



Evaluation of the Plant Growth-Promoting Microorganisms Potential for Drought Alleviation in Wheat Plants

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Abstract

One of the main abiotic stresses that threaten the survival of wheat (*Triticum* spp.) is drought. The present work aims to evaluate the ability of different plant growth promoting microorganisms (PGPMs) to alleviate the effects of dehydration in wheat and to understand the mechanisms involved. Wheat seeds (*Triticum aestivum* L. ssp. cv. ‘Galera’) inoculated with the strains *Priestia megaterium* CD_FICOS_01, *Peribacillus frigoritolerans* CD_FICOS_02, *Pseudomonas canadensis* CD_FICOS_03, *Azotobacter chroococcum* CD_FICOS_04 or *Pichia kluyveri* CD_FICOS_05 were exposed to prolonged drought by water shortage after which agronomic parameters, biochemical markers of abiotic stress and expression of genes related to plant response to water stress were measured. The behaviour of the PGPM strains evaluated varied in alleviating plant water stress, allowing two groups to be differentiated. The application of strains CD_FICOS_01, CD_FICOS_02 and CD_FICOS_03 resulted in an enhancement of crop production, with increases in aerial fresh biomass of 59%, 18%, and 38%, respectively, compared to the control. These strains also stimulated ethylene and/or abscisic acid metabolism. In contrast, strains CD_FICOS_04 and CD_FICOS_05 were also able to promote mechanisms related to the plant response to abiotic stress (MDA, proline, H₂O₂), but did not exert any physiological effect at plant level. In fact, they reduced the yield, with decreases in aerial fresh biomass of 43% and 21%, and in aerial dry biomass of 33% and 20%, respectively.

Keywords ABA · Abiotic stress · Drought response genes · PGPR · Proline · Wheat

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Introduction

Drought is considered to be one of the main abiotic stressors limiting agricultural productivity, and the frequency of droughts could increase in the coming years. Drought causes soil water stress for vegetation, which determines various morphological, physiological and biochemical consequences for agriculturally relevant plant species (Berg and Sheffield 2018).

For wheat (*Triticum* spp.), one of the most widely cultivated crops in the world, soil moisture deficit is associated with a disruption of normal plant growth through reduced nutrient assimilation, mobilisation and accumulation and a decrease in relative water content (Nyaupane et al. 2024; Sjulgård et al. 2025). This results in delayed seed germination, reduced seed and seedling vigour, reduced leaf area, accelerated leaf senescence and a decrease in dry aerial weight, plant height, number of tillers and leaves per plant (Ali et al. 2019). However, the root is not as affected as the

upper part as it expands in search of water (Ali et al. 2019). Drought also affects reproduction by altering gametogenesis, fertilisation, embryogenesis and endosperm development. Photosynthesis is also negatively influenced by water stress by decreasing chlorophyll content and slowing electron transport in photosystem II (Nyaupane et al. 2024). An inefficient electron transport chain results in increased production of reactive oxygen species (ROS) which include non-radical molecules such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$) and free radicals such as hydroxyl radical ($\text{OH}^\cdot$) and superoxide anion ($\text{O}_2^{\cdot-}$). These cause lipid peroxidation, mutations and fragmentation of peptide chains, oxidation, breakage and mutations in DNA, and ultimately lead to programmed cell death (Ahmad et al. 2018; Li et al. 2022).

On the other hand, the plant has adaptive mechanisms that allow it to cope with drought stress. ROS also act as messengers that trigger the expression of drought resistance genes such as those coding for antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, peroxidase, catalase and glutathione peroxidase. These enzymes act by neutralising or scavenging ROS thereby reducing oxidative damage (Ahmad et al. 2018; Nyaupane et al. 2024). Osmotic imbalance resulting from water deficit is counteracted by the accumulation of organic and inorganic solutes in the cytosol that cause a decrease in osmotic potential and maintain cell turgor. These are solutes such as glycine betaine, sugars, polyols and amino acids, with a prominent role for proline (Sallam et al. 2019). Plant hormones play an important regulatory role in increasing tolerance to water stress. Auxins, cytokinins and gibberellins mainly control cell differentiation and elongation by positively regulating plant development and growth (Abhinandan et al. 2018; Li et al. 2023; Wahab et al. 2022). However, their activity is normally suppressed in stressful conditions while resources are allocated to key processes as part of a strategy to alleviate the damage experienced (Abhinandan et al. 2018). At the same time, plant sensitivity to abscisic acid (ABA) which acts as a central regulator of abiotic stress tolerance in wheat is increased (Abhinandan et al. 2018; Zulfiqar et al. 2024). Signalling mediated by this phytohormone causes stomata closure to prevent water loss through transpiration which also has its negative effects on photosynthetic rate (Wahab et al. 2022). In addition, it stimulates the expression of stress response genes leading to the production of late embryogenesis abundant proteins and dehydrins and stimulates the synthesis of antioxidant enzymes and osmoprotectants such as the aforementioned proline (Chaves et al. 2003; Raza et al. 2019; Wahab et al. 2022). Exogenous application of salicylic acid also stimulates proline production leading to preservation of photosynthetic activity in wheat (Abhinandan et al. 2018; Talaat 2023). It also enhances the ROS-scavenging

system by promoting the production of the antioxidants glutathione and ascorbate, regulates transpiration rate, stomata movement and reduces membrane electrolyte leakage and lipid peroxidation (Abhinandan et al. 2018; Talaat 2023; Wahab et al. 2022). In contrast, the gaseous hormone ethylene can antagonise the regulatory effect of ABA under drought conditions by influencing stomata closure and grain filling (Abhinandan et al. 2018). Jasmonic acid, brassinosteroids and strigolactones play a positive role in response to water stress, but is still poorly understood in wheat (Abhinandan et al. 2018; Talaat 2023).

In recent years, these natural drought adaptation mechanisms have been enhanced mainly through two anthropogenic strategies such as conventional breeding or its variants like Mutation Breeding and Marker-Assisted Breeding and the transgenic approach (Gontia-Mishra et al. 2020; Srivastava 2019; Wang et al. 2025). However, crop improvement for abiotically stressed environments is limited by aspects such as its long duration and cost, the high degree of variability of selection environments under stress that vary from year to year and the large size of the wheat genome that hinders the breeding process by any genetic method (Srivastava 2019). In addition, the low frequency of transformation, the possibility of transgene silencing with consequent reduction of transgene expression and its possible negative effects on grain yield in the absence of stress together with ethical issues and restrictions on the subject in some parts of the world limit the use of genetic engineering (Srivastava 2019).

A cost-effective and eco-friendly alternative or complementary tool to these strategies is the use of plant growth-promoting microorganisms (PGPM), such as plant-growth promoting rhizobacteria (PGPR), actinomycetes and mycorrhiza (Aljeddani et al. 2024; Gontia-Mishra et al. 2020; Hanaka et al. 2021; Lastochkina et al. 2019). These microorganisms are able to mitigate the effects of drought on the plant by several mechanisms including regulation of phytohormonal activity, 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activity, accumulation of osmolytes, volatile compounds, antioxidant defence, exopolysaccharide production and a morphological change of the root (Lastochkina et al. 2019; Zhang et al. 2025). In the case of wheat, there are numerous studies reporting the ability of bacteria of the genera *Azospirillum*, *Bacillus*, *Paeinibacillus*, *Burkholderia*, *Ochrobactrum*, *Pseudomonas*, *Herbaspirillum*, *Rhizobium*, *Mesorhizobium*, *Burkholderia*, *Azotobacter*, *Arthrobacter* and *Dietzia* to stimulate plant tolerance to drought (Bouremani et al. 2024; Lastochkina et al. 2019; Salem et al. 2024). In this context, the objectives of this work were: (i) to comparatively evaluate the potential of four bacterial strains belonging to four different genera (*Azotobacter*, *Pseudomonas*, *Peribacillus* and *Priestia*) and

one fungal strain (*Pichia*) to enhance drought stress tolerance in wheat, measuring the agronomic response of plants under drought conditions inoculated with the microbes; (ii) to evaluate the mechanisms underlying the tolerance promoted by these strains. The evaluation of such mechanisms was carried out by measuring changes in biochemical markers of abiotic stress and in the expression profiles of genes related to plant response to water stress.

Materials and Methods

Bacterial Strains and Growth Conditions

Priestia megaterium strain CD_FICOS_01 and *Peribacillus frigoritolerans* strain CD_FICOS_02 were isolated from soil samples of irrigated pepper crops in Zamora (Spain), *Pseudomonas canadensis* strain CD_FICOS_03 and *Azotobacter chroococcum* strain CD_FICOS_04 were isolated from soil samples of citrus crops located in the Valencian Community (Spain) and *Pichia kluyveri* strain CD_FICOS_05 was isolated from the surface of grapes grown in León (Spain). The strains were selected for their different in-vitro and in-planta PGPM properties, as listed in Table 2, in previous assays among a broader battery of promising PGP bacteria. CD_FICOS_02, CD_FICOS_03 and CD_FICOS_04 were also evaluated for their ability to protect tomato plants against *Botrytis cinerea* (Mazuecos-Aguilera et al. 2025). However, strains CD_FICOS_01 and CD_FICOS_05 are cited for the first time in this work and are registered in the Spanish Type Culture Collection (Colección Española de Cultivos Tipo, CECT) by Ficosteria S.L. for patent purposes (CECT no. 30922, CD_FICOS_01; CECT no. 13224, CD_FICOS_05).

Taxonomic identification of the strains CD_FICOS_01 and CD_FICOS_05 involved amplification and sequencing of the 16 S rRNA gene by Macrogen (The Netherlands). The obtained sequences were processed and deposited at GenBank (accession no. PQ061798.1, *Priestia megaterium* CD_FICOS_01; accession no. PQ757234.1, *Pichia kluyveri* CD_FICOS_05) and compared with those from the EzTaxon-e server (<https://www.ezbiocloud.net/>; Chalita et al. 2024).

Strains CD_FICOS_01, CD_FICOS_02, CD_FICOS_03 and CD_FICOS_05 were routinely maintained on Tryptic Soy Agar (TSA) medium (Sigma-Aldrich, Madrid, Spain) while strain CD_FICOS_04 was maintained on Ashby-mannitol agar (5 g.L⁻¹ Mannitol, 0.2 g.L⁻¹ K₂HPO₄, 0.2 g.L⁻¹ MgSO₄·7H₂O, 0.2 g.L⁻¹ NaCl, 0.1 g.L⁻¹ CaSO₄, 0.1 g.L⁻¹ CaCO₃, 15 g.L⁻¹ agar).

Determination of PGP Traits

A range of assays was conducted for the five strains included in the study to assess their plant growth-promoting properties. Every assay was performed using three independent biological replicates. For plate-based methods, each biological replicate was further evaluated using three separate colony measurements. The dataset available for this study consisted of the final consolidated values for each isolate.

Siderophore Production

Siderophore production was determined by assessing the formation and size of an orange halo on chrome azurol S (CAS) agar medium after inoculation with cells of the strains and incubation at 28 °C for 72 h (Alexander and Zuberer 1991; Schwyn and Neilands 1987). The siderophore production index (SPI) was estimated by dividing the sum of colony diameter and orange halo diameter by colony diameter (Abirammi et al. 2018).

P Solubilisation

Qualitative measurement of inorganic phosphate solubilisation capacity was assessed on YED-P medium (2 g.L⁻¹ yeast extract, 10 g.L⁻¹ glucose, 4 g.L⁻¹ Ca₃(PO₄)₂ as the insoluble phosphorus source, 12 g.L⁻¹ agar) (Peix et al. 2004). Isolates were grown on YED-P medium and incubated at 28 °C for 5–7 days. The formation of a transparent halo in the culture medium around each colony was assessed to indicate the solubilisation of phosphate present in the medium. The phosphate solubilisation rate was calculated by measuring colony diameter and halo diameter using the formula of Edi-Premono et al. (1996).

