasters on global crop production. Nature 529, 84–87. https://doi.org/10.1038/nature16467.

Li, S., Yang, L., Huang, X., Zou, Z., Zhang, M., Guo, W., Addo-Danso, S.D., Zhou, L., 2023. Mineral nutrient uptake, accumulation, and distribution in *Cunninghamia lanceolata* in response to drought stress. Plants (Basel) 29 (12), 2140. https://doi.org/10.3390/plants12112140, 11.

Lisar, S.Y., Motafakkerazad, R., Hossain, M.M., Rahman, I.M.M., 2012. Water stress in plants: causes, effects and responses. In: Rahman, I.Md.M., Hasegawa, H. (Eds.), Water stress in plants: causes, effects and responses. Water stress. InTech Open 1–38. https://doi.org/10.5772/39363.

Liu, Y.X., Qin, Y., Chen, T., Lu, M., Qian, X., Guo, X., Bai, Y., 2021. A practical guide to amplicon and metagenomic analysis of microbiome data. Protein Cell 12 (5), 315–330. https://doi.org/10.1007/s13238-020-00724-8.

Maghsoudi, K., Emam, Y., Niazi, A., Pessarakli, M., Arvin, M.J., 2018. P5CS expression level and proline accumulation in the sensitive and tolerant wheat cultivars under control and drought stress conditions in the presence/absence of silicon and salicylic acid. J. Plant Interact. 13 (1), 461–471. https://doi.org/10.1080/17429145.2018.1506516.

Maia, P.S.P., Neto, C.F.O., Castro, D.S., Freitas, J.M.N., Lobato, A.K.S., Costa, R.C.L., 2007. Relative water content, proline content and total soluble carbohydrates in leaves of two corn cultivars subjected to water stress. Rev. Bras. Biociênc. 5, 918–920.

Malavolta, E., Vitti, G.C., Oliveira, S.A., 1997. Avaliação Do Estado Nutricional Das plantas: Princípios e Aplicações. POTAFOS, Piracicaba.

Martineau, E., Domec, J.C., Bosc, A., Denoroy, P., Fandino, V.A., Lavres, J., Meille, L.J., 2017. The effects of potassium nutrition on water use in field-grown maize (*Zea mays L.*). Environ. Exp. Bot. 134, 62–71. https://doi.org/10.1016/j.envexpbot.2016.11.004.

Martínez-Espinosa, R.M., 2020. Microorganisms and their metabolic capabilities in the context of the biogeochemical nitrogen cycle at extreme environments. Int. J. Mol. Sci. 21 (12), 4228. https://doi.org/10.3390/ijms21124228.

Mauad, M., Garcia, R.A., Silva, R.M.M.F., Silva, T.A.F., Schroeder, I.M., Knudsen, C.H., Quaresma, E.V.W.Q., 2015. Shoot dry matter and shoot macronutrient accumulation in Niger. Rev. Bras. Ciênc. Solo. 39 (2). https://doi.org/10.1590/01000683rbcs20140391.

Mendes, L.W., Braga, L.P.P., Navarrete, A.A., Souza, D.G., Silva, G.G.Z., Tsai, S.M., 2017. Using metagenomics to connect microbial community biodiversity and functions. Curr. Issues. Mol. Biol. 24, 103–118. https://doi.org/10.21775/cimb.024.103.

Mendes, L.W., Raaijmakers, J.M., de Hollander, M., Mendes, R., Tsai, S.M., 2018. Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. ISMe J. 12, 212–224. https://doi.org/10.1038/ismej.2017.158.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A., Raaijmakers, J.M., 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science (1979) 27 (332), 1097–1100. https://doi.org/10.1126/science.1203980, 6033.

Miyashita, K., Tanakamaru, S., Maitani, T., Kimura, K., 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ. Exp. Bot. 53, 205–214. https://doi.org/10.1016/j.envexpbot.2004.03.015.

