Journal of Plant Physiology 270 (2022) 153629

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

The cytokinin-producing plant beneficial bacterium *Pseudomonas fluorescens* G20-18 primes tomato (*Solanum lycopersicum*) for enhanced drought stress responses

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ARTICLE INFO

Keywords:
Drought stress
Plant growth-promoting rhizobacteria (PGPR)
Carbohydrate and antioxidant metabolism
Transcriptome analyses
P. fluorescens

ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are known for exerting beneficial effects on plant growth and tolerance to plant pathogens. However, their specific role in mediating protection against abiotic stress remains underexplored. The aim of this study was to characterise the ability of the cytokinin-producing beneficial bacterium *Pseudomonas fluorescens* G20-18 to enhance tomato growth and boost tolerance to drought stress. Tomato seedlings were root inoculated and their growth and physiological and molecular responses assessed under well-watered conditions and also in response to progressive drought stress and a subsequent recovery period. Root inoculation with G20-18 had a significant positive impact on tomato growth. Furthermore, G20-18 inoculated and drought-stressed plants showed higher leaf chlorophyll and abscisic acid (ABA) content and stomatal closure than non-inoculated controls. Root inoculation also increased the activity of different carbohydrate metabolism enzymes, which are important for root and leaf growth and development in drought stressed plants. A significant increase in the activity of different antioxidant enzymes and total antioxidant capacity correlated with elevated levels of relevant secondary metabolites, such as phenolics, anthocyanins and flavonoids. RNA sequencing revealed distinct qualitative and quantitative differences in gene regulation in response to G20-18. Notably, the number of genes differentially regulated in response to G20-18 was approximately sevenfold higher during drought stress, indicating that root inoculation with the bacteria primed the plants for a much stronger transcriptionally regulated systemic drought stress response. The regulated genes are related to phenylalanine metabolism and other key processes linked to plant growth, development and drought stress resilience. A role of the ability of G20-18 to produce the plant hormone cytokinin for interaction with tomato was established by the cytokinin-deficient biosynthesis mutants CNT1 and CNT2. In comparison with G20-18, the inoculation of plants with CNT1 resulted in a reduced number of differentially regulated genes. The relative change was most prominent under well-watered conditions with a 85 % reduction, corresponding to 462 genes. However, under drought conditions the absolute number of differentially regulated genes was reduced by even 2219 in response to the CNT1 mutant. The relevance of the ability of G20-18 to produce cytokinins for interaction with plants was also evident from differences in growth and specific cell and ecophysiological parameters in response to CNT1 and CNT2. These findings provide novel insights about G20-18's ability to improve drought stress responses and the role of interkingdom signalling by bacterial-derived cytokinins, and contribute to enhance the robustness of the practical application of these microorganisms to improve crop resilience in agricultural production.

1. Introduction

Abiotic stresses are one of the main constraints to crop production and global food security (Fahad et al., 2017). As agriculture continues to be impacted by climate change, drought, salinity and heat stress have become the most significant limiting factors to crop production and food security (Raza et al., 2019). Drought is expected to cause serious

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https://doi.org/10.1016/j.jplph.2022.153629
Received 23 June 2021; Received in revised form 24 December 2021; Accepted 19 January 2022
Available online 31 January 2022
0176-1617/© 2022 Published by Elsevier GmbH.

List of abbreviations:

ABA	abscisic acid
CK	cytokinin
WW	well watered
DS	drought stress
RCF	relative centrifugal force
OD	optical density
ROS	reactive oxygen species
PGPR	plant growth promoting rhizobacteria
SPAD	Soil Plant Analysis Development
ACC	1-aminocyclopropane-1-carboxylic acid
LB	Luria–Bertani medium
EDTA	Ethylenediaminetetraacetic acid
TRIS-HCl	Tris hydrochloride,
NADP	Nicotinamide adenine dinucleotide phosphate
AsA-GSH	Asada–Halliwell pathway
UGPase	UDP-glucose pyrophosphorylase
FK	fructokinase
HXK	hexokinase
PFK	phosphofructokinase
AGPase	ADP-glucose pyrophosphorylase
SuSy	sucrose synthase
cytInv	cytoplasmic invertase
vacInv	vacuolar invertase
cwInv	cell wall invertase
APX	ascorbate peroxidase
CAT	catalase
POX	cytoplasmic peroxidases
GR	glutathione reductase
SOD	superoxide dismutase
DHAR	dehydroascorbate reductase
MDHAR	monodehydroascorbate reductase
GST	glutathione S transferase
cwPOX	cell wall peroxidase
KEGG	Kyoto Encyclopedia of Genes and Genomes
TEAC	Trolox equivalent antioxidant capacity

plant-growth problems for more than 50 % of arable land by 2050 (Farooq et al., 2009; Hussain et al., 2018; Vinocur and Altman, 2005) and is considered the single most devastating environmental stress (Farooq et al., 2009). It leads to oxidative stress from increased levels of reactive oxygen species (ROS) as a direct result of the imbalance in electron transport rates and the metabolic consumer activity of the reductive power (Tripathy and Oelmuller, 2012).

Plants adapt to tolerate severe environmental conditions by adopting several sophisticated physiological, cellular and molecular mechanisms to maintain homeostasis (Kasim et al., 2012; Urano et al., 2010). To mitigate drought stress, plants employ different ecophysiological mechanisms, such as enhancing their photosynthetic rate, stomatal closure, and increasing leaf chlorophyll and abscisic acid (ABA) content (Brilli et al., 2019; Farooq et al., 2009; Mukhtar et al., 2020). Moreover, plants have developed advanced antioxidant defence systems in both enzymatic and non-enzymatic forms (Mukhtar et al., 2020). The enzymatic mechanisms include induction of antioxidant metabolism enzymes to remove excess ROS (Asada, 2006; Mukhtar et al., 2020). Among the non-enzymatic mechanisms, increasing levels of antioxidant secondary metabolites, such as phenolics, anthocyanin and flavonoids, serve to detoxify ROS (Asada, 2006). During progressive drought, plants face stress through the accumulation of high intracellular levels of osmoprotectant compounds to protect cellular components and restore the osmotic balance, altering their metabolism to better cope with stress rather than growth (Singh et al., 2015). Among these compatible osmolytes, sugars are the main components (Gurrieri et al., 2020). In plants, sugars are used as substrates for intermediate metabolism and as signalling molecules, thus connecting the carbon nutrient level with growth and development (Bolouri-Moghaddam et al., 2010).

Plant growth-promoting rhizobacteria (PGPR) are an indispensable part of the rhizospheric biota and are beneficial bacteria that colonise plant roots (Mukhtar et al., 2020; Rubin et al., 2017). Induced changes in different carbohydrate metabolism enzymes, ROS-scavenging antioxidant enzymes and enhanced photosynthetic efficiency have been reported in PGPR-inoculated plants, helping them cope with drought stress (Batool et al., 2020; Brilli et al., 2019; Mannino et al., 2020; Mukhtar et al., 2020; Sarker and Oba, 2018). In order to identify plant genes that play an essential role in the efficiency of the plant-microbe interaction, gene expression profiling has been used under drought stress (Kandasamy et al., 2009; SkZ et al., 2018; Yuwono et al., 2005). Furthermore, *P. simiae* WCS417 enhances growth by inducing the major transcriptional changes in plant sugar transport as well as several hormonal pathways linked not only to plant growth and development, but also to abiotic and biotic stress responses (Desrut et al., 2020). Notably, the PGPR strain not only triggers transcriptional changes in the pathways of the plant growth-related hormones, but also in the biosynthesis and signalling pathways of phytohormones commonly involved in plant responses to abiotic and biotic stress (Desrut et al., 2020; Zamioudis et al., 2013, 2015). Increased mRNA transcripts of a drought-responsive gene, carbohydrate metabolism-associated genes, and membrane transport genes have been found to enhance drought tolerance in PGPR-inoculated *A. thaliana* and *Zea mays* plants (SkZ et al., 2018; Timmusk and Wagner, 1999). Studies using Illumina sequencing (HiSeq 2000 system) have revealed that abscisic acid (ABA) signalling genes in sugarcane inoculated with *G. diazotrophicus* confer drought tolerance (Vargas et al., 2014).

Tomato (*Solanum lycopersicum* L.) is the world's most cultivated solanaceous vegetable crop and is of major importance to the agricultural industry (Mukhtar et al., 2020). Tomato production is currently constrained by numerous biotic and abiotic factors, including drought (Wang et al., 2019). Different options have been identified for conferring drought tolerance on agriculturally important crops (Brilli et al., 2019; Fleury et al., 2010; Niu et al., 2017; Timmusk et al., 2014), but drought tolerance mechanisms make the task of introducing new tolerant varieties very difficult, and there is poor acceptance of genetically-modified plants in some regions (Wahid et al., 2007).

An increased mechanistic understanding of the beneficial interactions between tomato and the PGPR associated with its roots may improve the future use of these bacteria for plant growth promotion and improvement of plant stress resilience (Ben Abdallah et al., 2018). Based on bioinformatic evidence and metagenome sequencing, the recognised ability of plant-beneficial *Pseudomonas* to produce the phytohormone cytokinin (CK) seems to be more widespread than previously thought. We have shown that the biocontrol ability of *P. fluorescens* G20-18 against a bacterial pathogen depends on the ability to produce CK (Akhtar et al., 2019; García de Salamone et al., 2001; Grosskinsky et al., 2016). Since CKs are known to be the key hormones for growth regulation and are also involved in drought stress protection in plants, the aim of this study was to explore the potential of the CK-producing bacterium *P. fluorescens* G20-18 to promote growth and enhance drought stress tolerance in tomato. Another objective was to functionally reveal the specific role of CKs in these processes by comparing the wild type strain with two TnphoA-derived mutants, CNT1 and CNT2, that have been shown to have a reduced capacity to produce CK (García de Salamone et al., 2001; Grosskinsky et al., 2016). It was hypothesised that CK produced by G20-18 affects plant growth positively and

improves the drought stress tolerance of tomato. In the current study, tomato roots were inoculated with the G20-18 wild type and the two CK deficient mutants, and plant growth as well as plant ecophysiological, metabolic, and biochemical changes against progressive drought stress were determined. These targeted approaches were complemented by non-targeted analyses of the impact on changes in the tomato transcriptome.