In those strains that showed a solubilization halo in the qualitative analysis, quantitative measurement was performed according to Murphy and Riley (1962). For this purpose, 160 µL of inoculum from each strain (1.8·10⁸ CFU.mL⁻¹) were inoculated into 40 mL of liquid NBRIP medium (10 g.L⁻¹ glucose, 5 g.L⁻¹ MgCl₂·6H₂O, 0.25 g.L⁻¹ MgSO₄·7H₂O, 0.2 g.L⁻¹ KCl, 0.1 g.L⁻¹ (NH₄)₂SO₄, 5 g.L⁻¹ Ca₃(PO₄)₂) at pH 7, in 250 mL flasks, and incubated at 30 °C at 120 rpm for 7 days. Every 24 h, 1.5 mL was extracted from each culture and centrifuged at 8000x g for 15 min. 1 mL of the supernatant was mixed with 9 mL of distilled water to estimate the soluble phosphate content using the molybdenum blue colorimetric method. To 10 mL of the mixture, 2 mL of reagent (10:3:6:1 sulfuric acid solution: ammonium molybdate solution: ascorbic acid solution: potassium antimonyl tartrate solution mixture (v/v) were added, and after standing for 10 min in the dark, the absorbance was measured at 882 nm. Molybdenum blue binds

to soluble phosphorus, forming phosphomolybdenum blue, which causes a measurable colour change at this wavelength. The solubilized phosphorus content was determined using a standard curve.

K Solubilisation

The solubilisation capacity of inorganic potassium was evaluated qualitatively in modified Aleksandrov medium (5 g.L⁻¹ glucose, 0.5 g.L⁻¹ MgSO₄·7H₂O, 0.005 g.L⁻¹ FeCl₃, 2 g.L⁻¹ Ca₃(PO₄)₂, 2 g.L⁻¹ AlK₂O₆Si₂, 15 g.L⁻¹ Agar, 0.1 g.L⁻¹ CaCO₃ and bromothymol blue (20 mL from a stock 50 mg/10 mL ethanol 70%) (Rajawat et al. 2016). Mica (potassium aluminium silicate) was used as a source of insoluble potassium. This method involves direct visualisation of a yellow halo around the potassium solubilising colonies on the plates by including bromothymol blue as an acid-base indicator and incubating the plates at 28 °C for 48–72 h. The potassium solubilisation index is based on the size and intensity of the yellow shift.

IAA Production and ACC Deaminase Activity

Auxin synthesis capacity was determined according to Khalid et al. (2004). For each isolate, 500 µL of a 3·10⁸ CFU.mL⁻¹ suspension in sterile saline, obtained from bacterial growth on Petri dishes with TSA medium, was transferred to a test tube containing 4 mL of Tryptic Soy Broth (TSB) liquid medium (Sigma-Aldrich, Madrid, Spain) and incubated for 3 days in the dark with shaking at 28 °C. Subsequently, 500 µL of the culture were transferred to test tubes with 4 mL of JMM medium (O'Hara et al. 1989) supplemented with 0.167 g.L⁻¹ L-tryptophan and incubated for a further 4 days in the dark with shaking at 28 °C. After incubation, bacterial concentration was estimated by measuring the optical density at 600 nm. Then, 1.5 mL from each test tube were centrifuged at 5000x g for 10 min. 1 mL of the supernatant was mixed with 1 mL of Salkowski reagent (0.01 M FeCl₃, 34.3% (v/v) perchloric acid). The mixture was allowed to stand for 30 min, and the optical density was measured at 535 nm. Auxin production was determined by quantifying IAA equivalents in µg.mL⁻¹, by comparison with a standard curve of known IAA concentrations.

The ACC deaminase activity of the isolates was assessed according to Penrose and Glick (2003), consisting of three parts: bacterial biomass production, ACC deaminase activity measurement, and protein concentration measurement.

For bacterial biomass production, strains were cultured in Petri dishes with DF salts medium (Dworkin and Foster 1958), using 0.5 M ACC as the sole nitrogen source, and incubated for 2 days at 28 °C. Strains that did not grow in this medium lacked ACC deaminase activity, while the

bacterial mass of those that did grow was subsequently cultured in TSB and incubated overnight at 28 °C and 200 rpm.

The bacterial mass was centrifuged at 8000x g at 4 °C, the supernatant was discarded, and the pellet was resuspended in 7.5 mL of liquid DF salts minimal medium. 45 µL of 0.5 M ACC were added, and the mixture was incubated overnight at 28 °C and 200 rpm. After this time, the cells were washed three times with 0.1 M Tris-HCl (pH 7.6) and centrifuged successively at 8000x g at 4 °C. The bacterial mass was stored at -20 °C.

The ACC deaminase activity of each strain was measured according to Honma and Shimomura (1978), which indirectly measures the enzyme's activity by measuring the production of α-ketobutyrate, a product of the enzyme's hydrolytic activity on ACC.

Each bacterial biomass obtained using the above procedure was resuspended in 1 mL of 0.1 M Tris-HCl (pH 7.6) and centrifuged at 16000x g for 5 min. The pellet was resuspended in 600 µL of 0.1 M Tris-HCl (pH 8.5), 30 µL of toluene were added, and an aliquot was stored at 4 °C for subsequent protein activity determination.

200 µL of the toluened cells were mixed with 20 µL of 0.5 M ACC, and after incubation at 30 °C for 15 min, the reaction was stopped with 1 mL of 0.56 M HCl. After centrifugation for 5 min at 16000x g at room temperature, 1 mL of the supernatant was transferred to a test tube, along with 800 µL of 0.56 M HCl and 300 µL of 2,4-dinitrophenylhydrazine (0.2% in 2 M HCl). The mixture was incubated at 30 °C for 30 min, 2 mL of 2 N NaOH were added, and the amount of α-ketobutyrate produced (in µmol) was determined by comparing the absorbance at 540 nm of the sample with an α-ketobutyrate standard curve (Sigma-Aldrich, Madrid, Spain).

Protein concentration was determined using the Bio-Rad Protein Assay kit (Hercules, CA, USA), based on the Bradford method (1976), which quantifies protein by measuring the colour change of Coomassie Brilliant Blue G-250 reagent at 595 nm compared to a bovine serum albumin standard curve. Aliquots of toluened cells stored at 4 °C were used for the analysis. 26.5 µL of these cells were mixed with 173.5 µL of 0.1 M Tris-HCl (pH 8) and 200 µL of 0.1 N NaOH. The mixture was shaken, boiled for 10 min, cooled, and mixed again. 2 mL of the previously diluted dye and 40 µL of the sample were added to a test tube, the tube was shaken, and the absorbance was measured.

Experimental Design with Wheat Plants

The wheat (*Triticum aestivum* L.) cultivar used in the experiments was 'Galera'. Seeds were disinfected with 70% (v/v) ethanol for 1 min and 20% (v/v) bleach for 5 min and pregerminated by incubation at 26 °C in the dark in a humidified

Petri dish for 48 h. The pregerminated seeds were planted in 1 L pots containing a mixture of washed sterile vermiculite and sterile soil (1:3) as a substrate and grown in a phytotron with a photoperiod of 16 h of light at 23 °C and 8 h of darkness at 15 °C. Before planting the pregerminated seeds, the pots of all treatments were watered with 200 mL of distilled water.

The treatments consisted of the independent inoculation of the four bacterial strains and one fungal strain (CD_FICOS_01, CD_FICOS_02, CD_FICOS_03, CD_FICOS_04 and CD_FICOS_05) in wheat plants exposed to drought water shortage, in order to evaluate the microbial protective activity by comparison with two controls consisting respectively of uninoculated and stressed (USC) and uninoculated and non-stressed (UNSC) wheat plants. The experimental design was completely randomised and each treatment consisted of eleven pots each containing five wheat plants.

Microbial inoculation was carried out immediately after transplanting the pregerminated seed to the pots with 1 mL per seed of the appropriate strain culture at $1.54 \cdot 10^7$ CFU. mL⁻¹. The inoculation of the control treatments was replaced by 1 mL of distilled water. The strains CD_FICOS_01, CD_FICOS_02, CD_FICOS_03 and CD_FICOS_05 were produced in TSB by incubation at 28 °C and 80 min⁻¹ for 48 h. Strain CD_FICOS_04 was produced in modified Ashby-mannitol liquid medium (5 g.L⁻¹ mannitol, 0.6 g.L⁻¹ K₂HPO₄, 0.2 g.L⁻¹ MgSO₄·7H₂O, 0.2 g.L⁻¹ NaCl, 0.1 g.L⁻¹ CaSO₄, 0.3 g.L⁻¹ CaCO₃) by incubation at 28 °C and 80 min⁻¹ for 72 h. The final concentrations of the inoculants were estimated by counting 10-fold serial dilutions on plates containing the same medium used in the culture.

Four days after sowing, all pots were watered with 200 mL of Hoagland solution at half the usual nutrient concentration (0.5 M Ca(NO₃)₂; 0.5 M KNO₃; 0.5 M MgSO₄·7H₂O; 0.5 M KH₂PO₄; 20 mg.L⁻¹ Fe EDTA; 0.5 M CaCl₂; 0.5 M KCl; 1.43 g.L⁻¹ H₃BO₃; 0.905 g.L⁻¹ MnCl₂·4H₂O; 0.055 g.L⁻¹ ZnCl₂; 0.025 g.L⁻¹ CuSO₄·5H₂O and 0.0125 g.L⁻¹ Na₂MoO₄·2H₂O). From this point on and for the remaining time that the experiment lasted, 24 more days, irrigation was stopped in the water-stressed treatments. In contrast, the non-stressed control pots were watered weekly until the end of the experiment, alternating between half concentration Hoagland solution and distilled water. All of the above watering was done on a bottom plate to avoid washing out the bacteria. Seven days before the end of the experiment, all inoculated plants received a booster inoculation with 1 mL of the corresponding strain culture at the same concentration as the first inoculation. The booster inoculation for the seedlings of both controls was 1 mL of distilled water.

At harvest (28 days after first inoculation), the eleven replicates of each treatment were divided into three groups

according to the subsequent analyses. In five replicates, the soil plant analysis development (SPAD) value of the chlorophyll index of the five plants of each replicate was determined using a SPAD-502 chlorophyll sensor (SPAD-502 Minolta Co. Ltd., Osaka, Japan) by measuring the last fully developed leaf. Fresh weight (FW) and dry weight (DW) of the whole plant were also measured. Dry weights were measured after drying the plant material in an oven at 70 °C to constant weight. The water content of the plants was calculated using the formula (FW - DW)/FW according to Zhang and Blumwald (2001).

The plants from the other three replicates were frozen in liquid nitrogen and ground into powdered shoot tissue (leaves and stems) to be subjected to biochemical analysis to determine the content of malondialdehyde (MDA), hydrogen peroxide, proline and ABA as biochemical parameters indicating the level of water stress in the plant.

Finally, the plant from the remaining three replicates was also frozen in liquid nitrogen and ground into powdered shoot tissue and the expression levels of four genes involved in the plant response to drought stress were evaluated by quantitative reverse transcription PCR (RT-qPCR).

Biochemical Analysis

Lipid Peroxidation (MDA Content)

The level of lipid peroxidation in shoot tissue was performed by the thiobarbituric acid (TBA) test according to Velikova et al. (2000) and Heath and Packer (2022) which determines MDA as the end product of lipid peroxidation. Three biological replicates were analysed per treatment. A total of 0.1 g of pulverised shoot tissue was homogenised in 1 mL of a 0.1% (w/v) trichloroacetic acid (TCA) solution. After centrifuging the mixture for 20 min at 10000x g, 0.5 mL of the supernatant was transferred to a tube containing 1 mL of 0.5% (w/v) TBA in 20% (w/v) TCA. The resulting volume was incubated at 100 °C for 30 min after which the reaction was stopped by placing the tube on ice and centrifuged for 5 min at 10000x g. The absorbance of the supernatant was measured at 532 and 600 nm. The amount of MDA, in $\mu\text{mol MDA.g}^{-1}$ fresh weight, was determined by Lambert-Beer's law with a molar extinction coefficient of 155 mM.cm⁻¹ (Heath and Packer 2022), an optical path length of 1 cm and the absorbance value resulting from the subtraction between the absorbances at 600 and 532 nm.

Hydrogen Peroxide Content

The hydrogen peroxide content was determined according to Velikova et al. (2000). Each treatment was analysed using three biological replicates. For the assay, 0.1 g of powdered

shoot tissue was homogenised in an ice bath with 1 mL of a 0.1% (w/v) TCA solution. After centrifuging the mixture for 15 min at 12000x g, 0.5 mL of the supernatant was added to a mixture of 0.5 mL 10 mM K_2HPO_4 (pH 7.0) and 1 mL 1 M KI. After incubation in the dark for 1 h, the absorbance of the supernatant was measured at 390 nm. The hydrogen peroxide content, in mM H_2O_2 ·100mg⁻¹ fresh weight, was obtained using a standard curve.