Møller, I.M., Jensen, P.E., Hansson, A., 2007. Oxidative modifications to cellular components in plants. Annu Rev. Plant Biol. 58, 459–481. https://doi.org/10.1146/annurev.arplant.58.032806.103946.

Monakhova, O.F., Cherniad'ev, I.I., 2002. Protective role of kartolin-4 in soil drought in wheat plants. Prikl. Biokhim. Mikrobiol. 38, 433–440.

Naylor, D., Coleman-Derr, D., 2018. Drought stress and root-associated bacterial communities. Front. Plant Sci. 9 (8), 2223. https://doi.org/10.3389/fpls.2017.02223.

Oksanen, J., Kindt, R., Legendre, P., 2007. The vegan package. Community Ecol. Packag. 10, 631–637.

Oliveira, J.S.S., Santos, G.S., Moraes, J.A., Saliba, A.M., Barja-Fidalgo, T.C., Mattos-Guaraldi, A.L., Nagao, P.E., 2015. Reactive oxygen species generation mediated by NADPH oxidase and PI3K/akt pathways contribute to invasion of *Streptococcus agalactiae* in human endothelial cells. Mem. Inst. Oswaldo Cruz. 113 (6), 140421. https://doi.org/10.1590/0074-02760170421.

Oliveira, M.A.J., Bovi, M.L.A., Machado, E.C., Gomes, M.M.A., Habermann, G., Rodrigues, J.D., 2002. Fotossíntese, condutância estomática e transpiração em pupunheira sob deficiência hídrica. Sci. Agric. 59 (1), 59–63. https://doi.org/10.1590/S0103-90162002000100008.

Padhi, Y., Chatterjee, S., 2023. XdfA, a novel membrane-associated DedA family protein of *Xanthomonas campestris*, is required for optimum virulence, maintenance of magnesium, and membrane homeostasis. mBio 14 (4), 0136123. https://doi.org/10.1128/mbio.01361-23.

Pantin, F., Simonneau, T., Rolland, G., Dauzat, M., Muller, B., 2011. Control of leaf expansion: a developmental switch from metabolics to hydraulics. Plant Physiol. 156 (2), 803–815. https://doi.org/10.1104/pp.111.176289.

Paraizo, E.A., David, A.M.S.S., Conceição, E.R.S., Silva, R.R.P., Silva, C.D., Nobre, D.A.C., 2021. Tiamina como atenuador do estresse salino em sementes de arroz. Agron. Crop J. 30, 123–135. https://doi.org/10.32929/2446-8355.2021v30n2p123-135.

Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 30 (21), 3123–3124. https://doi.org/10.1093/bioinformatics/btu494.

Pereira, M.J.Z., Ramalho, M.A.P., Abreu, A.F.B., 2011. Reação de linhagens de feijoeiro ao fungo *Fusarium oxysporum* f. sp. phaseoli em condições controladas. Cienc. Agrotec 35 (5), 940–947. https://doi.org/10.1590/S1413-70542011000500011.

Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic. Acids. Res. 30 (9), 36. https://doi.org/10.1093/nar/30.9.e36.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nat. Ver. Microbiol. 11, 789–799. https://doi.org/10.1038/nrmicro3109.

Polania, J., Rao, I.M., Cajiao, C., Grajales, M., Rivera, M., Velasquez, F., Raatz, B., Beebe, S.E., 2017. Shoot and root traits contribute to drought resistance in recombinant inbred lines of MD 23–24 × SEA 5 of common bean. Front. Plant Sci. 8, 296. https://doi.org/10.3389/fpls.2017.00296.

Polania, J., Rao, I.M., Cajiao, C., Rivera, M., Raatz, B., Beebe, S.E., 2016. Physiological traits associated with drought resistance in Andean and Mesoamerican genotypes of common bean (*Phaseolus vulgaris L.*). Euphytica 210, 17–29. https://doi.org/10.1007/s10681-016-1691-5.