2. Materials and methods

2.1. Plant growth, bacterial strains and root inoculation

Tomato (*Solanum lycopersicum* L.) variety Moneymaker (supplied by Kings Seed, UK) was used in this study. Seeds were sown on seedling trays filled with peat soil and allowed to germinate for two weeks under greenhouse conditions. The relative humidity ranged between 65 % and 75 %, the temperature was 22 ± 2 °C, and a 16/8 h day/night and light regime was used. After two weeks from the date of sowing, uniformly-sized plants were selected and used for root inoculation. Cytokinin-producing *P. fluorescens* G20-18 and the two TnphoA-derived mutants (CNT1 and CNT2) were obtained from strain collections at the Department of Plant and Environmental Sciences, University of Copenhagen, Denmark. Bacterial strains were removed from glycerol stock (−80 °C) and plated on Lysogeny Broth (LB) medium supplemented with 20 mg L^{-1} gentamycin, and routinely grown at 28 °C for 48 h for various experiments. The liquid bacterial cultures were prepared by inoculating liquid LB medium with a freshly grown colony of bacteria and incubating the culture overnight under agitation at 200 rpm at 28 °C. The broth culture was centrifuged at 3077 rcf for 10 min and the supernatant was discarded. The pellet was resuspended in 10 mM $MgCl_2$. The optical density at 600 nm (OD_{600}) was adjusted to 0.2 (corresponding to ca. 1X 10^9 CFU/mL). Root inoculation was performed by taking the two-week-old seedlings after shaking away the soil adhering to the roots, and dipping the roots into the bacterial solution. For control plants, seedlings were inoculated with 10 mM $MgCl_2$ (Mangmang et al., 2016). Then plants were immediately transferred to 4-L pots containing non-sterilised field soil (sandy loam soil from the University of Copenhagen field station, Taastrup, Denmark) and allowed to grow in the above-mentioned greenhouse conditions. Two separate experiments were conducted to evaluate the growth-promoting effect of the bacterial strains by taking biomass and plant height measurements after four weeks of root inoculation from five plants per treatment.

2.2. Drought stress exposure

After three weeks (from the time of root inoculation), the weight of all pots (24 plants in each treatment) were measured and the plants were exposed to well-watered (WW) or drought stress (DS) treatments. Two separate drought experiments were conducted in a similar way. In the WW treatment, plants were irrigated at 90 % of pot water-holding capacity, while the irrigation was withheld in the DS plants until the daily water loss was 10 % of the water consumed by the WW control plants. Irrigation was subsequently resumed for the DS plants to 90 % of water-holding capacity for five days of drought stress recovery. During the treatment period, leaf chlorophyll content (SPAD) measurements were taken before drought conditions were initiated, at the end of DS, and after drought recovery in both WW and DS treated plants. Stomatal conductance was measured every third day throughout the period of progressive drought. Root and shoot fresh weight were measured immediately after sampling, and dry weight was measured after 72 h at 70 °C. During these harvests, leaf (the third and fourth leaves from the top) and rhizosphere (soil adhered with root tissue) sampling was also undertaken. All samples were snap frozen with liquid nitrogen and subsequently stored at −80 °C.

2.3. Carbohydrate and antioxidant metabolic enzyme assays

2.3.1. Sample extractions

Sample extractions were performed according to the protocol of Jammer et al. (Jammer et al., 2015) from samples of three biological replicates from each treatment. Briefly, frozen tomato leaf samples were homogenised in liquid nitrogen with 0.1 % Polyvinylpolypyrrolidone (PVPP). Approximately 500 mg of plant material was used for extractions and all subsequent steps were performed on ice and/or in cooled incubation rooms (4 °C), using pre-cooled liquids. The ground plant material was extracted with 1 mL of extraction buffer (40 mM TRIS-HCl pH 7.6, 1 mM EDTA, 0.1 mM PMSF (phenylmethylsulfonyl fluoride), 1 mM benzamidine, 24 μM NADP, 14 mM β-mercaptoethanol) on ice. The homogenate was centrifuged at 4 °C and 13,680 rcf for 10 min until a solid pellet and clear supernatant were obtained, and the pellet was kept on ice. The supernatant (crude extract) containing the soluble proteins was subsequently centrifuged at 4 °C and 13,680 rcf to remove all remaining particles. The original pellet, which was kept on ice, was washed three times in distilled water by centrifugation at 4 °C and 11, 400 rcf for 5 min, and then re-suspended in 1 mL of high salt buffer (1 M NaCl, 40 mM TRIS-HCl pH 7.6, 3 mM $MgCl_2$ and 1 mM EDTA). The sample was then mixed by continuous shaking at 4 °C overnight and the homogenate was centrifuged twice at 4 °C and 13,680 rcf for 10 min. This supernatant was the cell wall extract containing proteins that were ionically bound to the cell walls. Lastly, the crude extract and cell wall extract were dialysed overnight against 20 mM potassium phosphate buffer (pH 7.4) at 4 °C (changing the buffer three times). All extracts were stored at −20 °C in small aliquots until further use.

2.3.2. Carbohydrate and antioxidant metabolic enzyme assays

Nine carbohydrate metabolic enzymes were selected to determine their activity in leaf tissue. The dialysed crude extract was used to estimate UDP-glucose pyrophosphorylase (UGPase), fructokinase (FK), hexokinase (HXK), phosphofructokinase (PFK), ADP-glucose pyrophosphorylase (AGPase), sucrose synthase (SuSy), cytoplasmic invertase (cytInv) and vacuolar invertase (vacInv), and the cell wall extract was used to assess the activity of cell wall invertase (cwInv). Their activities were determined following the methods of Jammer et al. (Jammer et al., 2015). Either an endpoint or a kinetic assay was used (Gasperl et al., 2015; Jammer et al., 2015; Li et al., 2019).

The methodology described by Fimognari et al. (Fimognari et al., 2020) was used to determine the activities of nine antioxidant metabolism enzymes, applying the modifications described by Garcia-Lemos et al. (2019), Akhtar et al. (2020) and Shokat et al. (2020). The activities of the antioxidative enzymes ascorbate peroxidase (APX), catalase (CAT), cytoplasmic peroxidases (POX), glutathione reductase (GR), superoxide dismutase (SOD), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione S transferase (GST) were determined photometrically by kinetic assays in a miniaturised 96-well plate format, following the approach presented in Fimognari et al. (Fimognari et al., 2020), from the dialysed crude extract, and cell wall peroxidase (cwPOX) activity was determined from the cell wall extract.

For both carbohydrate and antioxidant metabolic enzymes, the dynamics of absorbance at a given wavelength was monitored using an Epoch Take3 spectrophotometer (Biotek, Bad Friedrichshall, Germany) with a 96-well microtitre format. The analyses of enzyme activities for each sample were run in triplicates with blanks. The specific enzyme activity normalised by plant tissue fresh weight (FW) was calculated within the linear range of substrate conversion and expressed in nkat mg^{-1}FW.

2.4. Total antioxidant capacity and phenolic, flavonoid and anthocyanin content

From frozen tomato leaf samples, 400 mg homogenised powder was

mixed with 100 % methanol, vortexed for 10 s, and subsequently incubated with shaking/agitating for 1 h at 4 °C. After centrifugation for 5 min at 13,680 rcf and 4 °C, the supernatant was taken as the first extract. The procedure was repeated, and the second methanol extract was mixed with the first extract and stored at −20 °C to determine antioxidant capacity levels and total anthocyanin, flavonoid and phenolic contents (Francesca et al., 2020). Total non-enzymatic antioxidant capacity was measured using Trolox equivalent antioxidant capacity (TEAC) assays. These were performed according to a modified version of Cai et al. (2004), using gallic acid as a reference material. Briefly, 2.5 µL extract was diluted by adding 7.5 µL methanol. Then 190 µL of 50 µM ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was added and the sample was incubated for 6 min at room temperature. Thereafter the absorbance was measured at 734 nm in 96 well-microtitre formats (same settings as the enzyme assays).

To determine the total phenolic content, 10 µL of methanol extract was diluted (1:15) by adding 140 µL MilliQ water, followed by the addition of 12.5 µL Folin (Folin-Ciocalteu's phenol reagent). Then 37.5 µL Na_2CO_3 were added in all wells. After incubation for 2 h at room temperature, the absorbance was measured at 765 nm (Cai et al., 2004; Khan et al., 2020). To determine the total flavonoid content, 10 µL of methanol extract was diluted (1:2) by adding 10 µL methanol. Then 60 µL $NaNO_2$ was added to all wells, followed by 6 min incubation at room temperature. Following incubation, 60 µL $AlCl_3$ was added followed by a further incubation for 5 min at room temperature. Finally, 60 µl 0.7 M NaOH was added and absorbance was measured at 510 nm (Khodaie et al., 2012). To determine the total anthocyanin content, 5 µL of methanol extract was diluted (1:4) by adding 15 µL methanol. Then 180 µL KCl and 180 µL of sodium acetate was added. The absorbance was measured at first maximum (520 nm) and second maximum (700 nm) (Sohail Akh et al., 2020). All the capacity/contents were calculated based on the fresh weight of sample material used.