Proline Content

The analysis was performed on three biological replicates for each treatment. 0.1 g of powdered shoot tissue was mixed with 1 mL of ethanolic extract (70:30 ethanol: water mixture (v/v)). Then, 500 µL of the homogenate was pooled in a screw-cap tube with 1000 µL of the reaction mix (ninhydrin 1% (w/v) in acetic acid 60% (v/v), ethanol 20% (v/v) and after mixing, the tube was incubated at 95 °C for 20 min. Finally, the tube was allowed to cool to room temperature, centrifuged at 10000 rpm for 1 min and the absorbance of the supernatant was measured at 520 nm. The concentration of proline, in µg.gFW⁻¹, was obtained using a standard curve for which 500 µL of ethanolic extract was replaced by 100 µL of 0.2, 0.5, 1, 2 or 5 mM proline completed with up to 400 µL of ethanol: water (40:60 (v/v) (Carillo and Gibon 2011).

ABA Content

To measure the concentration of ABA in plant sample tissues, three biological replicates were processed per treatment, each prepared from 0.1 g of powdered shoot tissue that was mixed with 1.6 mL of extraction solution (80% methanol, 100 mg.L⁻¹ butylated hydroxytoluene (BHT), and 0.5 g.L⁻¹ citric acid monohydrate). The mixture was incubated under stirring and at 4 °C overnight after which it was centrifuged at 1000x g for 20 min and the supernatant was transferred to a new tube where it was dried under vacuum. The pellet was resuspended in 1 mL of a mixture

of 10% methanol and 90% Tris-buffered saline (TBS) buffer (pH 7.8) (Barnawal et al. 2017). Phytohormone levels in the resulting mixture were determined by Enzyme-linked immunosorbent assay (ELISA) using the Phytodetek[®] ABA Test Kit (Agdia Inc., Elkhart, IN, USA), according to the manufacturer's instructions.

RNA Extraction and Purification and Analysis of Gene Expression

Total RNA was extracted from pulverised shoot tissue using the Direct-zol[™] RNA MiniPrep kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's recommendations. Subsequently, RNA was treated with the TURBO DNA-free[™] Kit (Invitrogen, ThermoFisher Scientific Inc., Waltham, MA, USA) to remove trace DNA and its integrity and purity was checked by electrophoresis on 1% (w/v) agarose gels and by quantification on a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific Inc., Waltham, MA, USA). Treated, DNA-free RNA was used for cDNA synthesis, following the protocol of the Reverse Transcription System A3500 kit (Promega, Madison, WI, USA) and then checked for integrity by PCR. Samples from three different biological replicates were obtained.

Gene expression analysis was performed by RT-qPCR. The qPCR reaction components and thermocycling conditions followed the Universal qPCR Master Mix Luna[®] (New England Biolabs[®] Inc., Ipswich, MA, USA) using the *T. aestivum* actin gene as endogenous reference gene. All primers used in RT-qPCR experiments are listed in Table 1 and the efficiency of each pair was calculated using six serial dilutions of cDNA as template and all showed efficiencies greater than 97%. All reactions were performed on a Stratagene Mx3005P thermal cycler (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions for the use of SYBR[®] Green as quantifier and the QuantStudio[™] Design & Analysis Software v1.5.1 (ThermoFisher Scientific Inc., Waltham, MA, USA) was used for the analysis of the data. The 2^{-ΔΔCt} method (Pfaffl 2001) was

Table 1 Oligonucleotides used in RT-qPCR

Oligonucleotide	Ace. No.*	Sequence (5'-3')
<i>TaAct</i> -Fwd	AB181991 ^a	CGAAACCTTCAGTTGCCAGCAAT
<i>TaAct</i> -Rev	AB181991 ^a	ACCATCACCAGAGTCGAGCACAAT
<i>TaDreb2</i> - Fwd	DQ021908 ^a	CGGAGATGCAGCTTCTTGATT
<i>TaDreb2</i> - Rev	DQ021908 ^a	GATCTCGAGCGACGGGTACTT
<i>TaCat1</i> - Fwd	E16461 ^a	CCATCTGGCTCTCCTACTGG
<i>TaCat1</i> - Rev	E16461 ^a	AGAACTTGGACGGCCCTGA
<i>TaCtrl</i> - Fwd	100286402 ^b	GCTGCTCTTGTGAATCCTGTTG
<i>TaCtrl</i> - Rev	100286402 ^b	ATCCACAATGCTTGAAACGAA
<i>TaCYP707A1</i> - Fwd	EU430344 ^a	GCGCACCTCTTCAAGCCTA
<i>TaCYP707A1</i> - Rev	EU430344 ^a	CGAAGATGGACAGCAATGC

*GenBank accession number ^(a) or GenBank gene ID ^(b)

Table 2 Plant growth-promoting activities of the isolates used in the plant drought stress assay

Strain	Siderophore production index (SPI)	Phosphate solubilization		ACC deaminase activity ($\mu\text{mol ACC. mg}^{-1} \text{ prot.h}^{-1}$)	IAA production ($\mu\text{g IAA.10}^{-8} \text{ CFU}$)	Potassium solubilization (scale 0–4)
		Phosphate solubilization index (PSI)	Solubilized <i>P</i> ($\mu\text{g.mL}^{-1}$)			
CD_FICOS_01	2.30	ND	57.02	107.60	10.68	3.00
CD_FICOS_02 ^a	2.34	2.22	56.61	115.32	9.72	2.00
CD_FICOS_03 ^a	2.68	3.13	63.54	99.29	5.61	4.00
CD_FICOS_04 ^a	2.86	2.41	64.41	15.90	4.64	3.00
CD_FICOS_05	ND	2.74	ND	ND	ND	4.00

^a The strains CD_FICOS_02, CD_FICOS_03 and CD_FICOS_04 were analysed by Mazuecos-Aguilera et al. (2025). ND: no activity detected for the corresponding strain under the assay conditions. Values shown correspond to the consolidated result from three independent replicates

Table 3 Mean $\pm$ standard deviation per pot for fresh biomass, dry biomass, plant water content and SPAD chlorophyll index for each treatment. Treatments: USC=uninoculated and stressed control; UNSC=uninoculated and non-stressed control; CD_FICOS_01–05=plants inoculated with strains CD_FICOS_01–05. Percentage variation (Δ (%)) is expressed relative to USC. Data were analysed by one-way ANOVA followed by LSD test. Different letters indicate statistically significant differences among treatments ($p < 0.05$). Five pots were used per treatment ($n = 5$)

Treatment	Fresh weight		Dry weight		Water content		Chlorophyll index	
	g	Δ (%)	g	Δ (%)	%	Δ (%)	SPAD value	Δ (%)
CD_FICOS_01	1.11 ^b $\pm$ 0.06	+58.78	0.35 ^d $\pm$ 0.01	-12.59	68.29 ^a $\pm$ 2.07	+68.30	46.63 ^a $\pm$ 1.61	+45.81
CD_FICOS_02	0.83 ^{cd} $\pm$ 0.06	+18.30	0.45 ^b $\pm$ 0.02	+13.08	44.91 ^b $\pm$ 3.10	+10.69	37.65 ^{cd} $\pm$ 1.62	+17.72
CD_FICOS_03	0.97 ^{bc} $\pm$ 0.03	+38.36	0.31 ^e $\pm$ 0.01	-21.64	67.71 ^a $\pm$ 2.05	+66.90	35.51 ^{de} $\pm$ 1.82	+11.05
CD_FICOS_04	0.40 ^f $\pm$ 0.06	-43.21	0.27 ^f $\pm$ 0.02	-33.03	27.44 ^c $\pm$ 9.87	-32.38	24.88 ^f $\pm$ 1.43	-22.21
CD_FICOS_05	0.56 ^e $\pm$ 0.03	-20.52	0.32 ^{de} $\pm$ 0.01	-19.88	41.71 ^{bc} $\pm$ 4.35	+2.81	42.59 ^{ab} $\pm$ 1.91	+33.16
USC	0.70 ^{de} $\pm$ 0.08		0.40 ^e $\pm$ 0.01		40.57 ^{bc} $\pm$ 5.78		31.98 ^e $\pm$ 2.27	
UNSC	7.14 ^a $\pm$ 0.06	+919.05	1.37 ^a $\pm$ 0.01	+244.28	80.71 ^a $\pm$ 0.20	+98.93	41.69 ^{bc} $\pm$ 0.97	+30.3

employed for the calculation of relative expression levels of each gene. Three replicate techniques of each cDNA were analysed to calculate the mean and standard deviation.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics v29. Data were expressed as mean $\pm$ standard deviation and the Shapiro-Wilk test and Levene's test were used to test for fit to a normal distribution and homoscedasticity of variances, respectively. Differences among treatments were analysed by one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) post hoc test when significant effects were detected. Differences were considered statistically significant at $p < 0.05$.

Results

Physiological Properties of Selected Isolates

The strain CD_FICOS_01 showed the capacity to produce siderophores, solubilise phosphates, solubilise potassium, ACC deaminase activity and with IAA production being the highest of all the strains considered (Table 2). On the other hand, CD_FICOS_05 only presented potassium and phosphate solubilisation capacity, but in similar

values to the other strains. Therefore, CD_FICOS_01 and CD_FICOS_05 were selected together with the other three strains for this plant drought stress assay among a battery of bacteria due to their PGP characteristics and their ability to enhance plant biomass production in previous trials (data not shown).

Effect of PGPM Inoculation on Plant Growth and Other Physiological Parameters

Drought stress conditions reduced growth, measured as fresh and dry plant weight, water content and chlorophyll content of wheat plants. The observed differences between uninoculated stressed control (USC) and UNSC were statistically significant for all parameters (Table 3; Fig. 1a–d). Under water stress conditions, only CD_FICOS_02 significantly increased the aerial dry biomass with respect to USC, while interestingly the others reduced it (Table 3; Fig. 1b). On the other hand, the treatments CD_FICOS_01, CD_FICOS_02, CD_FICOS_03 and CD_FICOS_05 increased the water content of the plants with respect to the USC, although only for CD_FICOS_01 and CD_FICOS_03 the increase was statistically significant, resulting in a statistically significant increase in aerial fresh biomass (Fig. 2). Conversely, CD_FICOS_04 produced a non-statistically significant reduction of water content with respect to USC, but a significant reduction of the FW (Table 3; Fig. 1c and