Poudel, M., Mendes, R., Costa, L.A.S., Bueno, C.G., Meng, Y., Folimonova, S.Y., Garrett, K.A., Martins, S.J., 2021. The role of plant-associated bacteria, fungi, and viruses in drought stress mitigation. Front. Microbiol. 12, 743512. https://doi.org/10.3389/fmicb.2021.743512.

Putpeerawit, P., Sojikul, P., Thitamadee, S., Narangajavana, J., 2017. Genome-wide analysis of aquaporin gene family and their responses to water-deficit stress conditions in cassava. Plant Physiol. Biochem. 121, 118–127. https://doi.org/10.1016/j.plaphy.2017.10.025.

Recchia, G.H., Konzen, E.R., Cassieri, F., Caldas, D.G.G., Tsai, S.M., 2018. Arbuscular mycorrhizal symbiosis leads to differential regulation of drought-responsive genes in tissue-specific root cells of common bean. Front. Microbiol. https://doi.org/10.3389/fmicb.2018.01339.

Ribeiro, N.D., Mezzomo, H.C., Santos, G.G., 2019. Genetic parameters and combined selection for seed coat color and macrominerals in Mesoamerican common bean lines. Genet. Mol. Res. 18 (2), 1–14. https://doi.org/10.4238/gmr18224.

Rosales-Serna, R., Koshashi, J., Acosta-Gallegos, J.A., Trejo-Lõpeza, C., Ortiz-Cereceres, J., Kelly, J.D., 2004. Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. Field. Crops. Res. 85 (2-3), 203–211. https://doi.org/10.1016/S0378-4290(03)00161-8.

Ruijter, J.M., Lorenz, P., Tuomi, J.M., 2014. Fluorescent increase kinetics of different fluorescent reporters used for qPCR depends on monitoring chemistry, targeted sequence, type of DNA input and PCR efficiency. Microchim. Acta. https://doi.org/10.1007/s00604-013-1155-8.

Ruijter, J.M., Pfaffl, M.W., Zhao, S., Spiess, A.N., Boggy, G., Blom, J., Rutledge, R.G., Sisti, D., Lievens, A., De Preter, K., Derveaux, S., Hellemans, J., Vandesompele, J., 2013. Evaluation of qPCR curve analysis methods for reliable biomarker discovery: bias, resolution, precision, and implications. Methods 59, 32–46. https://doi.org/10.1016/j.ymeth.2012.08.011.

Ruijter, J.M., Ramakers, C., Hoogaars, W.M., Karlen, Y., Bakker, O., van den Hoff, M.J., Moorman, A.F., 2009. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res. 37, 45. https://doi.org/10.1093/nar/gkp045.

Sankar, B., Jaleel, C.A., Manivannan, P., Kishorekumar, A., Somasundaram, R., Panneerselvam, R., 2007. Effect of paclobutrazol on water stress amelioration through antioxidants and free radical scavenging enzymes in Arachis hypogaea L. Colloids. Surf. B Biointerfaces. 60, 229–235. https://doi.org/10.1016/j.colsurfb.2007.06.016.

Schwarz, S., Van Dikck, P., 2016. Trehalose metabolism: a sweet spot for *Bulkholderia pseudomallei* virulence. Virulence 8, 5–7. https://doi.org/10.1080/21505594.2016.1216295.

Seleiman, M.F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H.H., Battaglia, M.L., 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants (Basel) 10 (2), 259. https://doi.org/10.3390/plants10020259.

Silva, G.R., Silva Jr, M.L., Melo, V.S., 2006. Effect of land use and management on chemical properties of a yellow latosol in Pará State. Brazil. Acta Amazon. 36 (2), 151–158. https://doi.org/10.1590/S0044-59672006000200004.

Taiz, L., Zeiger, E., 2004. Fisiologia Vegetal, 3. ed. Artmed.