2.5. Determination of abscisic acid (ABA) content

For this analysis, 30 mg of frozen leaf powder samples was incubated in 1.5 mL distilled water overnight at 4 °C with agitation at 50 rpm. The samples were then centrifuged at 15,960 rcf for 5 min at 4 °C, and the supernatant was taken for ABA determination. ABA concentration in leaf samples was determined through an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody for ABA (AFRCMAC252) (Asch, 2000).

2.6. RNA extraction and transcriptional analysis by RNA sequencing

Total RNA was isolated from WW, DS, DS recovery and bacterial inoculated (G20-18 and CNT1) WW, DS, DS recovery leaf samples using the TRItidy G™ (AppliChem GmbH, Darmstadt, Germany) and Qiagen RNeasy Plant Mini kit (Qiagen), according to the manufacturers' protocol. The integrity and quality of total RNA were assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA) and RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA).

RNA-seq was performed by the Novogene company (Cambridge, United Kingdom) on three biological replicates for each treatment. One µg RNA per sample was used to generate sequencing libraries with the NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA), following the manufacturer's recommendations. Purified mRNA was isolated from total RNA using poly-T oligo-attached magnetic beads. Following purification, the mRNA was fragmented in NEBNext First Strand Synthesis Reaction Buffer (5×), and the cleaved RNA fragments were copied into the first-strand cDNA using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H-). Second-strand cDNA synthesis was performed using DNA Polymerase I and RNase H. Blunt ends were generated using exonuclease/polymerase activities for remaining overhangs. After adenylation, the NEBNext Adaptor with a hairpin loop structure was ligated to prepare for hybridisation. Purified library fragments were then enriched by PCR to create the final cDNA library. Lastly, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system. The filtered reads were mapped to the *S. lycopersicum* genome. The gene expression levels were estimated by counting the read numbers mapped for each gene, including known and novel genes. In order to compare the gene expression levels between two conditions/groups with three biological replicates per condition/group, the DESeq2 R package was used. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis was used to identify significantly enriched metabolic pathways or signalling pathways associated with differentially expressed genes compared with the whole genome background.

2.7. Statistical analysis

Analysis of variance (one-way ANOVA) for biomass, plant height, stomatal conductance, leaf chlorophyll and ABA content was performed using Statistix v.8 from five biological replicates (differences were considered significant at $p \leq 0.05$ by Tukey's HSD test). The same software was used to perform LSD All-Pairwise comparisons test of all enzymatic and non-enzymatic data based on the mean values of three biological replicates. Differences were considered statistically significant at $p \leq 0.05$. The functions *devtools, factoextar and fviz_pca_biplot* were used to undertake principal component analysis for all carbohydrate and antioxidant metabolism enzyme activities in leaves in RStudio 1.0.153. The function *PerformanceAnalytics* was used to perform the combined Pearson correlation using the same software.

3. Results

3.1. Growth promotion effect of G20-18

To determine the effect of *P. fluorescens* G20-18 (wild type) and the two derived mutants CNT1 and CNT2 with impaired CK production on plant growth, biomass and plant height were determined four weeks after root inoculation of seedlings. A general trend of higher shoot and root weight compared with non-inoculated plants was observed following root inoculation with the G20-18 wild type strain (Fig. 1A, Fig. 1B, Fig. S1A and Fig. S1B). However, only the positive effect on shoot fresh weight was statistically significant. Inoculation with the mutant strains CNT1 and CNT2 resulted in significantly increased shoot dry weight (Fig. 1A) and shoot fresh weight (Fig. S1A) compared with non-inoculated plants. These mutant strains also led to increased root dry weight (Fig. 1B) and significantly increased root fresh weight (Fig. S1B). A positive bacterial effect on shoot growth was also observed as a significant effect on plant height after inoculation with G20-18, CNT1 and CNT2 (Fig. 1C), but the effect of CNT1 was significantly smaller than that of G20-18 and CNT2. These data show that both the wild type and the two mutant strains stimulated growth although the positive impact was stronger in plants inoculated with any of the two CK deficient strains.

3.2. Impact of G20-18 on ecophysiological parameters under well-watered, drought and drought recovery conditions

To assess the impact of G20-18 on drought stress responses, the tomato plants were subjected to a progressive drought stress three weeks after root inoculation of seedlings. Watering was resumed when the daily water loss was 10% of the water consumption of the WW control plants and plant responses were assessed till day five of the recovery period.

3.2.1. Leaf chlorophyll content

Relative leaf chlorophyll content, a parameter used to evaluate stress intensity, was determined using SPAD before DS, after DS and after DS

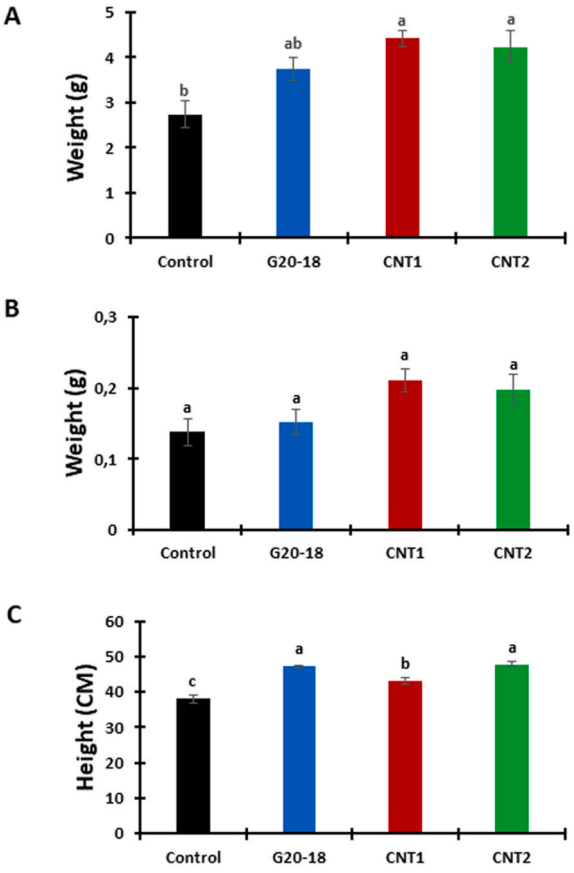


Fig. 1. Growth promotion effect of the bacterial strains (*Pseudomonas fluorescens* G20-18, CNT1 and CNT2) under standard greenhouse conditions four weeks after root inoculation, where (A) is shoot dry weight, (B) root dry weight and (C) plant height. The values are mean ±SE (standard error) and different letters show significant differences at $p \leq 0.05$ by Tukey's HSD test.

recovery. There was no significant difference in chlorophyll content between G20-18, CNT1 and CNT2 inoculated and non-inoculated plants before DS (Fig. 2A). However, after DS, all G20-18, CNT1 and CNT2 inoculated and stressed plants showed significant increases in chlorophyll content compared with the control plants. In the well-watered (WW) plants, the difference in chlorophyll content was less prominent (Fig. 2B). During recovery from drought stress, all G20-18, CNT1 and CNT2 inoculated and previously drought-stressed plants also showed significant increases in their leaf chlorophyll content, and here the same differences were seen as for WW plants (Fig. 2C). Across all experiments, there were no significant differences between plants inoculated with the CK-producing G20-18 and CK-deficient mutants CNT1 and CNT2, suggesting that CK production does not have a major impact on the relative leaf chlorophyll content.

3.2.2. Stomatal conductance

Stomatal aperture plays a major role in adjusting photosynthetic and transpiration rates in plants. Stomatal conductance was measured using a leaf porometer every third day, starting from the day of the start of DS up to the end of DS recovery. During the progressive drought, the DS plants (Fig. 3B) decreased stomatal conductance compared with the WW plants (Fig. 3A). Towards the end of drought stress (D9 and D11), G20-18 inoculated plants had lower stomatal conductance than control DS plants, but CNT1 and CNT2 inoculated DS plants showed far lower stomatal conductance than G20-18 inoculated and control DS plants. Overall, during progression of DS, CNT2 inoculated DS plants showed a more rapid decrease in stomatal conductance compared with other inoculated and non-inoculated DS plants (D5, D7, D9 and D11). When

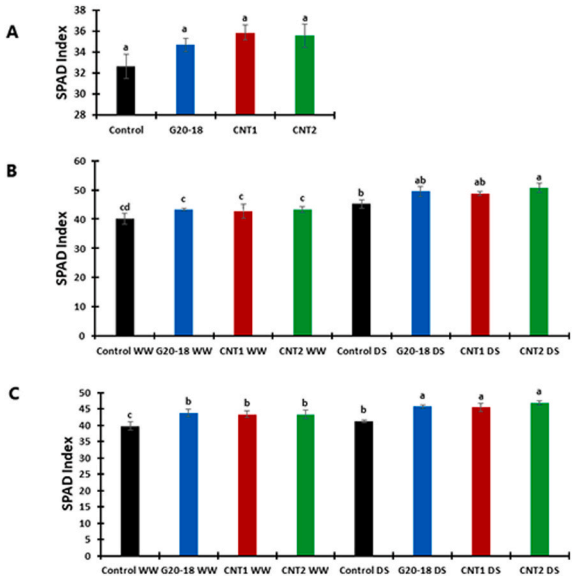


Fig. 2. Leaf chlorophyll content of tomato plants inoculated with different bacterial strains (*Pseudomonas fluorescens* G20-18, CNT1 and CNT2) under well-watered and drought-stress conditions (A) before drought, (B) after drought stress, and (C) after drought stress recovery from five plants in each treatment. The values are mean ±SE (standard error) and different letters show significant differences at $p \leq 0.05$ by Tukey's HSD test.