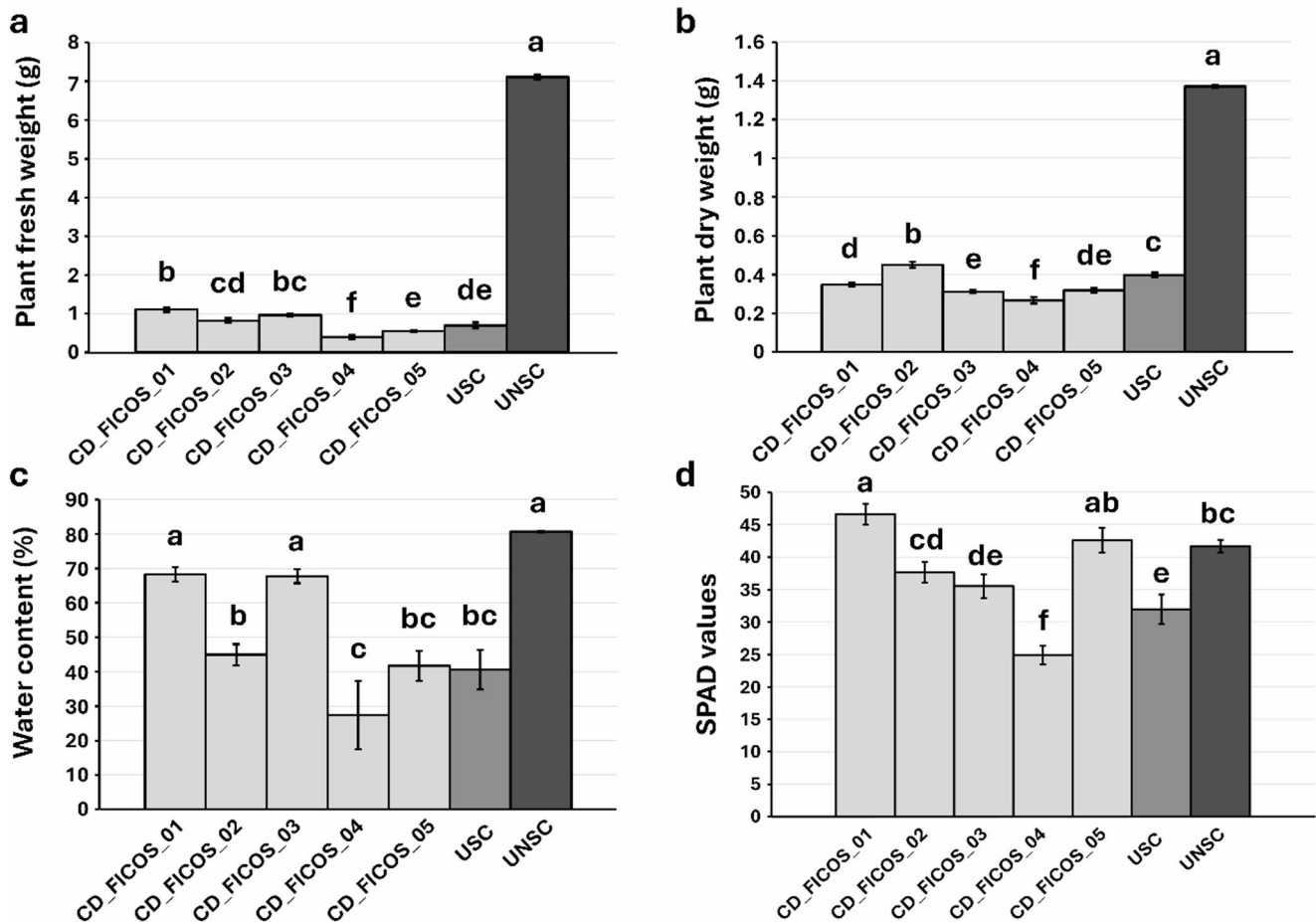


Fig. 1 Effect of inoculation of the five strains on (a) fresh weight, (b) dry weight, (c) water content and (d) chlorophyll of wheat plants under water-stress conditions at the early vegetative stage (CD_FICOS_01: *P. megaterium*, CD_FICOS_02: *P. frigiditolerans*, CD_FICOS_03: *P. canadensis*, CD_FICOS_04: *A. chroococcum*, CD_FICOS_05: *P. kluyveri*). Non-inoculated wheat plants under water-stress condi-

tions (USC) and non-inoculated wheat plants under non-stress conditions (UNSC). Values are expressed per pot. Each bar represents the mean $\pm$ standard deviation of five replicates. Different letters indicate statistically significant differences among treatments according to one-way ANOVA followed by Fisher's least significant difference (LSD) test ($p < 0.05$)

a). A statistically significant effect of CD_FICOS_01, CD_FICOS_02, CD_FICOS_04 and CD_FICOS_05 treatments with respect to USC was observed on the chlorophyll levels of the stressed plants, which were in all cases, except CD_FICOS_04, higher than those of the stressed control (Table 3; Fig. 1d).

Effect of PGPM Inoculation on the Biochemical Parameters of the Plant

Drought stress conditions increased the accumulation of proline, MDA and ABA in USC plants compared to UNSC plants. This increase was statistically significant for these parameters, except for ABA (Table 4; Fig. 3b–d). In contrast, H_2O_2 accumulation was not affected by drought stress (Table 4; Fig. 3a). Inoculation of plants under drought conditions resulted in proline accumulation (Table 4; Fig. 3c), through an increase in the amino acid concentration,

compared to USC, and this was statistically significant in all cases. For MDA (Table 4; Fig. 3b), all inoculation treatments except CD_FICOS_01 caused an increase in the accumulation of the compound compared to USC plants, but this was only statistically significant in treatments CD_FICOS_03, CD_FICOS_04 and CD_FICOS_05. Regarding the ABA concentration (Table 4; Fig. 3d), the results of CD_FICOS_02 and CD_FICOS_05 were not statistically significant, although the tendency was that these two strains caused a decrease in hormone accumulation compared to the USC, while CD_FICOS_01, CD_FICOS_03 and CD_FICOS_04 had the opposite effect and this was statistically significant for CD_FICOS_01 and CD_FICOS_03. In relation to H_2O_2 , the dispersion of the results led to a lack of statistically significant differences in the case of all treatments and the inoculation with CD_FICOS_05 caused the most intense decrease of peroxide compared to USC, but it was not statistically significant either (Table 4; Fig. 3a).

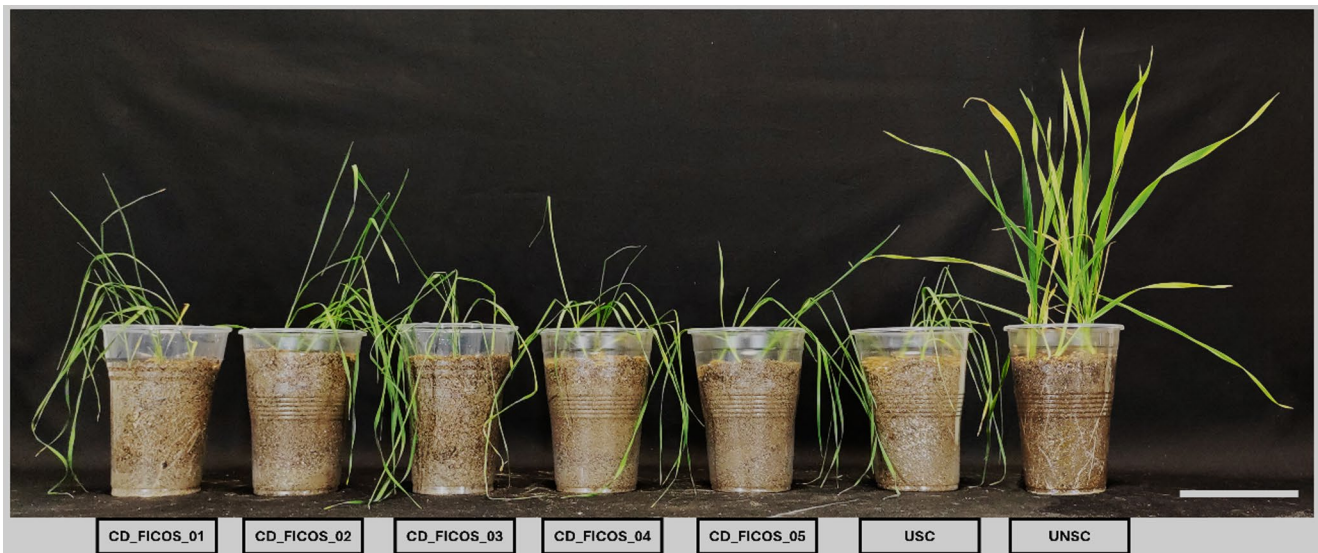


Fig. 2 Representative wheat plants from each treatment at the early vegetative stage, photographed at the end of the experiment: non-inoculated wheat plants under water-stress conditions (USC), non-inoculated wheat plants under non-stress conditions (UNSC), and

plants inoculated with strains CD_FICOS_01 (*P. megaterium*), CD_FICOS_02 (*P. frigiditolerans*), CD_FICOS_03 (*P. canadensis*), CD_FICOS_04 (*A. chroococcum*) and CD_FICOS_05 (*P. kluyveri*). Scale bar = 10 cm

Table 4 Mean values $\pm$ standard deviation per pot of H_2O_2 , MDA, proline and ABA levels in wheat plants under each treatment. Treatments: USC=uninoculated and stressed control; UNSC=uninoculated and non-stressed control; CD_FICOS_01–05=plants inoculated with strains CD_FICOS_01–05. Percentage variation (% Δ) is expressed relative to USC. Data were analysed by one-way ANOVA followed by LSD test. Different letters indicate statistically significant differences among treatments ($p < 0.05$). Three pots were used per treatment ($n = 3$)

Treatment	H_2O_2		MDA		Proline		ABA	
	mM.100mg ⁻¹ FW	Δ (%)	nmol.g ⁻¹ FW	Δ (%)	μ g.g ⁻¹ FW	Δ (%)	picomol.mL ⁻¹	Δ (%)
CD_FICOS_01	0.12 ^{ab} $\pm$ 0.07	-6.34	29.35 ^b $\pm$ 0.52	-1.80	88.27 ^a $\pm$ 0.40	+59.15	2297.66 ^a $\pm$ 389.92	+805.58
CD_FICOS_02	0.18 ^a $\pm$ 0.09	+36.42	30.80 ^b $\pm$ 0.56	+3.02	76.82 ^a $\pm$ 0.65	+38.50	144.31 ^{cd} $\pm$ 26.48	-43.12
CD_FICOS_03	0.12 ^{ab} $\pm$ 0.04	-10.35	36.71 ^a $\pm$ 0.98	+22.81	86.96 ^a $\pm$ 4.24	+56.79	836.79 ^b $\pm$ 102.65	+229.80
CD_FICOS_04	0.10 ^{ab} $\pm$ 0.03	-21.86	37.25 ^a $\pm$ 1.38	+24.60	82.20 ^a $\pm$ 1.85	+48.20	598.13 ^{bc} $\pm$ 47.07	+135.74
CD_FICOS_05	0.05 ^b $\pm$ 0.01	-63.50	38.17 ^a $\pm$ 1.19	+27.70	84.99 ^a $\pm$ 15.10	+53.24	174.38 ^{cd} $\pm$ 8.63	-31.27
USC	0.13 ^{ab} $\pm$ 0.03		29.89 ^b $\pm$ 0.14		55.46 ^b $\pm$ 3.02		253.72 ^{cd} $\pm$ 82.72	
UNSC	0.14 ^{ab} $\pm$ 0.02	+6.53	9.59 ^c $\pm$ 0.21	-67.91	1.05 ^c $\pm$ 0.01	-98.10	6.50 ^d $\pm$ 0.53	-97.44

Effect of PGPM Inoculation on the Expression of Plant *TaCat1*, *TaCtr1*, *TaCYP707A1* and *TaDreb2* Genes

Among the non-inoculated plants, water stress caused a statistically significant increase in *TaCat1*, *TaCtr1*, *TaCYP707A1* and *TaDreb2* gene expression (Fig. 4a–d). Inoculation of stressed plants resulted in a statistically significant reduction of *TaCYP707A1* expression levels compared to USC in all cases except for CD_FICOS_05, where it increased, although this change was not statistically significant (Fig. 4c). All PGPM treatments caused a reduction in *TaCat1* transcript accumulation compared to USC (Fig. 4a), although the differences were only significant for CD_FICOS_01, CD_FICOS_02 and CD_FICOS_03, with the first one causing the greatest reduction of all. In the case of *TaCtr1* (Fig. 4b), CD_FICOS_01, CD_FICOS_02 and CD_FICOS_03 treatments showed a significant reduction

in gene transcript levels compared to USC. CD_FICOS_05 treatment also showed the same behaviour, but this result was not significant. However, CD_FICOS_04 had the opposite effect and was not significant. With respect to *TaDreb2* (Fig. 4d), inoculation with CD_FICOS_04 and CD_FICOS_05 increased the transcript level of the gene compared to USC, while inoculation with CD_FICOS_01, CD_FICOS_02 and CD_FICOS_03 decreased it, although the differences were only statistically significant when comparing CD_FICOS_01 and CD_FICOS_03 with USC. Mean Ct values for all genes and treatments are provided in Supplementary Table S1.