Tie, Z., Wang, P., Chen, W., Tang, B., Yu, Y., Liu, Z., Zhao, S., Khan, F.H., Zhang, X., Xi, H., 2023. Different responses of the rhizosphere microbiome to *verticillium dahliae* infection in two cotton cultivars. Front. Microbiol. 14, 1229454. https://doi.org/10.3389/fmicb.2023.1229454.

Tondo, M.L., de Pedro-Jové, R., Vandecaveye, A., Piskulic, L., Orellano, E.G., Valls, M., 2020. *kat*E from the bacterial plant pathogen *Ralstonia solanacearum* is a monofunctional catalase controlled by HrpG that plays a major role in bacterial survival to hydrogen peroxide. Front. Plant Sci. 11, 1156. https://doi.org/10.3389/fpls.2020.01156.

Upadhyay, S.K., Srivastava, A.K., Rajput, V.D., Chauhan, P.K., Bhojiya, A.A., Jain, D., Chaubey, G., Dwivedi, P., Sharma, B., Minkina, T., 2022. Root exudates: mechanistic insight of plant growth promoting rhizobacteria for sustainable crop production. Front. Microbiol. 13, 916488. https://doi.org/10.3389/fmicb.2022.916488.

Vendruscolo, E.P., Rodrigues, A.H.A., Oliveira, P.R., Leitão, R.A., Campos, L.F.C., Seleguini, A., Lima, S.F., 2020. Exogenous application of thiamine on upland rice summited to water déficit. Rev. Ciênc. Agroveterin. 19 (1), 48–53. https://doi.org/10.5965/223811711912020048.

Venturi, V., Keel, C., 2016. Signaling in the rhizosphere. Trends. Plant Sci. 21, 187–198. https://doi.org/10.1016/j.tplants.2016.01.005.

Xue, C.X., Lin, H., Zhu, X.Y., Liu, J., Zhang, Y., Rowley, G., Todd, J.D., Li, M., Zhang, X. H., 2021. DiTing: a pipeline to infer and compare biogeochemical pathways from metagenomic and metatranscriptomic data. Front. Microbiol. 12, 698286. https://doi.org/10.3389/fmicb.2021.698286.

Yang, H., Sun, Y., Wang, H., 2021. Genome-wide identification and functional analysis of the ERF2 gene family in response to disease resistance against *stemphylium lycopersici* in tomato. BMC. Plant Biol. 21, 72. https://doi.org/10.1186/s12870-021-02848-3.

Yang, Y., Tang, M., Sulpice, R., 2014. Arbuscular mycorrhizal fungi alter fractal dimension characteristics of *Robinia pseudoacacia* L. seedlings through regulating plant growth, leaf water status, photosynthesis, and nutrient concentration under drought stress. J. Plant Growth Regul. 33, 612–625. https://doi.org/10.1007/s00344-013-9410-0.

Ye, J.Y., Tian, W.H., Jin, C.W., 2022. Nitrogen in plants: from nutrition to the modulation of abiotic stress adaptation. Stress. Biol. 2 (4). https://doi.org/10.1007/s44154-021-00030-1.

Zhang, L., Sun, W., Gao, W., 2024. Genome-wide identification and analysis of the *GGCT* gene family in wheat. BMC. Genomics. 25, 32. https://doi.org/10.1186/s12864-023-09934-w.

Zhou, R., Kozlov, A.G., Roy, R., Zhang, J., Korolev, S., Lohman, T.M., Ha, T., 2011. SSB functions as a sliding platform that migrates on DNA via reptation. Cell 146, 222–232. https://doi.org/10.1016/j.cell.2011.06.036.

Zupin, M., Sedlar, A., Kidrič, M., Meglič, V., 2017. Drought-induced expression of aquaporin genes in leaves of two common bean cultivars differing in tolerance to drought stress. J. Plant Res. 130 (4), 735–745. https://doi.org/10.1007/s10265-017-0920-x.