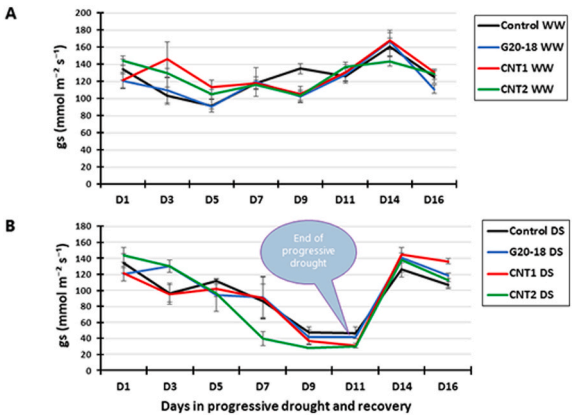


Fig. 3. Stomatal conductance (gs) of tomato plants as influenced by different bacterial strains under (A) well-watered (WW) and (B) drought-stressed (DS) conditions (D1: before drought stress starts, D11: end of drought stress, D16: end of drought stress recovery). The only statistically significant differences in drought-stressed plants were found at D7 (CNT2), D9 and D11, where inoculated plants had significantly less stomatal conductance than control DS plants at $p \leq 0.05$ by Tukey's HSD test.

watering was resumed for DS plants, stomatal conductance increased compared with the level measured for WW plants (D14 and D16). Across all experiments, root inoculation with G20-18 resulted in lower leaf stomatal conductance and faster recovery. This specific impact on stomata aperture was even more pronounced in plants inoculated with the two cytokinin deficient mutants CNT1 and CNT2.

3.2.3. Abscisic acid (ABA) content

ABA plays a key role in regulating stomatal conductance and molecular-mediated responses under DS (Saradadevi et al., 2017). The ABA content was quantified from leaf samples taken before DS, after DS and after DS recovery. After DS, a significant increase was observed for G20-18 inoculated plants compared with non-inoculated plants; CNT1 and CNT2 inoculation also resulted in significant increases in ABA

content (Fig. 4). No significant differences were seen between plants inoculated with G20-18 and mutants CNT1 and CNT2. For the WW plants, there was no significant effect of inoculation on the ABA content. Moreover, no significant effects of inoculation were observed before DS or after DS recovery (Figs. S2A and S2B).

3.3. Impact of G20-18 on cell physiological parameters under well-watered, drought and drought recovery conditions

3.3.1. Activities of key enzymes of carbohydrate metabolism

The activities of nine key enzymes of the primary carbohydrate metabolism were determined. These catalyse cleavage of the main transport sugar, sucrose, and are involved in further metabolism of the resulting hexose by glycolysis and the oxidative pentose phosphate pathway. With the method used one single extraction enables determination of all the enzyme activities listed in the material and methods section (Jammer et al., 2015). The enzyme activity assays were carried out in a microtiter plate format that allow 96 kinetic assays to be performed in parallel. Data for each enzyme activity are presented in a table with colour schemes and statistical significance indicated (Table 1). Before DS, all inoculated plants showed significantly lower activity of vacInv than non-inoculated plants. Furthermore, CNT2 inoculated plants had significantly lower activities of cwInv and vacInv. Overall, there were some significant differences between G20-18, CNT1 and CNT2 inoculated plants since G20-18 inoculation resulted in higher activities of PFK, UGPase, cwInv and cytInv.

After DS, G20-18 inoculated plants showed significantly higher activity of PFK, UGPase and cytInv than non-inoculated DS plants. Inoculation with CNT2 caused a significantly higher activity of AGPase, UGPase and vacInv, while CNT1 inoculation had a significant positive effect on UGPase and SuSy activity at the same stage compared with the control DS plants. There were significant differences between G20-18, CNT1 and CNT2 inoculated plants in the activity of PFK, vacInv and cytInv, while UGPase was the only enzyme significantly increased by all the three strains compared with non-inoculated DS plants (Table 1 and Fig. 5A). After DS recovery, G20-18 inoculated plants showed significantly higher activity of AGPase, UGPase cwInv and cytInv than non-inoculated DS plants. For comparison, CNT1 inoculation also showed significantly higher activity of AGPase, UGPase and cwInv, while this strain had unique effects on SuSy and vacInv activities, which were significantly stimulated. Lastly, CNT2 had a significant positive effect on the activity of cwInv and vacInv at the same stage compared with control DS plants. There were significant differences between G20-18, CNT1 and CNT2 inoculated plants in the activity of AGPase, vacInv and cytInv, while cwInv was the only enzyme significantly increased by all the three strains compared with non-inoculated DS plants (Table 1 and Fig. 5B).

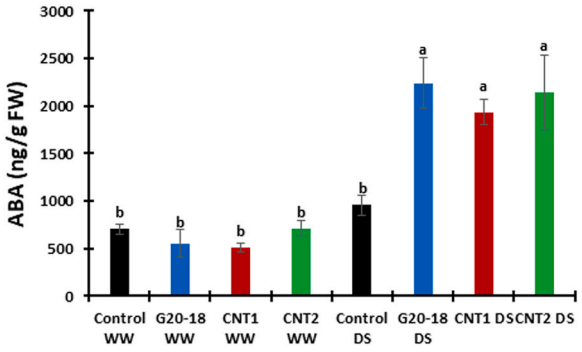


Fig. 4. ABA content of tomato leaf as influenced by different bacterial strains, G20-18, CNT1 and CNT2, under well-watered (WW) and drought stressed (DS) conditions. Different letters show significant differences at $p \leq 0.05$ by Tukey's HSD test.

3.3.2. Antioxidant metabolism

The activity of nine enzymes involved in antioxidant metabolism, specifically by detoxifying O^{-2} and H_2O_2 to H_2O or by maintaining the redox buffer condition, was determined based on the methodology described by Fimognari et al. (Fimognari et al., 2020), employing the same principle of a semi-high throughput approach and extracts, as for the carbohydrate metabolism enzymes (Jammer et al., 2015). The activity of enzymes was studied from leaf samples taken before DS, after DS and at the end of DS recovery. Data on each enzyme activity are presented in a table, with statistical significances highlighted in colour (Table 2). Before DS, G20-18 inoculated plants showed significantly higher activity of CAT and GR, but lower activity of DHAR than the control plants. CNT1 inoculated plants also showed significantly higher activity of CAT than non-inoculated control plants, while the increase caused by CNT2 inoculation was not significant. Before plants were stressed, there was a statistically significant difference between G20-18, CNT1 and CNT2 inoculated plants in the activity of GR and DHAR.

After DS, G20-18 inoculated plants showed significantly higher activity of SOD, POX and CAT than non-inoculated DS plants. CNT2 inoculated plants showed significantly higher activity of these three enzymes and in addition GR, cwPOX and APX at the same stage compared with control DS plants. After plants were stressed, there was a statistically significant difference between G20-18, CNT1 and CNT2 inoculated plants in the activity of cwPOX, SOD and MDHAR in WW plants and GR, POX, cwPOX, SOD and APX in DS plants (Table 2 and Fig. 5C). After DS recovery, G20-18 inoculated plants showed significantly higher activity of GR, CAT and DHAR and lower activity of SOD than non-inoculated DS plants. CNT2 inoculated plants showed significantly higher activity of GR and CAT, whereas CNT1 resulted in higher activities of CAT and DHAR than non-inoculated DS plants. After DS recovery, there was a statistically significant difference between G20-18, CNT1 and CNT2 inoculated plants in the activity of MDHAR and DHAR in WW plants and in the activity of DHAR in DS plants. CAT was the only enzyme whose activity was significantly increased by all the three strains in DS plants after recovery (Table 2 and Fig. 5D).

Based on the analysis of total non-enzymatic antioxidants (Table 2, Fig. 5C and D), all inoculated strains increased the total phenolic content before DS, but only CNT1 inoculated plants had a significantly higher phenolic content than the control plants. After DS, G20-18 and CNT1 inoculated plants showed a significantly higher anthocyanin content than non-inoculated DS plants. After DS recovery, G20-18 and CNT2 inoculated WW plants showed a significantly lower anthocyanin content. G20-18 inoculated DS plants showed significantly higher flavonoid levels than non-inoculated DS plants after recovery. There was a statistically significant difference between G20-18, CNT1 and CNT2 inoculated plants in flavonoid content before drought and after drought in WW and DS plants. Plants inoculated with G20-18, CNT1 and CNT2 showed significant increases in Trolox equivalent antioxidant capacity (TEAC) before DS and by trend, even when inoculated plants were exposed to DS. However, after DS recovery, no clear differences were observed between inoculated plants and non-inoculated control plants.

3.3.3. Multivariant analyses of cell physiological responses

To obtain a global view of the impact of inoculant on plant physiology, a principle component analyses (PCA) was performed for carbohydrate metabolism enzymes, antioxidant metabolism enzymes, total phenolic (Phen), flavonoid (Flav) and anthocyanin (Anth) content, and total antioxidant activity (TEAC) after DS (Fig. 6A) and DS recovery (Fig. 6B). For data obtained after DS, the analysis clearly showed that the values for G20-18 inoculated plants clustered separately from those for control plants. Values for the CNT1 and CNT2 inoculated DS plants also clustered separately. After DS recovery, the PCA showed distinct clustering of G20-18, CNT1, CNT2 and control plants as well. Hence, G20-18 plus the two mutant strains had each distinct and unique effects on plant cell physiology.