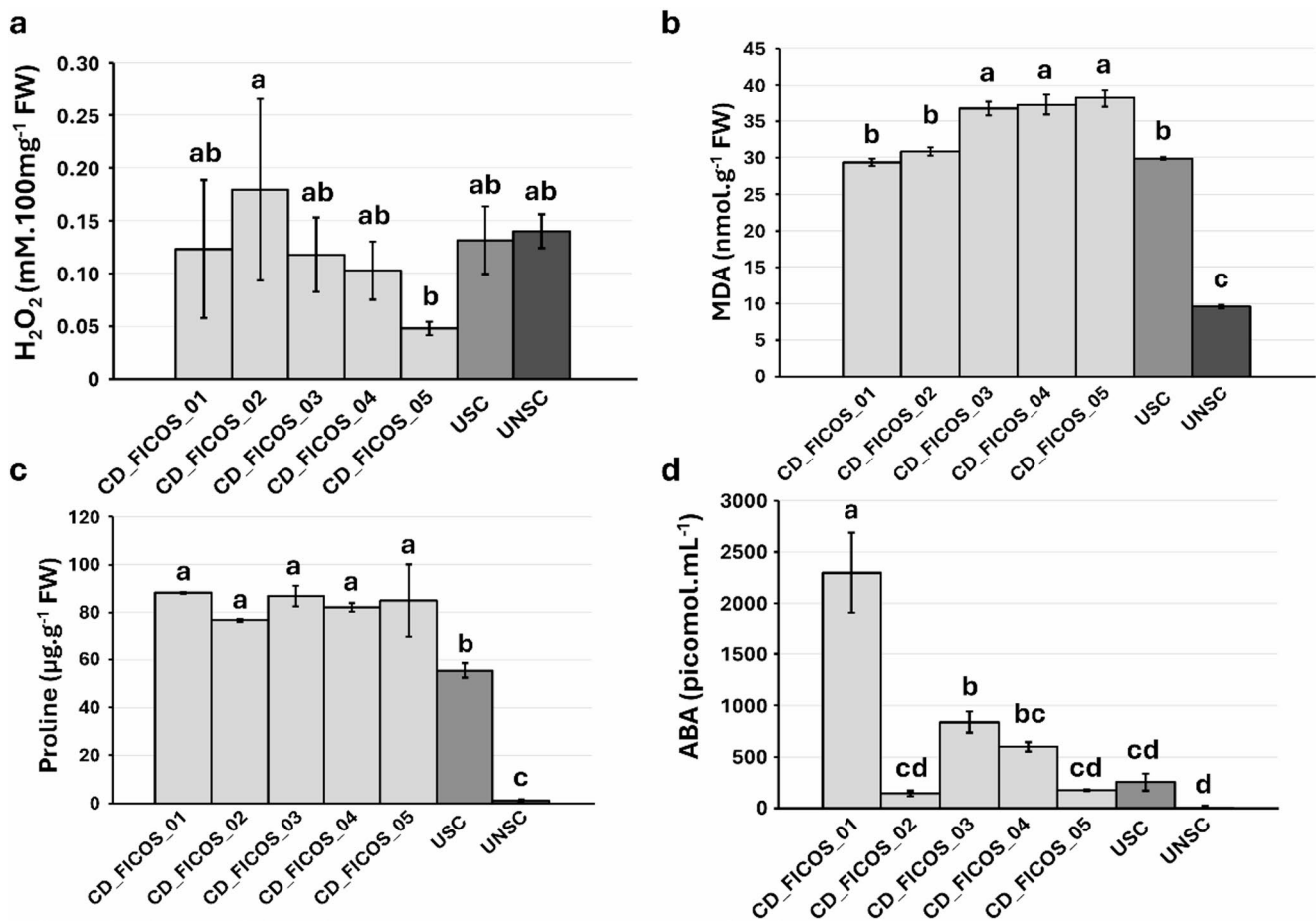


Fig. 3 Effect of inoculation of the five strains on the concentrations of (a) H_2O_2 , (b) MDA, (c) proline and (d) ABA in the shoot tissue of wheat plants under water stress conditions (CD_FICOS_01: *P. megaterium*, CD_FICOS_02: *P. frigiditolerans*, CD_FICOS_03: *P. canadensis*, CD_FICOS_04: *A. chroococcum*, CD_FICOS_05: *P. kluveri*). Non-inoculated wheat plants under water stress conditions (USC) and

non-inoculated wheat plants under non-stress conditions (UNSC). Values are expressed per pot. Each bar represents the mean $\pm$ standard deviation of three replicates. Different letters indicate statistically significant differences among treatments according to one-way ANOVA followed by Fisher's least significant difference (LSD) test ($p < 0.05$)

Discussion

Morphological and Physiological Aspects

Drought stress conditions affect various molecular, biochemical, physiological, morphological and ecological traits and processes in plants. At the agronomic level, crop yield and biomass quality are severely affected. In our work, the comparison between UNSC and USC revealed a sharp decrease in aerial biomass and water content as a result of the drought stress reflecting a common drought adaptation strategy of plants (Seleiman et al. 2021). However, PGPM can promote plant water uptake and retention by stimulating root development, production of different extracellular polysaccharides, osmolytes and proteins (Gontia-Mishra et al. 2020; Zhang et al. 2025). In our work, CD_FICOS_01 (*Priestia megaterium*) and CD_FICOS_03 (*Pseudomonas canadensis*) significantly increased plant water content

compared to USC plants resulting in higher fresh biomass, although interestingly, only CD_FICOS_02 (*Peribacillus frigiditolerans*) increased the aerial dry biomass (Table 3; Fig. 1). Different strains of *Priestia* and *Pseudomonas* genera have been shown to have a positive effect on relative water content or fresh biomass in wheat or mung bean under water or salt stress (Kour et al. 2020; Prasad et al. 2025; Sarma and Saikia 2014; Shahid et al. 2022). *Peribacillus* also increased shoot dry weight and leaf water content in *Arabidopsis* under dehydration stress (Nourashrafeddin et al. 2024).

However, the significant reduction of aerial biomass, fresh and dry, and the non-significant reduction of water content as a result of the inoculation with *Azotobacter chroococcum* (CD_FICOS_04) indicates that this strain, that is a good N-fixer, is not a good option for helping the plant to cope with water stress situations (Table 3; Fig. 1). In the literature it is described that other strains of *A. chroococcum*

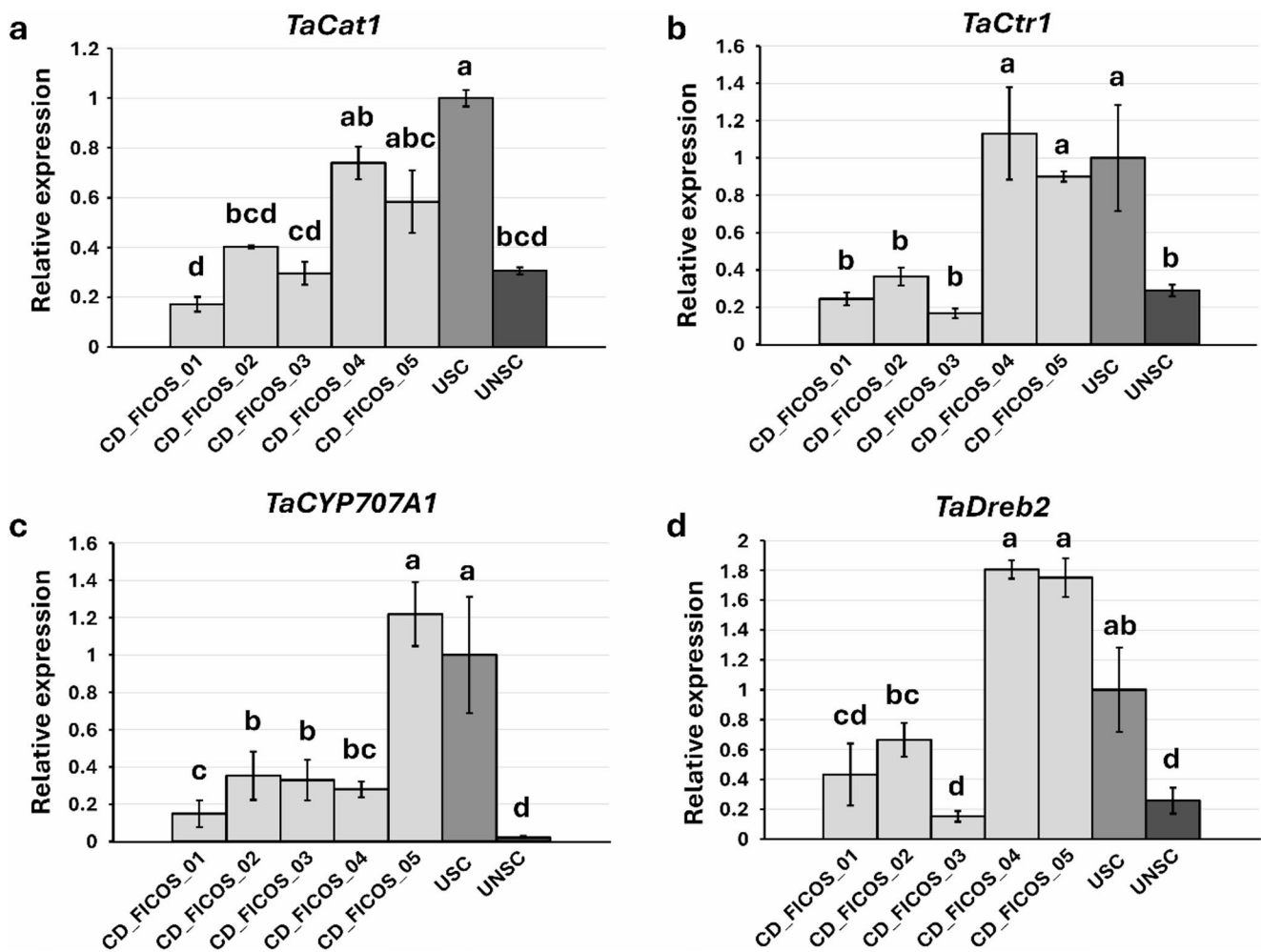


Fig. 4 Effect of inoculation of the five strains on gene expression of (a) *TaCat1*, (b) *TaCtrl*, (c) *TaCYP707A1* and (d) *TaDreb2* in the shoot tissue of wheat plants under water stress conditions (CD_FICOS_01: *P. megaterium*, CD_FICOS_02: *P. frigiditolerans*, CD_FICOS_03: *P. canadensis*, CD_FICOS_04: *A. chroococcum*, CD_FICOS_05: *P. kluyveri*). Non-inoculated wheat plants under water stress conditions (USC) and non-inoculated wheat plants under non-stress conditions (UNSC). Expression ratios were normalised by using the *T. aestivum*

actin gene as an endogenous control. The value of 1.0 was denoted for the transcript level of all genes in USC plants. Each bar represents the mean $\pm$ standard deviation of three technical replicates for each of the three biological replicates per treatment. Different letters indicate statistically significant differences among treatments according to one-way ANOVA followed by Fisher's least significant difference (LSD) test ($p < 0.05$)

and *A. vinelandii* can increase the relative water content in eggplant grown under drought stress (Kiran et al. 2022), although with no influence on shoot fresh and dry weights. However, in other cases several strains of *Azotobacter* increased shoot dry weight or plant height in maize under drought stress (Shirinbayan et al. 2019).

The fungal strain CD_FICOS_05 from *Pichia kluyveri* had a negligible effect on water content and fresh biomass, but a reduction of dry biomass (Table 3; Fig. 1). However, it has been reported that strains of the genus *Pichia* had a positive influence on the total water stable aggregates in inoculated soils which has been associated with increased water availability to plants and could be caused by the production of extracellular polysaccharides (Ramya et al. 2021).

However, due to the substrate used in the experiment, no soil structure improvement parameters were evaluated in our experiment.

Under water stress conditions, chlorophyll content is known to decrease due to the activation of chlorophyllase, impairment of synthesis enzymes or oxidative damage to the chloroplast (Rijal et al. 2021). In our experiment, USC plants showed a significant decrease in chlorophyll content while plants inoculated with CD_FICOS_01, CD_FICOS_02 and CD_FICOS_05 showed a significant increase compared to USC plants (Table 3; Fig. 1d). The recovery of chlorophyll levels in these cases could be explained by the accumulation of proline in the inoculated plants, since a protective effect of proline on chlorophyll has been reported. The amino acid

acts as a scavenger of ROS inside the chloroplast and to increases the stability of the membranes of this organelle under abiotic stress conditions (Gadallah 1999; Spormann et al. 2023). Inoculation with these strains seems to increase proline accumulation compared to the USC control, with the differences being significant in all of them. The exception would be CD_FICOS_04 as it caused a significant increase in proline levels, but a reduction in SPAD measures, also significant, compared to USC (Table 3; Fig. 3c). This could point to differences in the mechanisms of action involved in this strain compared to the others. Although the improvement of the chlorophyll level as a result of inoculation with PGPM in plants under water or salt stress conditions is quite common (Abiala et al. 2023; Al-Shammari et al. 2025; Marik et al. 2024; Pradhan et al. 2018; Shahid et al. 2022; Shirinbayan et al. 2019), sometimes it is related with an increase of proline level (Abiala et al. 2023; Marik et al. 2024; Kiran et al. 2022; Kour et al. 2020; Ma et al. 2020; Pradhan et al. 2018) and sometimes with a decrease (Ma et al. 2020; Shahid et al. 2022). In concrete, Shahid et al. (2022) found a reduction in proline concentration concomitantly with an increase in chlorophyll levels and leaf relative water content in wheat. Proline acts as a stress signal as well as an osmoprotectant and these differences may depend on the bacterial and plant species involved, their interaction, and the type and degree of stress (Shahid et al. 2022).

In addition, proline could improve the water status of stressed plants by inhibiting water flow through its effect on membrane stability and reducing transpiration through its effect on stomatal regulation (Gadallah 1999). In this way, the significant improvement of the water status of stressed plants inoculated with CD_FICOS_01 and CD_FICOS_03 could be explained by the significant increase of proline.

Oxidative Stress

H₂O₂ and MDA are commonly used as markers of oxidative damage, the latter being the end product of polyunsaturated fatty acid peroxidation in cells (Zhang et al. 2021). In general, H₂O₂ levels are related to MDA levels and both increase under water stress, as H₂O₂ promotes lipid peroxidation.

It is interesting to note that H₂O₂ levels remained unchanged in controls and most treatments (Table 3; Fig. 3a). This result could be due to the activation of the ROS detoxification systems by the plant in response to stress, which would have brought the levels of the molecule in the stressed plants in line with those of the unstressed plants without an apparent influence of the treatment with PGPM. However, MDA levels showed a slightly different pattern of variation than H₂O₂, as MDA was significantly higher in all drought-stressed plants whether inoculated or not, and MDA was also significantly higher in CD_FICOS_03,

CD_FICOS_04 and CD_FICOS_05 treatments than in the USC (Table 3; Fig. 3b). There is some controversy on how to interpret the MDA levels, because on the one hand a reduction in MDA and H₂O₂ synthesis is considered an indicator of plant tolerance to drought conditions (Abiala et al. 2023; Crespo-Barreiro et al. 2025; Marik et al. 2024; Kiran et al. 2025; Kiran et al. 2022; Shahid et al. 2022), and in this sense MDA can be used as an indicator of oxidative damage to plant membranes if its level remains high. On the other hand, an increase in MDA may indicate acclimation processes rather than damage if its removal and the regulation of redox signalling are working properly (Morales and Munné-Bosch 2019), as MDA can play a beneficial role by activating regulatory genes involved in plant defence and development under oxidative stress conditions acting as a protective mechanism. Similar to our results, a transient increase in MDA levels was observed in rosemary plants under saline stress, but no signs of oxidative damage due to activation of an antioxidant system were observed (Tounekti et al. 2011). Therefore, the role of MDA as harmful or protective depends on its production, removal and modulation of signalling (Morales and Munné-Bosch 2019). Thus, in our study, the observed increase in MDA levels in drought-stressed plants, exacerbated by the inoculation with CD_FICOS_03, CD_FICOS_04 and CD_FICOS_05 could have different meanings. In CD_FICOS_03 could be a sign of plant protection against drought stress, because it is associated with a better fresh biomass production and water status, whereas in CD_FICOS_04 and in CD_FICOS_05 it could be a sign of a stress situation that reduced plant growth.

TaCat1 encodes for a catalase enzyme that plays an important role in controlling ROS homeostasis by catalysing the decomposition of H₂O₂, and it is also associated with oxidative stress. In our work, transcript levels of this gene were increased in USC compared to UNSC and the difference was statistically significant. Similarly, previous reports suggest an increase in *TaCat1* transcription in wheat grown under drought stress (Barquero et al. 2022; Gontia-Mishra et al. 2016). The application of CD_FICOS_01, CD_FICOS_02 and CD_FICOS_03 induced a significant reduction of gene expression in the plants under drought stress while the other inoculums did not induce statistically significant changes, but a tendency to decrease transcript levels was observed (Fig. 4a). This result correlates with previous studies in which a down-regulation of *TaCat1* or a decrease in the activity of the enzyme was observed in PGPM-inoculated wheat plants under drought or salinity stress ((Ahmad Ansari et al. 2023; Barquero et al. 2022; Gontia-Mishra et al. 2016). The decrease in *TaCat1* expression is compatible with the results of H₂O₂ levels that we observed in the inoculated plants. A decrease in *TaCat1* transcript accumulation would have resulted in a lower catalase activity, insufficient

to reduce the levels of the compound. However, *TaCat1* belongs to a gene family consisting of numerous genes in *T. aestivum* L. each of which responds differently depending on the tissue, the developmental stage of the plant and environmental stress conditions (Al Khallaf et al. 2025; Zhang et al. 2022). Therefore, the decreased expression of *TaCat1* in inoculated plants could have been compensated by the increased expression of one or more other members of the family while keeping the H_2O_2 concentration at a stable level or causing the tendency to decrease observed in the treatment with CD_FICOS_05.

Ethylene Metabolism

Under abiotic stress conditions, the ethylene-mediated plant response participates in osmotic adjustments, oxidative stress management and on development to arrest or redirect plant growth and nutrient distribution thereby increasing stress tolerance (Abhinandan et al. 2018). Furthermore, depending on the severity and duration of stress, the stress-induced ethylene responses can have a detrimental effect on crop yield (Abhinandan et al. 2018). *Ctrl* encodes for Constitutive Triple Response 1, a serine/threonine kinase that plays a key role in ethylene signal transduction through its interaction with ethylene receptors. *Ctrl* is found interacting directly with receptors which keeps it in an active state and can inactivate downstream components by phosphorylation thereby negatively regulating signalling pathway. In contrast, in the presence of ethylene, it is sensed by the receptors and its binding leads to inactivation of *Ctrl*. As a consequence, the transcriptional cascade response is activated resulting in the expression of ethylene-responsive genes (Barnawal et al. 2017; Jakubowicz et al. 2019). In our study, *TaCtrl* expression was induced by the effect of water stress while treatments with CD_FICOS_01, CD_FICOS_02 and CD_FICOS_03 downregulated it (Fig. 4b). These results are in agreement with those obtained by Barquero et al. (2022) where *TaCtrl* was induced in wheat after prolonged exposure to drought while inoculation of stressed plants with two strains of *Rhizobium leguminosarum* caused a decrease in its expression and a concomitant increase in growth parameters compared to stressed and non-inoculated plants, the same as we observed for CD_FICOS_01, CD_FICOS_02 and CD_FICOS_03, which could reflect the ability of *Priestia*, *Peribacillus* and *Pseudomonas* strains to alleviate drought stress through their influence on the ethylene signalling pathway. However, different or opposite behaviours to this have also been described for *TaCtrl* under drought stress conditions (Barnawal et al. 2017). The fact that *TaCtrl* is a member of a family of genes that can function differently in different regulatory pathways could explain these results (Bi et al. 2010).

Some PGPBs can play an important role in stress-induced ethylene responses via indole-3-acetic acid (IAA) production and ACC deaminase activity. Ethylene is derived from methionine, which is converted to S-adenosyl-L-methionine (SAM) by SAM synthetase, and then ACC synthetase converts SAM to ACC, which will ultimately give rise to ethylene. In plants inoculated with ACC deaminase-producing PGPBs under abiotic stress, bacterial ACC deaminase is induced in response to plant stress-induced ACC and hydrolyses excess ACC in the plant to ammonia and α -ketobutyrate and reduces deleterious ethylene levels. At the same time, some ACC deaminase-producing PGPBs can utilise tryptophan and other small molecules present in root exudates and convert them into IAA, which is used by plant roots together with their endogenous IAA and can regulate plant development. IAA can also induce transcription of ACC synthase leading to increased levels of ACC and ultimately ethylene or ammonia and α -ketobutyrate depending on bacterial ACC deaminase activity (Kang et al. 2019; Naing et al. 2021). In our work, strains CD_FICOS_01, CD_FICOS_02, CD_FICOS_03 and CD_FICOS_04 are IAA producers and have ACC deaminase activity. It is possible that the positive influence observed in CD_FICOS_01 and CD_FICOS_03 on plant growth parameters is due to their ACC deaminase activity which would have reduced plant ethylene levels, compensating for the stimulation of this molecule produced by bacterial IAA, and contributed to drought tolerance. On the other hand, in CD_FICOS_04 with lower ACC deaminase activity compared to the other three strains, but similar IAA synthesis activity, ethylene would have reached levels detrimental to plant growth (Table 2).

ABA Metabolism

Ctrl expression is also influenced by ABA, the production of which is stimulated by drought stress, as we observed when we compared the ABA rate of USC and UNSC, although this result was not statistically significant. This hormone acts as a primary signal for plants to respond to drought by triggering a variety of physiological processes (Aslam et al. 2022). In our experiment, considering only the stressed plants, whether inoculated or not, the downregulation of *TaCtrl* was associated with higher levels of ABA; particularly CD_FICOS_01 and CD_FICOS_03 showed the lowest expression of *TaCtrl*, as low as the unstressed control, and concomitantly the higher levels of ABA (Table 3; Fig. 3d). Bi et al. (2010) also observed that ABA application in wheat and *Arabidopsis* inhibited *TaCtrl* expression, which recovered after ABA removal. It has been reported in the literature (Arkhipova et al. 2020; Barnawal et al. 2017; Barquero et al. 2022; Crespo-Barreiro et al. 2025) that the

inoculation of wheat with different strains of the genera *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Rhizobium* counteracted the increase in ABA caused by salt or water stress in the aerial part of the plant while a strain of the genus *Dietzia* had no significant effect on the hormone. In our experiment, plants inoculated with the PGPM showed either a slight but not significant reduction in the ABA rate, or an increase, which was statistically significant for CD_FICOS_01 and CD_FICOS_03, the PGPM strains that better maintained the plant's water status.

CYP707A1 is a gene encoding for a key 8'-hydroxylase which is involved in the catabolism ABA to maintain endogenous ABA levels within a tolerable range for the plant during drought stress. Therefore, its expression generally increases in response to drought stress as in peanut (Long et al. 2019) and in soybean (Zheng et al. 2012), or *CYP707A3* in *Arabidopsis* (Umezawa et al. 2006). The same was observed in our work. Moreover, *CYP707A1* was induced upon salt stress in tomato regardless the presence of an inoculum of *Pseudomonas* (Mellidou et al. 2021). However, the gene was downregulated upon drought stress in wheat and inoculation of stressed plants with *Rhizobium* upregulated it (Barquero et al. 2022). The opposite effect had *Pseudomonas* inoculation on *CYP707A1*, *CYP707A2* and *CYP707A3* in tomato seedlings under salt stress (Dong et al. 2024). Our results for CD_FICOS_01 and CD_FICOS_03 point in the same direction as Dong et al. (2024) and indicate that these strains enhanced the drought tolerance by increasing the accumulation of ABA and decreasing the expression of the gene *TaCYP707A1* involved in the degradation of the hormone (Fig. 4c).

Water stress also caused an increase in *TaDreb2* expression, while its expression pattern in the treatments depended on the inoculated species, with CD_FICOS_01 and CD_FICOS_03 showing the lowest mean relative expression level of all treatments with respect to USC (Fig. 4d). *Dreb2* codes for a dehydration-responsive element binding protein, a transcription factor that regulates the expression of a set of genes that respond to abiotic stress and increase plant tolerance to stress (Komal et al. 2025). These results are consistent with previously reported studies in which PGPM inoculation resulted in water stress-induced gene repressing in several plant species (Barquero et al. 2022; Gontia-Mishra et al. 2016). Moreover, since *TaDreb2* induction may be either ABA-dependent or ABA-independent manner, the increased levels of the hormone present in *Priestia* and *Pseudomonas* treatments would point to an ABA-independent regulation of this transcription factor (Aslam et al. 2022; Barquero et al. 2022). However, there are also studies reporting the opposite effect by both water deficit stress and PGPM inoculation (Barnawal et al. 2017; Ferrante et al. 2024; Gagné-Bourque et al. 2015; Kim et al.

2014; Manjunatha et al. 2022; Sarma and Saikia 2014). In any case, as with *TaCt1*, the expression pattern of *TaDreb2* indicates that gene expression can be modulated in different ways during drought stress and by inoculation with PGPMs.