From the combined correlation analysis for carbohydrate and

Table 1
Summary table for activity of carbohydrate metabolism enzymes of tomato leaves as influenced by different bacterial strains under well-watered (WW) and drought-stressed (DS) conditions.

		FK	HXK	PFK	AGPase	UGPase	SuSy	cwInv	vacInv	cytInv
Before drought	Control	0.11 ± 0.055 (a)	0.04 ± 0.017 (a)	0.09 ± 0.05 (ab)	0.73 ± 0.118 (a)	1.58 ± 0.151 (ab)	0.19 ± 0.048 (b)	43.89 ± 7.913 (a)	13.975 ± 1.584 (a)	2.462 ± 0.827 (a)
	G20-18	0.25 ± 0.111 (a)	0.01 ± 0.001 (a)	0.149 ± 0.007 (a)	0.71 ± 0.087 (a)	1.88 ± 0.251 (a)	0.28 ± 0.108 (ab)	45.81 ± 7.221 (a)	8.977 ± 0.914 (b)	3.632 ±2.349 (a)
	CNT1	0.19 ± 0.056 (a)	0.03 ± 0.014 (a)	0.102 ± 0.02 (ab)	0.64 ± 0.103 (a)	1.68 ± 0.078 (ab)	0.56 ± 0.12 (a)	32.96 ± 3.821 (ab)	9.058 ± 1.714 (b)	0.548 ± 3.181 (b)
	CNT2	0.18 ± 0.05 (a)	0.02 ± 0.007 (a)	0.06 ± 0.007 (b)	0.62 ± 0.09 (a)	1.29 ± 0.087 (b)	0.20 ± 0.073 (b)	23.10 ± 5.587 (b)	6.389 ± 0.443 (b)	1.294 ± 1.645 (a)
After drought stress	Control WW	0.12 ± 0.009 (ab)	0.04 ± 0.013 (a)	0.021 ± 0.009 (b)	0.48 ± 0.021 (ab)	2.50 ± 0.125 (bc)	0.10 ± 0.121 (ab)	21.03 ± 1.297 (ab)	2.884 ± 1.18 (c)	0.108 ± 0.383 (bc)
	G20-18 WW	0.07 ± 0.025 (b)	0.06 ± 0.023 (a)	0.029 ± 0.004 (ab)	0.53 ± 0.061 (ab)	2.49 ± 0.17 (bc)	0.20 ± 0.052 (ab)	13.55 ± 4.175 (b)	2.14 ± 0.745 (c)	2.033 ± 0.582 (a)
	CNT1 WW	0.11 ± 0.005 (ab)	0.02 ± 0.008 (a)	0.006 ± 0.002 (b)	0.59 ± 0.057 (ab)	2.07 ± 0.014 (c)	0.00 ± 0.063 (b)	23.66 ± 2.083 (ab)	2.426 ± 0.947 (c)	3.242 ± 1.288 (a)
	CNT2 WW	0.14 ± 0.058 (ab)	0.03 ± 0.005 (a)	0.031 ± 0.012 (ab)	0.69 ± 0.027 (ab)	2.74 ± 0.259 (b)	0.02 ± 0.015 (b)	16.55 ± 4.968 (ab)	5.812 ± 0.736 (bc)	0.041 ± 0.265 (c)
	Control DS	0.11 ± 0.034 (ab)	0.03 ± 0.009 (a)	0.022 ± 0.013 (b)	0.44 ± 0.055 (b)	2.75 ± 0.328 (b)	0.03 ± 0.055 (b)	23.77 ± 9.066 (a)	6.363 ± 0.452 (bc)	0.298 ± 0.35 (bc)
	G20-18 DS	0.13 ± 0.004 (ab)	0.09 ± 0.07 (a)	0.06 ± 0.014 (a)	0.66 ± 0.125 (ab)	3.42 ± 0.198 (a)	0.15 ± 0.046 (ab)	25.89 ± 1.247 (ab)	7.309 ± 0.767 (bc)	3.645 ± 0.517 (a)
	CNT1 DS	0.08 ± 0.021 (b)	0.04 ± 0.035 (a)	0.03 ± 0.024 (ab)	0.71 ± 0.209 (ab)	3.76 ± 0.141 (a)	0.28 ± 0.114 (a)	31.48 ± 10.606 (a)	10.448 ± 4.49 (ab)	0.307 ± 1.182 (bc)
	CNT2 DS	0.21 ± 0.061 (a)	0.05 ± 0.022 (a)	0.022 ± 0.008 (b)	0.78 ± 0.169 (a)	3.87 ± 0.284 (a)	0.06 ± 0.053 (b)	31.49 ± 5.94 (a)	13.893 ± 1.496 (a)	0.729 ± 2.077 (b)
After drought stress recovery	Control WW	0.08 ± 0.022 (ab)	0.11 ± 0.049 (a)	0.021 ± 0.009 (b)	0.32 ± 0.03 (ab)	1.92 ± 0.127 (c)	-0.20 ± 0.111 (abc)	13.46 ± 1.378 (bc)	5.044 ± 0.682 (bc)	0.002 ± 0.042 (b)
	G20-18 WW	0.06 ± 0.03 (ab)	0.07 ± 0.041 (a)	0.014 ± 0.004 (b)	0.29 ± 0.054 (ab)	2.12 ± 0.085 (bc)	-0.09 ± 0.064 (abc)	13.80 ± 0.612 (bc)	1.548 ± 0.324 (c)	0.424 ± 0.056 (ab)
	CNT1 WW	0.12 ± 0.014 (ab)	0.08 ± 0.013 (a)	0.013 ± 0.001 (b)	0.31 ± 0.072 (ab)	2.14 ± 0.104 (bc)	0.31 ± 0.144 (ab)	22.16 ± 4.511 (ab)	6.156 ± 4.094 (bc)	0.049 ± 0.45 (b)
	CNT2 WW	0.03 ± 0.006 (b)	0.01 ± 0.005 (a)	0.039 ± 0.022 (ab)	0.24 ± 0.009 (ab)	2.11 ± 0.272 (bc)	-0.31 ± 0.175 (bc)	10.98 ± 2.984 (bc)	10.159 ± 3.001 (ab)	0.096 ± 0.014 (b)
	Control DS	0.12 ± 0.088 (ab)	0.15 ± 0.098 (a)	0.033 ± 0.003 (ab)	0.21 ± 0.036 (b)	2.01 ± 0.163 (c)	-0.42 ± 0.259 (c)	7.17 ± 2.176 (c)	6.024 ± 0.684 (bc)	0.075 ± 0.032 (b)
	G20-18 DS	0.19 ± 0.069 (a)	0.17 ± 0.09 (a)	0.029 ± 0.015 (ab)	0.35 ± 0.032 (a)	2.57 ± 0.125 (ab)	-0.13 ± 0.045 (abc)	28.93 ± 1.268 (a)	4.471 ± 0.424 (bc)	1.02 ± 0.517 (a)
	CNT1 DS	0.13 ± 0.038 (ab)	0.04 ± 0.026 (a)	0.03 ± 0.023 (ab)	0.34 ± 0.04 (a)	2.75 ± 0.246 (a)	0.49 ± 0.235 (a)	24.13 ± 11.062 (ab)	13.53 ± 0.745 (a)	0.141 ± 0.058 (b)
	CNT2 DS	0.10 ± 0.016 (ab)	0.04 ± 0.024 (a)	0.068 ± 0.019 (a)	0.20 ± 0.056 (b)	2.32 ± 0.199 (abc)	0.28 ± 0.531 (abc)	23.74 ± 4.27 (ab)	15.224 ± 2.538 (a)	0.129 ± 0.05 (b)

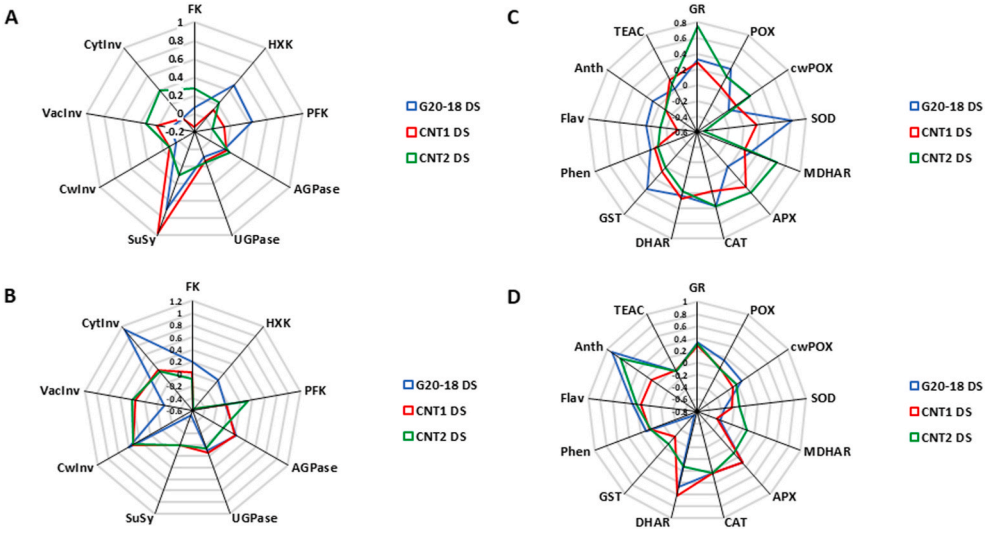


Fig. 5. Activity of carbohydrate metabolism enzymes in DS plants after drought stress (A) and after drought recovery (B). Antioxidant metabolism in DS plants after drought stress (C) and after drought stress recovery (D). The values used are based on the ratio of mean activity of the enzymes (nkat mg FW^{-1}) in response to the bacterial treatment versus the control, and indicated after log2 transformation in WW and DS plants respectively. UDP-glucose pyrophosphorylase (UGPase), fructokinase (FK), hexokinase (HXK), phosphofructokinase (PFK), ADP-Glucose pyrophosphorylase (AGPase), sucrose synthase (SuSy), cytoplasmic invertase (cytInv), vacuolar invertase (vacInv), cell wall invertase (cwInv), ascorbate peroxidase (APX), catalase (CAT), cytoplasmic peroxidases (POX), glutathione reductase (GR), superoxide dismutase (SOD), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione S transferase (GST) and cell wall peroxidases (cwPOX)).