Conclusions

This work shows the variation in the different roles and strategies that PGPMs play to communicate with the host under water stress depending on the plant and bacterial species involved and the severity of the environmental stress. The behaviour of the five PGPM strains tested varied in alleviating water stress in wheat plants according to the species, ultimately producing positive or neutral or negative effects on plant growth. On the one hand, treatments with strains CD_FICOS_01, CD_FICOS_02 and CD_FICOS_03 yielded improvements in fresh and/or dry biomass of wheat plants which could be attributed to their simultaneous potential to produce IAA and ACC deaminase. In addition, ABA signalling would also have been involved in the improvement in growth rate of stressed plants stimulated by CD_FICOS_01 and CD_FICOS_03. Conversely, inoculation with CD_FICOS_04 and CD_FICOS_05 resulted in a reduction of agronomic parameters. Our results suggest that these isolates have the capacity to promote mechanisms related to the plant response to abiotic stress, but they did not have a determinant effect on drought stress.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests Alejandro Martinez-Quesada and Luis Lombana-Larrea are employed by Ficosteria S.L. Luis Lombana-Larrea has patents CECT 30922, CECT 30923, CECT 30930, CECT 30924 and CECT 13224 pending to FICOSTERRA S.L. The other authors declare that they have no known competing financial interests or personal relationships that could have influenced the work presented in this article.

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References

- Abhinandan K, Skori L, Stanic M, Hickerson NMN, Jamshed M, Samuel MA (2018) Abiotic stress signaling in wheat—an inclusive overview of hormonal interactions during abiotic stress responses in wheat. *Front Plant Sci* 9:734. <https://doi.org/10.3389/fpls.2018.00734>
- Abiala M, Sadhukhan A, Muthuvel J et al (2023) Rhizosphere *Priestia* species altered cowpea root transcriptome and enhanced growth under drought and nutrient deficiency. *Planta* 257:11. <https://doi.org/10.1007/s00425-022-04047-2>
- Abirami TV, Suman A, Singh R, Gond S (2018) Siderophore—the iron chelator production potential of bacteria associated with diverse crops and growth medium optimization for its high production. *Int J Curr Microbiol Appl Sci* 7:1052–1062. <https://doi.org/10.20546/ijemas.2018.707.128>
- Ahmad Z, Waraich EA, Akhtar S et al (2018) Physiological responses of wheat to drought stress and its mitigation approaches. *Acta Physiol Plant* 40:80. <https://doi.org/10.1007/s11738-018-2651-6>
- Ahmad Ansari F, Ahmad I, Pichtel J (2023) Synergistic effects of biofilm-producing PGPR strains on wheat plant colonization, growth and soil resilience under drought stress. *Saudi J Biol Sci* 30:103664. <https://doi.org/10.1016/j.sjbs.2023.103664>
- Al Khallaf H, Rashad HM, Alsamadany H (2025) Biofertilizers mitigate salinity stress: insights from spring wheat physiology and gene expression. *Front Plant Sci* 16:1697160. <https://doi.org/10.3389/fpls.2025.1697160>
- Al-Shammari W, Alshammery K, Lotfi S et al (2025) Wheat salinity tolerance is enhanced by application of *Bacillus megaterium* or arbuscular mycorrhizal fungi via improving physio-biochemical and anatomical characteristics. *BMC Plant Biol* 25:835. <https://doi.org/10.1186/s12870-025-06845-8>
- Alexander D, Zuberer D (1991) Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol Fertil Soils* 12:39–45. <https://doi.org/10.1007/BF00369386>
- Ali OAM (2019) Wheat responses and tolerance to drought stress. In: Hasanuzzaman M, Nahar K, Hossain M (eds) *Wheat production in changing environments*. Springer, Singapore, pp 129–138. http://doi.org/10.1007/978-981-13-6883-7_5
- Aljeddani GS, Hamouda RA, Abdelsattar AM, Heikal YM (2024) Stress-Responsive Gene Expression, Metabolic, Physiological and Agronomic Responses by Consortium Nano-Silica with *Trichoderma* against Drought Stress in Bread Wheat. *Int J Mol Sci* 25(20):10954. <https://doi.org/10.3390/ijms252010954>
- Arkhipova T, Martynenko E, Sharipova G, Kuzmina L, Ivanov I, Garipova M, Kudoyarova G (2020) Effects of plant growth promoting rhizobacteria on the content of abscisic acid and salt resistance of wheat plants. *Plants (Basel)* 9:1429. <https://doi.org/10.3390/plants9111429>
- Aslam M, Waseem M, Jakada BH, Okal EJ, Lei Z, Saqib HSA, Yuan W, Xu W, Zhang Q (2022) Mechanisms of abscisic acid-mediated drought stress responses in plants. *Int J Mol Sci* 23:1084. <https://doi.org/10.3390/ijms23031084>
- Barnawal D, Bharti N, Pandey SS, Pandey A, Chanotiya CS, Kalra A (2017) Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and *TaCTR1/TaDREB2* expression. *Physiol Plant* 161:502–514. <https://doi.org/10.1111/ppl.12614>
- Barquero M, Poveda J, Laureano-Marín AM, Ortiz-Liébana N, Brañas J, González-Andrés F (2022) Mechanisms involved in drought stress tolerance triggered by rhizobia strains in wheat. *Front Plant Sci* 13:1036973. <https://doi.org/10.3389/fpls.2022.1036973>
- Berg A, Sheffield J (2018) Climate change and drought: the soil moisture perspective. *Curr Clim Change Rep* 4:180–191. <https://doi.org/10.1007/s40641-018-0095-0>
- Bi C, Wen X, Zhang X, Liu X (2010) Cloning and characterization of a putative *CTR1* gene from wheat. *Agric Sci China* 9:1241–1250. [https://doi.org/10.1016/s1671-2927\(09\)60213-3](https://doi.org/10.1016/s1671-2927(09)60213-3)
- Bouremani N, Cherif-Silini H, Silini A et al (2024) Osmotolerant plant growth-promoting bacteria mitigate adverse effects of drought stress on wheat growth. *AIMS Microbiol* 10:507–541. <https://doi.org/10.3934/microbiol.2024025>
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Carillo P, Gibon Y (2011) Extraction and determination of proline. *PrometheusWiki*. https://www.researchgate.net/publication/211353600_PROTOCOL_Extraction_and_determination_of_proline. Accessed 10 Jan 2024
- Chalita M, Kim YO, Park S, Oh HS, Cho JH, Moon J, Baek N, Moon C, Lee K, Yang J, Nam GG, Jung Y, Na SI, Bailey MJ, Chun J (2024) EzBioCloud: a genome-driven database and platform for microbiome identification and discovery. *Int J Syst Evol Microbiol* 74:006421. <https://doi.org/10.1099/ijsem.0.006421>
- Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—From genes to the whole plant. *Funct Plant Biol* 30:239–264. <https://doi.org/10.1071/FP02076>
- Crespo-Barreiro A, Mazuecos-Aguilera I, Anta-Fernández F et al (2025) Enhancing drought resistance in olive trees: understanding the synergistic effects of the combination of PGPR and biochar. *J Plant Growth Regul* 44:4383–4395. <https://doi.org/10.1007/s00344-025-11693-9>
- Dong H, Wang Y, Di Y, Qiu Y, Ji Z, Zhou T, Shen S, Du N, Zhang T, Dong X, Guo Z, Piao F, Li Y (2024) Plant growth-promoting rhizobacteria *Pseudomonas aeruginosa* HG28-5 improves salt tolerance by regulating Na⁺/K⁺ homeostasis and ABA signaling pathway in tomato. *Microbiol Res* 283:127707. <https://doi.org/10.1016/j.micres.2024.127707>
- Dworkin M, Foster JW (1958) Experiments with some microorganisms which utilize ethane and hydrogen. *J Bacteriol* 75:592–603. <https://doi.org/10.1128/jb.75.5.592-603.1958>
- Edi-Premono M, Moawad AM, Vlek PLG (1996) Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian J Crop Sci* 11:13–23

- Ferrante R, Campagni C, Vettori C, Checcucci A, Garosi C, Paffetti D (2024) Meta-analysis of plant growth-promoting rhizobacteria interaction with host plants: implications for drought stress response gene expression. *Front Plant Sci* 14:1282553. <https://doi.org/10.3389/fpls.2023.1282553>
- Gadallah M (1999) Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress. *Biol Plant* 42:249–257. <https://doi.org/10.1023/A:1002164719609>
- Gagné-Bourque F, Mayer BF, Charron J-B, Vali H, Bertrand A, Jabaji S (2015) Accelerated growth rate and increased drought stress resilience of the model grass *Brachypodium distachyon* colonized by *Bacillus subtilis* B26. *PLoS ONE* 10:e0130456. <https://doi.org/10.1371/journal.pone.0130456>
- Gontia-Mishra I, Sapre S, Sharma A, Tiwari S (2016) Amelioration of drought tolerance in wheat by the interaction of plant growth-promoting rhizobacteria. *Plant Biol J* 18:992–1000. <https://doi.org/10.1111/plb.12505>
- Gontia-Mishra I, Sapre S, Deshmukh R, Sikdar S, Tiwari S (2020) Microbe-mediated drought tolerance in plants: current developments and future challenges. In: Yadav A, Singh J, Rastegari A, Yadav N (eds) *Plant microbiomes for sustainable agriculture. Sustainable Development and Biodiversity*, vol 25. Springer, Cham, pp 351–379. https://doi.org/10.1007/978-3-030-38453-1_12
- Hanaka A, Ozimek E, Reszczyńska E, Jaroszek-Ścisł J, Stolarz M (2021) Plant tolerance to drought stress in the presence of supporting bacteria and fungi: an efficient strategy in horticulture. *Horticulturae* 7:390. <https://doi.org/10.3390/horticulturae7100390>
- Heath RL, Packer L (2022) Reprint of: Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 726:109248. <https://doi.org/10.1016/j.abb.2022.109248>
- Honma M, Shimomura T (1978) Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric Biol Chem* 42:1825–1831. <https://doi.org/10.1080/00021369.1978.10863261>
- Jakubowicz M, Nowak W, Gałgański Ł, Babula-Skowrońska D (2019) Expression profiling of CTR1-like and EIN2-like genes in buds and leaves of *Populus tremula*, and in vitro study of the interaction between their polypeptides. *Plant Physiol Biochem* 139:660–671. <https://doi.org/10.1016/j.plaphy.2019.04.029>
- Kang SM, Shahzad R, Bilal S et al (2019) Indole-3-acetic-acid and ACC deaminase producing *Leclercia adecarboxylata* MO1 improves *Solanum lycopersicum* L. growth and salinity stress tolerance by endogenous secondary metabolites regulation. *BMC Microbiol* 19:80. <https://doi.org/10.1186/s12866-019-1450-6>
- Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J Appl Microbiol* 96:473–480. <https://doi.org/10.1046/j.1365-2672.2003.02161.x>
- Kim K, Jang YJ, Lee SM, Oh BT, Chae JC, Lee KJ (2014) Alleviation of salt stress by *Enterobacter* sp. EJ01 in tomato and *Arabidopsis* is accompanied by up-regulation of conserved salinity responsive factors in plants. *Mol Cells* 37:109–117. <https://doi.org/10.14348/molcells.2014.2239>
- Kiran S, Furtana GB, Ellialtıoglu ŞŞ (2022) Physiological and biochemical assay of drought stress responses in eggplant (*Solanum melongena* L.) inoculated with commercial inoculant of *Azotobacter chroococcum* and *Azotobacter vinelandii*. *Scientia Hort* 305:111394. <https://doi.org/10.1016/j.scienta.2022.111394>
- Kiran S, Baysal Furtana G, Ellialtıoglu ŞŞ (2025) Inoculation with *Azotobacter* spp. improves eggplant tolerance to combined salt and drought stress. *J Plant Growth Regul* 44:5496–5511. <https://doi.org/10.1007/s00344-025-11775-8>
- Komal L, Kamran A, Jahan S et al (2025) Molecular insights into drought tolerance in wheat through *in-silico* genome-wide analysis of DREB1 transcription factor and peroxidase interactions. *BMC Plant Biol* 25:1158. <https://doi.org/10.1186/s12870-025-06938-4>
- Kour D, Rana KL, Kaur T, Sheikh I, Yadav AN, Kumar V, Dhaliwal HS, Saxena AK (2020) Microbe-mediated alleviation of drought stress and acquisition of phosphorus in great millet (*Sorghum bicolor* L.) by drought-adaptive and phosphorus-solubilizing microbes. *Biocatal Agric Biotechnol* 23:101501. <https://doi.org/10.1016/j.bcab.2020.101501>
- Lastochkina O, Aliniaefard S, Seifikalhor M, Yuldashev R, Pusenkova L, Garipova S (2019) Plant growth-promoting bacteria: biotic strategy to cope with abiotic stresses in wheat. In: Hasanuzzaman M, Nahar K, Hossain M (eds) *Wheat Production in Changing Environments*. Springer, Singapore, pp 579–614. https://doi.org/10.1007/978-981-13-6883-7_23
- Li BB, Zhang SB, Lv YY, Wei S, Hu YS (2022) Reactive oxygen species-induced protein carbonylation promotes deterioration of physiological activity of wheat seeds. *PLoS ONE* 17:e0263553. <https://doi.org/10.1371/journal.pone.0263553>
- Li Y, Han S, Qi Y (2023) Advances in structure and function of auxin response factor in plants. *J Integr Plant Biol* 65:617–632. <https://doi.org/10.1111/jipb.13392>
- Long H, Zheng Z, Zhang Y, Xing P, Wan X, Zheng Y, Li L (2019) An abscisic acid (ABA) homeostasis regulated by its production, catabolism and transport in peanut leaves in response to drought stress. *PLoS ONE* 14:e0213963. <https://doi.org/10.1371/journal.pone.0213963>
- Ma Y, Dias MC, Freitas H (2020) Drought and salinity stress responses and microbe-induced tolerance in plants. *Front Plant Sci* 11:591911. <https://doi.org/10.3389/fpls.2020.591911>
- Manjunatha BS, Nivetha N, Krishna GK, Elangovan A, Pushkar S, Chandrashekar N, Aggarwal C, Asha AD, Chinnusamy V, Rairupia RK, Watts A, Bandeppa S, Dukare AS, Paul S (2022) Plant growth-promoting rhizobacteria *Shewanella putrefaciens* and *Cronobacter dublinensis* enhance drought tolerance of pearl millet by modulating hormones and stress-responsive genes. *Physiol Plant* 174:e13676. <https://doi.org/10.1111/ppl>
- Marik D, Sharma P, Chauhan NS, Jangir N, Shekhawat RS, Verma D, Mukherjee M, Abiala M, Roy C, Yadav P, Sadhukhan A (2024) *Peribacillus frigoritolerans* T7-IITJ, a potential biofertilizer, induces plant growth-promoting genes of *Arabidopsis thaliana*. *J Appl Microbiol* 135:lxae066. <https://doi.org/10.1093/jambio/lxae066>
- Mazuecos-Aguilera I, Anta-Fernández F, Crespo-Barreiro A, Martínez-Quesada A, Lombana-Larrea L, González-Andrés F (2025) Plant growth-promoting rhizobacteria enhanced induced systemic resistance of tomato against *Botrytis cinerea* phytopathogen. *Front Plant Sci* 16:1570986. <https://doi.org/10.3389/fpls.2025.1570986>
- Mellidou I, Ainalidou A, Papadopoulou A, Leontidou K, Genitsaris S, Karagiannis E, Van de Poel B, Karamanoli K (2021) Comparative transcriptomics and metabolomics reveal an intricate priming mechanism involved in PGPR-mediated salt tolerance in tomato. *Front Plant Sci* 12:713984. <https://doi.org/10.3389/fpls.2021.713984>
- Morales M, Munné-Bosch S (2019) Malondialdehyde: facts and artifacts. *Plant Physiol* 180:1246–1250. <https://doi.org/10.1104/pp.19.00405>
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- Naing AH, Maung TT, Kim CK (2021) The ACC deaminase-producing plant growth-promoting bacteria: Influences of bacterial strains and ACC deaminase activities in plant tolerance to abiotic stress. *Physiol Plant* 173:1992–2012. <https://doi.org/10.1111/ppl.13545>
- Nourashrafeddin SM, Ramandi A, Seifi A (2024) Rhizobacteria isolated from xerophyte *Haloxylon ammodendron* manipulate root