antioxidant metabolism enzyme activities and physiological parameters after DS (Table 3) and after DS recovery (Table S1), several significant positive or negative correlations were found. After DS, the ABA content was positively correlated with total antioxidant activity (TEAC), anthocyanin, and phenolic contents, as well as with the activities of CAT, APX, POX, GR, vacInv, UGPase, HXK and PFK. Stomatal conductance was negatively correlated with the activities of CAT, POX, UGPase, HXK and PFK. Leaf chlorophyll content was positively correlated with phenolic, anthocyanin and flavonoid contents and the activities of DHAR, CAT, APX, cwPOX, cytInv, SuSy, HXK, PFK and AGPase. Relatively fewer correlations were found after DS recovery as ABA was negatively correlated with SuSy and cwInv activities. Stomatal conductance correlated positively with POX, cwPOX, GR and cytInv activities, and finally leaf chlorophyll content was positively correlated with the activities of CAT, POX, GR, cwInv and cytInv.

3.4. Global transcriptome analyses reveals major qualitative and quantitative differences in the impact of G20-18 in well-watered, drought and drought recovery conditions

Several mechanisms have been proposed for PGPR-mediated abiotic stress tolerance in plants, but little is known about the molecular regulatory mechanisms involved in mediating the drought tolerance induced or primed by PGPR. This study therefore addressed the differential gene expression response between bacterial strains G20-18 and the CK-deficient mutant strain CNT1 and *S. lycopersicum* interaction under drought stress using Illumina sequencing. RNA-seq libraries were generated from leaf tissue of *S. lycopersicum* plants with and without

Table 2
Summary table for activity of antioxidant metabolism enzymes of tomato leaves as influenced by different bacterial strains under well-watered (WW) and drought-stressed (DS) conditions.

		GR	POX	cwPOX	SOD	MDHAR	APX	CAT	DHAR	GST	Phen	Flav	Anth	TEAC
Before drought	Control	3.56 ± 0.637 (b)	1.136 ± 0.149 (ab)	0.654 ± 0.149 (ab)	55.67 ± 17.393 (a)	4.349 ± 0.558 (a)	0.212 ± 0.161 (a)	0.156 ± 0.045 (b)	36.82 ± 7.8 (a)	0.597 ± 0.137 (a)	8.06 ± 1.579 (b)	0.08 ± 0.021 (ab)	0.15 ± 0.02 (a)	0.09 ± 0.02 (b)
	G20-18	7.77 ± 1.374 (a)	1.396 ± 0.519 (ab)	0.706 ± 0.259 (ab)	42.03 ± 7.907 (a)	5.734 ± 1.152 (a)	0.987 ± 0.288 (a)	0.33 ± 0.053 (a)	4.22 ± 2.677 (b)	0.687 ± 0.220 (a)	11.32 ± 0.816 (ab)	0.06 ± 0.015 (b)	0.10 ± 0.03 (a)	0.15 ± 0.03 (ab)
	CNT1	4.19 ± 0.626 (b)	1.93 ± 0.429 (a)	1.194 ± 0.257 (a)	64.23 ± 4.664 (a)	5.612 ± 0.982 (a)	1.105 ± 0.37 (a)	0.338 ± 0.025 (a)	23.09 ± 3.054 (a)	0.320 ± 0.113 (a)	12.93 ± 0.619 (a)	0.11 ± 0.023 (ab)	0.16 ± 0.09 (a)	0.18 ± 0.001 (a)
	CNT2	3.81 ± 0.357 (b)	0.759 ± 0.117 (b)	0.536 ± 0.062 (b)	51.83 ± 4.608 (a)	4.753 ± 0.704 (a)	1.071 ± 0.481 (a)	0.253 ± 0.025 (ab)	36.26 ± 0.883 (a)	0.394 ± 0.076 (a)	11.26 ± 0.916 (ab)	0.12 ± 0.005 (a)	0.26 ± 0.021 (a)	0.18 ± 0.01 (a)
After drought stress	Control WW	2.83 ± 0.31 (b)	3.46 ± 0.659 (de)	1.094 ± 0.284 (cd)	28.39 ± 9.499 (b)	0.43 ± 0.241 (bc)	0.53 ± 0.187 (c)	0.03 ± 0.012 (c)	53.67 ± 3.468 (ab)	0.119 ± 0.045 (c)	14.48 ± 2.779 (ab)	0.09± 0.014 (cd)	0.04 ± 0.046 (ab)	0.33 ± 0.04 (ab)
	G20-18 WW	2.09 ± 0.194 (b)	3.58 ± 0.194 (de)	2.557 ± 0.286 (ab)	82.67 ± 7.265 (a)	0.40 ± 0.15 (bc)	1.38 ± 0.416 (bc)	0.04 ± 0.006 (bc)	34.78 ± 4.594 (abc)	0.418 ± 0.090 (abc)	10.61 ± 0.152 (c)	0.22 ± 0.019 (a)	0.04 ± 0.007 (b)	0.23 ± 0.04 (ab)
	CNT1 WW	1.36 ± 0.238 (b)	3.5 ± 0.247 (de)	1.728 ± 0.236 (bc)	77.22 ± 5,566 (a)	0.21 ± 0.052 (c)	0.81 ± 0.351 (c)	0.04 ± 0.015 (bc)	57.95 ± 1.259 (a)	0.434 ± 0.164 (ab)	11.69 ± 1.110 (bc)	0.07 ± 0.017 (d)	0.04 ± 0.045 (ab)	0.03 ± 0.07 (b)
	CNT2 WW	2.87 ± 1.434 (b)	1.66 ± 0.425 (e)	0.508 ± 0.063 (d)	28.43 ± 0.359 (b)	2.00 ± 1.502 (ab)	0.96 ± 0.554 (c)	0.06 ± 0.006 (bc)	24.25 ± 9.352 (c)	0.150 ± 0.035 (bc)	10.45 ± 0.264 (c)	0.14 ± 0.022 (bc)	0.0006 ± 0.008 (b)	0.01 ± 0.05 (b)
	Control DS	1.28 ± 0.523 (b)	5.35 ± 1.63 (cd)	1.768 ± 0.329 (bc)	23.22 ± 4.923 (bc)	0.81 ± 0.303 (abc)	1.31 ± 0.591 (bc)	0.06 ± 0.012 (bc)	28.40 ± 14.754 (bc)	0.206 ± 0.016 (abc)	14.72 ± 0.786 (ab)	0.13 ± 0.019 (bc)	0.09 ± 0.0005 (b)	0.30 ± 0.06 (ab)
	G20-18 DS	2.69 ± 0.219 (b)	10.97 ± 0.874 (a)	1.373 ± 0.777 (cd)	96.13 ± 4.546 (a)	1.07 ± 0.17 (abc)	1.28 ± 0.415 (bc)	0.14 ± 0.006 (a)	48.03 ± 8.521 (abc)	0.476 ± 0.071 (a)	16.10 ± 0.616 (a)	0.15 ± 0.022 (b)	0.11 ± 0.109 (a)	0.32 ± 0.08 (a)
	CNT1 DS	2.47 ± 1.278 (b)	6.01 ± 0.089 (c)	1.8 ± 0.478 (abc)	33.83 ± 10.955 (b)	0.89 ± 0.322 (abc)	2.86 ± 0.377 (ab)	0.09 ± 0.044 (ab)	53.13 ± 12.58 (ab)	0.244 ± 0.163 (abc)	13.94 ± 1.034 (abc)	0.06 ± 0.015 (d)	0.07 ± 0.094 (a)	0.43 ± 0.04 (a)
	CNT2 DS	7.18 ± 0.911 (a)	8.39 ± 0.557 (b)	2.928 ± 0.029 (a)	7.13 ± 2.326 (c)	2.50 ± 0.296 (a)	3.53 ± 1.148 (a)	0.14 ± 0.022 (a)	42.17 ± 10.003 (abc)	0.212 ± 0.114 (abc)	12.66 ± 0.937 (abc)	0.10 ± 0.009 (bcd)	0.07± 0.032 (ab)	0.38 ± 0.01 (a)
After drought stress recovery	Control WW	0.95 ± 0.173 (c)	1.83 ± 0.185 (cd)	0.673 ± 0.07 (cd)	53.34 ± 12.896 (ab)	2.53 ± 1.097 (ab)	0.34 ± 0.074 (a)	0.04 ± 0.008 (b)	52.33 ± 9.975 (a)	0.712 ± 0.184 (a)	12.81 ± 1.925 (a)	0.12 ± 0.026 (ab)	0.15 ± 0.113 (ab)	0.51 ± 0.04 (ab)
	G20-18 WW	2.33 ± 0.459 (abc)	1.73 ± 0.637 (cd)	0.687 ± 0.348 (cd)	30.43 ± 11.935 (b)	0.42 ± 0.171 (b)	0.42 ± 0.152 (a)	0.07 ± 0.005 (a)	27.34 ± 8.814 (bc)	0.367 ± 0.241 (ab)	12.75 ± 1.632 (a)	0.11 ± 0.015 (ab)	0.15 ± 0.048 (cd)	0.39 ± 0.05 (bc)
	CNT1 WW	1.82 ± 0.259 (abc)	1.51 ± 0.604 (d)	0.444 ± 0.148 (d)	29.15 ± 3.225 (b)	0.86 ± 0.194 (ab)	0.25 ± 0.14 (a)	0.07 ± 0.018 (a)	53.09 ± 12.156 (a)	0.287 ± 0.074 (ab)	13.21 ± 0.492 (a)	0.11 ± 0.020 (ab)	0.04 ± 0.146 (a)	0.36 ± 0.06 (bc)
	CNT2 WW	2.56 ± 0.371 (ab)	3.15 ± 0.426 (bc)	1.026 ± 0.056 (bcd)	53.31 ± 2.369 (ab)	4.27 ± 2.614 (a)	0.26 ± 0.134 (a)	0.05 ± 0.008 (ab)	13.40 ± 6.911 (c)	0.702 ± 0.248 (a)	11.40 ± 0.610 (a)	0.08 ± 0.006 (b)	0.08 ± -0.065 (d)	0.56 ± 0.09 (a)
	Control DS	1.42 ± 0.331 (bc)	3.55 ± 0.549 (ab)	1.376 ± 0.283 (ab)	63.68 ± 14.871 (a)	3.82 ± 1.787 (ab)	0.26 ± 0.13 (a)	0.04 ± 0.004 (b)	16.31 ± 7.245 (c)	0.432 ± 0.163 (ab)	10.88 ± 1.093 (a)	0.08 ± 0.022 (b)	0.03 ± 0.088 (abc)	0.42 ± 0.01 (abc)
	G20-18 DS	3.07 ± 0.635 (a)	4.86 ± 0.604 (a)	1.685 ± 0.231 (a)	27.81 ± 5.12 (b)	1.54 ± 0.568 (ab)	0.53 ± 0.112 (a)	0.07 ± 0.01 (a)	49.38 ± 7.216 (ab)	0.082 ± 0.032 (b)	13.92 ± 1.251 (a)	0.15 ± 0.028 (a)	0.24 ± 0.01 (abcd)	0.36 ± 0.04 (c)
	CNT1 DS	2.75 ± 1.006 (ab)	3.62 ± 0.034 (ab)	1.121 ± 0.053 (abc)	37.60 ± 2.317 (ab)	1.31 ± 0.583 (ab)	0.55 ± 0.216 (a)	0.07 ± 0.007 (a)	67.36 ± 6.41 (a)	0.240 ± 0.103 (ab)	11.85 ± 0.955 (a)	0.11 ± 0.018 (ab)	0.04 ± 0.006 (bcd)	0.38 ± 0.01 (bc)
	CNT2 DS	2.94 ± 0.199 (a)	3.42 ± 0.573 (ab)	1.321 ± 0.251 (ab)	48.97 ± 6.471 (ab)	4.38 ± 0.873 (a)	0.33 ± 0.047 (a)	0.07 ± 0.011 (a)	21.66 ± 5.306 (c)	0.353 ± 0.168 (ab)	11.72 ± 0.993 (a)	0.13 ± 0.016 (ab)	0.16 ± 0.021 (abcd)	0.37 ± 0.05 (bc)

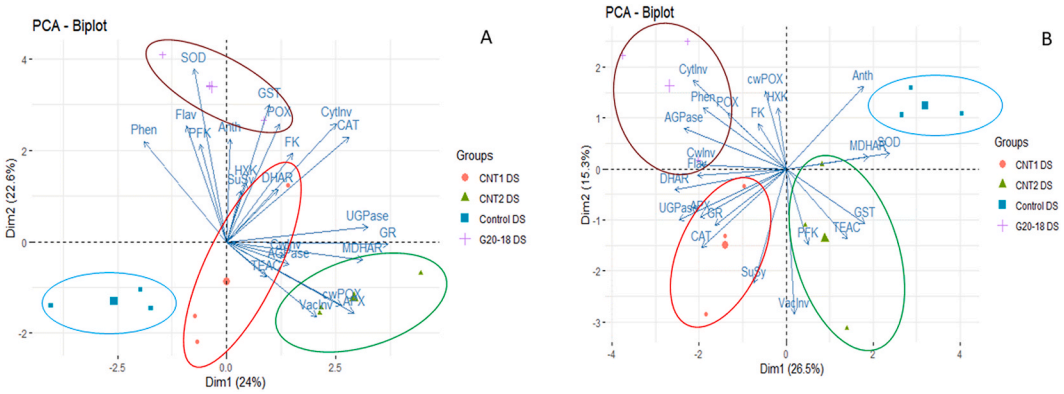
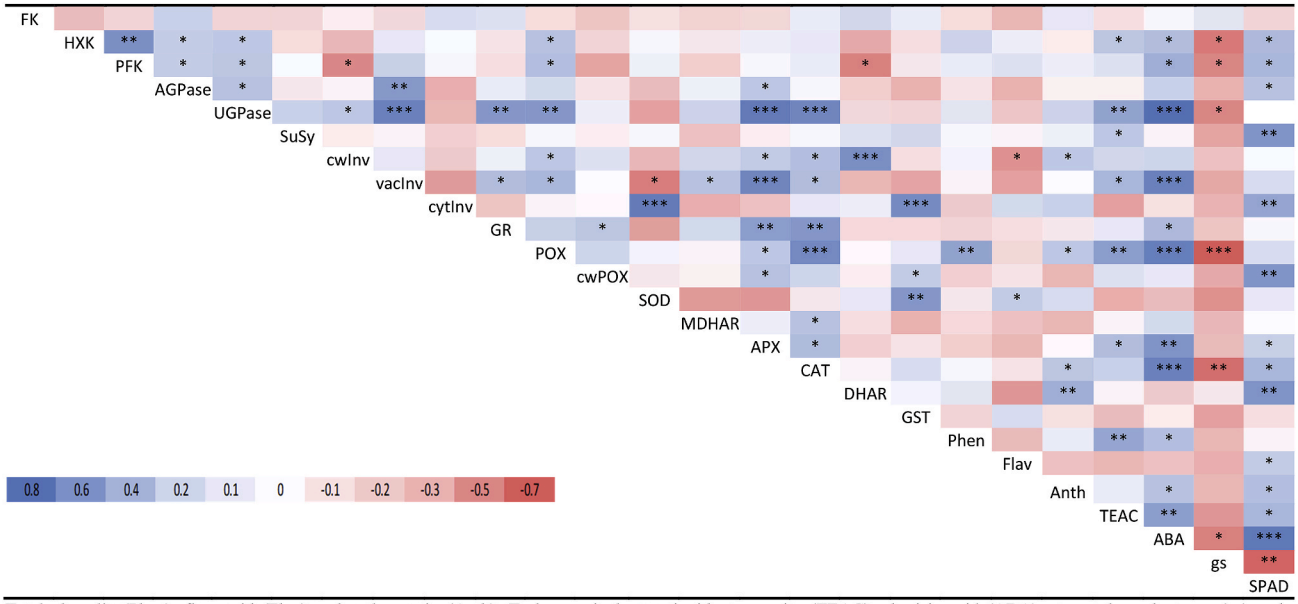


Fig. 6. Biplot of combined principal component analysis carbohydrate metabolism enzymes, antioxidant metabolism enzymes and total phenolic (Phen), flavonoid (Flav) and anthocyanin (Anth) content and Trolox equivalent antioxidant capacity (TEAC) in plants after drought stress (A) and after drought stress recovery (B).

G20-18 and CNT1 inoculations from well-watered plants (pre-drought stress) and plants subjected to drought stress and drought stress recovery. The libraries were mapped with the *S. lycopersicum* genome database for the identification of differentially expressed genes (DEGs). The expression study of G20-18 inoculated plants revealed the upregulation and downregulation respectively of 320 and 227 genes in pre-drought treated seedlings, 1581 and 2242 genes in DS plants, 1134 and 641 genes in DS recovered plants, when compared with the corresponding non-inoculated controls (Fig. 7). The expression studies of plants inoculated with the CK deficient CNT1 mutant showed a much lower number of regulated genes compared with the wild type strain G20-18. The data showed upregulation and down-regulation in response to CNT1 of only 36 and 49 genes in pre-drought treated plants, 1078 and 531 genes in DS plants, 512 and 989 genes in DS recovered plants, respectively, compared with their non-inoculated controls (Fig. 7). The analysis revealed 270 and 209 differentially regulated genes in G20-18 and CNT1 inoculated pre-drought treated seedlings, respectively, when they were compared with each other, while differentially regulated genes increased in DS plants (305 and 451 genes) and the smallest number of differentially regulated genes (202 and 178) were observed in DS recovered plants, respectively, when G20-18 and CNT1 inoculated strains were compared (Fig. 8A–C). To explore the interaction between the studied treatments as well as the number of differentially regulated gene numbers of different samples, a PCA was performed. The PCA analysis revealed that the differentially regulated gene numbers in drought stressed and inoculated seedlings separated into different clusters, whereas the cluster of pre-drought and drought-recovered seedlings fell into the same cluster (Fig. 8D). These findings highlight the ability of the gram-negative bacterium G20-18 to produce the plant hormone CK, which is of particular relevance in DS conditions and thus for improving the abiotic stress resilience of the host plant, which is apparently related to a priming effect of G20-18 in mitigating drought stress and enhancing the resilience of tomato. A substantial difference in the number of genes regulated by the wild type and CNT1 mutant was observed under well-watered conditions (ca. sixfold higher than the mock control) in contrast to around a twofold difference in DS and DR, indicating that only 50 % of DS and DR specific gene regulation depends on CK. The list of genes retrieved from the KEGG PATHWAY database was used to elucidate the biological processes that are regulated in response to G20-18 and CNT1 in well-watered plants, DS plants and

Table 3
Combined Pearson correlation for carbohydrate and antioxidant metabolism enzymes and physiological parameters after drought stress (DS).