- system architecture and enhance drought and salt tolerance in *Arabidopsis thaliana*. *Int Microbiol* 27:337–347. <https://doi.org/10.1007/s10123-023-00394-6>
- Nyaupane S, Poudel MR, Panthi B, Dhakal A, Paudel H, Bhandari R (2024) Drought stress effect, tolerance, and management in wheat – a review. *Cogent Food Agric* 10. <https://doi.org/10.1080/23311932.2023.2296094>
- O'Hara GW, Goss TJ, Dilworth MJ, Glenn AR (1989) Maintenance of intracellular pH and acid tolerance in *Rhizobium meliloti*. *Appl Environ Microbiol* 55:1870–1876. <https://doi.org/10.1128/aem.55.8.1870-1876.1989>
- Peix A, Rivas R, Santa-Regina I (2004) *Pseudomonas lutea* sp. nov., a novel phosphate solubilizing bacterium isolated from the rhizosphere of grasses. *Int J Syst Evol Microbiol* 54:847–850. <https://doi.org/10.1099/ijs.0.02966-0>
- Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol Plant* 118:10–15. <https://doi.org/10.1034/j.1399-3054.2003.00086.x>
- Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45. <https://doi.org/10.1093/nar/29.9.e45>
- Pradhan M, Sahoo RK, Swain DM, Dangar TK, Mohanty S (2018) Inoculation of *Azotobacter vinelandii* (SRI Az3) to rice plant increases stress tolerance in rice plant during drought stress. *ORYZA - Int J Rice* 55:406. <https://doi.org/10.5958/2249-5266.2018.00049.8>
- Prasad JK, Dey R, Pandey P, Raghuwanshi R (2025) Exploring drought tolerance in PGPR-protected wheat plants through physiological and molecular expression. *J Plant Growth Regul* 44:3681–3696. <https://doi.org/10.1007/s00344-025-11649-z>
- Rajawat MVS, Singh S, Tyagi SP, Saxena AK (2016) A modified plate assay for rapid screening of potassium-solubilizing bacteria. *Pedosphere* 26:768–773. [https://doi.org/10.1016/S1002-0160\(15\)60080-7](https://doi.org/10.1016/S1002-0160(15)60080-7)
- Ramya P, Gomathi V, Devi RP et al (2021) *Pichia kudriavzevii*—A potential soil yeast candidate for improving soil physical, chemical and biological properties. *Arch Microbiol* 203:4619–4628. <https://doi.org/10.1007/s00203-021-02447-8>
- Raza A, Mehmood SS, Tabassum J, Batool R (2019) Targeting plant hormones to develop abiotic stress resistance in wheat. In: Hasanuzzaman M, Nahar K, Hossain M (eds) *Wheat Production in Changing Environments*. Springer, Singapore, pp 557–577. https://doi.org/10.1007/978-981-13-6883-7_22
- Rijal B, Baduwal P, Chaudhary M, Chapagain S, Khanal S, Khanal S, Poudel PB (2021) Drought stress impacts on wheat and its resistance mechanisms. *Malays J Sustain Agric* 5:67–76. <https://doi.org/10.26480/mjsa.02.2021.67.76>
- Salem MA, Ismail M, Radwan KH, Abd-Elhalim HM (2024) Unlocking the potential of plant growth-promoting rhizobacteria to enhance drought tolerance in Egyptian wheat (*Triticum aestivum*). *Sustainability* 16:4605. <https://doi.org/10.3390/su16114605>
- Sallam A, Alqudah AM, Dawood MFA, Baenziger PS, Börner A (2019) Drought stress tolerance in wheat and barley: Advances in physiology, breeding, and genetics research. *Int J Mol Sci* 20:3137. <https://doi.org/10.3390/ijms2013137>
- Sarma RK, Saikia R (2014) Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. *Plant Soil* 377:111–126. <https://doi.org/10.1007/s11104-013-1981-9>
- Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* 160:47–56. [https://doi.org/10.1016/0003-2697\(87\)90612-9](https://doi.org/10.1016/0003-2697(87)90612-9)
- Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH, Battaglia ML (2021) Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants (Basel)* 10:259. <https://doi.org/10.3390/plants10020259>
- Shahid M, Zeyad MT, Syed A, Singh UB, Mohamed A, Bahkali AH, Elgorban AM, Pichtel J (2022) Stress-tolerant endophytic isolate *Priestia aryabhatai* BPR-9 modulates physio-biochemical mechanisms in wheat (*Triticum aestivum* L.) for enhanced salt tolerance. *Int J Environ Res Public Health* 19:10883. <https://doi.org/10.3390/ijerph191710883>
- Shirinbayan S, Khosravi H, Malakouti MJ (2019) Alleviation of drought stress in maize (*Zea mays*) by inoculation with *Azotobacter* strains isolated from semi-arid regions. *Appl Soil Ecol* 133:138–145. <https://doi.org/10.1016/j.apsoil.2018.09.015>
- Sjulgård H, Graf LV, Colombi T et al (2025) Earth observation reveals reduced winter wheat growth and the importance of plant available water during drought. *Biogeosciences* 22:1341–1354. <https://doi.org/10.5194/bg-22-1341-2025>
- Spormann S, Nadais P, Sousa F, Pinto M, Martins M, Sousa B, Fidalgo F, Soares C (2023) Accumulation of proline in plants under contaminated soils—Are we on the same page? *Antioxid (Basel)* 12:666. <https://doi.org/10.3390/antiox12030666>
- Srivastava N (2019) Molecular and biotechnological tools in developing abiotic stress tolerance in wheat. In: Hasanuzzaman M, Nahar K, Hossain M (eds) *Wheat Production in Changing Environments*. Springer, Singapore, pp 283–341. https://doi.org/10.1007/978-981-13-6883-7_13
- Talaat NB (2023) Role of phytohormones in regulating abiotic stresses in wheat. In: Khan MK, Pandey A, Hamurcu M, Gupta OP, Gezgün S (eds) *Abiotic Stresses in Wheat*. Academic, London, pp 111–130. <https://doi.org/10.1016/B978-0-323-95368-9.00019-9>
- Tounekti T, Vadel AM, Oñate M, Khemira H, Munné-Bosch S (2011) Salt-induced oxidative stress in rosemary plants: Damage or protection? *Environ Exp Bot* 71:298–305. <https://doi.org/10.1016/j.envexpbot.2010.12.016>
- Umezawa T, Okamoto M, Kushihiro T, Nambara E, Oono Y, Seki M, Kobayashi M, Koshiba T, Kamiya Y, Shinozaki K (2006) CYP707A3, a major ABA 8'-hydroxylase involved in dehydration and rehydration response in *Arabidopsis thaliana*. *Plant J* 46:171–182. <https://doi.org/10.1111/j.1365-3113X.2006.02683.x>
- Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sci* 151:59–66. [https://doi.org/10.1016/S0168-9452\(99\)00197-1](https://doi.org/10.1016/S0168-9452(99)00197-1)
- Wahab A, Abdi G, Saleem MH, Ali B, Ullah S, Shah W, Mumtaz S, Yasin G, Muresan CC, Marc RA (2022) Plants' physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. *Plants (Basel)* 11:1620. <https://doi.org/10.3390/plants11131620>
- Wang YL, Xu RG, He ZH (2025) Strategies to improve wheat's drought and heat resistance. *Triticeae Genomics Genet* 16:166–174. <https://doi.org/10.5376/tgg.2025.16.0018>
- Zhang HX, Blumwald E (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nat Biotechnol* 19:765–768. <https://doi.org/10.1038/90824>
- Zhang Y, Luan Q, Jiang J, Li Y (2021) Prediction and utilization of malondialdehyde in exotic pine under drought stress using near-infrared spectroscopy. *Front Plant Sci* 12:735275. <https://doi.org/10.3389/fpls.2021.735275>
- Zhang Y, Zheng L, Yun L, Ji L, Li G, Ji M, Shi Y, Zheng X (2022) Catalase (CAT) gene family in wheat (*Triticum aestivum* L.): Evolution, expression pattern and function analysis. *Int J Mol Sci* 23:542. <https://doi.org/10.3390/ijms23010542>
- Zhang L, Pan Y, Qi Y, Bai J, Han D (2025) Study on the isolation of rhizosphere bacteria and the mechanism of growth promotion in winter wheat in response to drought stress. *Front Plant Sci* 16:1595554. <https://doi.org/10.3389/fpls.2025.1595554>

- Zheng Y, Huang Y, Xian W, Wang J, Liao H (2012) Identification and expression analysis of the *Glycine max* CYP707A gene family in response to drought and salt stresses. Ann Bot 110:743–756. <https://doi.org/10.1093/aob/mcs133>
- Zulfiqar B, Raza MAS, Saleem MF et al (2024) Absciscic acid improves drought resilience, growth, physio-biochemical and quality

attributes in wheat (*Triticum aestivum* L.) at critical growth stages. Sci Rep 14:20411. <https://doi.org/10.1038/s41598-024-71404-4>

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