Total phenolic (Phen), flavonoid (Flav) and anthocyanin (Anth), Trolox equivalent antioxidant capacity (TEAC), abscisic acid (ABA), stomatal conductance (gs) and chlorophyll content (SPAD), UDP-glucose pyrophosphorylase (UGPase), fructokinase (FK), hexokinase (HXK), phosphofructokinase (PFK), ADP-glucose pyrophosphorylase (AGPase), sucrose synthase (SuSy), cytoplasmic invertase (cytInv), vacuolar invertase (vacInv), cell wall invertase (cwInv), ascorbate peroxidase (APX), catalase (CAT),

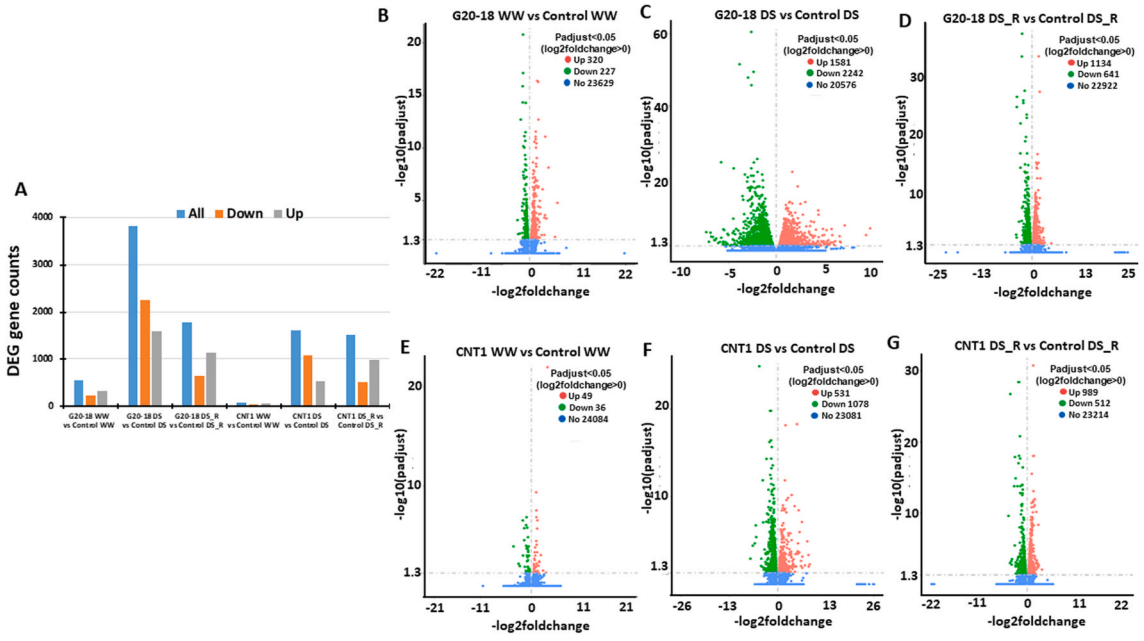


Fig. 7. Comparison of differentially expressed genes. (A) The number of all differentially expressed genes as well as the number of differentially expressed genes that are upregulated and downregulated in inoculated and non-inoculated control plants in well-watered (pre-drought, WW), drought-stressed (DS) and drought stress recovery (DS_R) conditions. Volcano plot for differentially expressed genes in response to G20-18 (B, C, D) and CNT1 (E, F, G). X-axis represents the fold change in gene expression between different samples; the y-axis shows the statistical significance of the differences. Significantly upregulated and downregulated genes are highlighted in red and green respectively. Genes that did not express differently between the treatment group and control group are shown in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

plants that have recovered from DS. G20-18 inoculated DS plants showed downregulation in a comprehensive set of genes of photosynthesis, photosynthesis antenna proteins, porphyrin and chlorophyll metabolism, carbon fixation in photosynthetic organisms, glyoxylate and dicarboxylate metabolism, carbon metabolism, carotenoid biosynthesis pathways and phenylalanine metabolism. Except for phenylalanine metabolism, all these genes were upregulated in G20-18 inoculated DS recovered plants along with sulfur metabolism, fructose and mannose metabolism, glycine, serine and threonine metabolism, pentose phosphate pathway, biosynthesis of amino acids, glycolysis/gluconeogenesis, nitrogen metabolism and aminoacyl-tRNA biosynthesis. Except for phenylalanine metabolism, the same pathways were

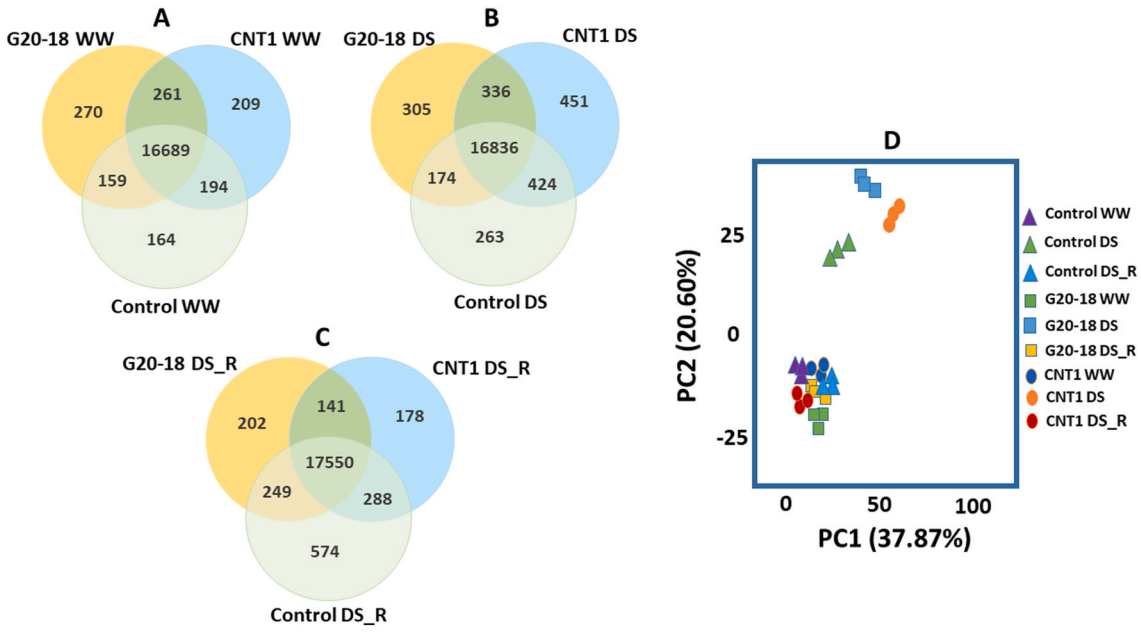


Fig. 8. Venn diagrams representing the number of unique and common number of genes in G20-18 and CNT1 inoculated and non-inoculated control plants under well-watered (A), drought-stressed (B) and drought stress recovery conditions (C). (D) Principal component analyses (PCA) on variables related to the gene number (proportion) of different samples.

downregulated in CNT1 inoculated DS plants as in G20-18 inoculated plants (Table 4). All these pathways were upregulated in CNT1 inoculated drought-recovered plants along with glycolysis/gluconeogenesis, fructose and mannose metabolism, nitrogen metabolism, biosynthesis of amino acids, pentose phosphate pathway and aminoacyl-tRNA biosynthesis pathways. Genes of flavonoid biosynthesis along with phenylpropanoid biosynthesis, steroid biosynthesis, stilbenoid, diarylheptanoid and gingerol biosynthesis, alpha-linolenic acid metabolism were upregulated in CNT1 inoculated drought-stressed plants. During the following DS recovery, an upregulation in CNT1 inoculated plants was observed only in flavonoid biosynthesis and circadian rhythm.

4. Discussion

4.1. Growth promotion by G20-18 in well-watered conditions

This study shows that inoculation of tomato with the CK-deficient mutants CNT1 or CNT2 led to even better promotion of shoot and root growth than the corresponding wild type strain G20-18. This finding disproves the hypothesis that CK production by root rhizosphere bacteria positively affects the growth of tomato and might be related to the inhibitory effects of higher concentrations of CK on root growth and the recently shown effect of CK on the microbiome composition (Gupta et al., 2022). Moreover, the finding indirectly supports the ability of *Pseudomonas* strains to produce CK in direct association with plant roots in the presence of root exudates (Karnwal and Kaushik, 2011) and thus affecting the host plant and possibly also the microbiome composition. These results are in overall agreement with the results of a previous study revealing that inoculation with G20-18 resulted in only a small stimulation of wheat root growth (Volkmar and Bremer, 1998).

In plant cell physiological responses of the host plants overall, there were some significant differences in the enzyme activity signatures between G20-18, CNT1 and CNT2 inoculated plants, as G20-18 inoculation resulted in higher activities of PFK, UGPase, cwInv and cytInv. Similarly, there was a statistically significant difference between G20-18, CNT1 and CNT2 inoculated plants in the activity of CAT, GR and DHAR. There was also a statistically significant difference between G20-18, CNT1 and CNT2 inoculated plants in flavonoid content in inoculated plants. G20-18 increased the activity of carbohydrate metabolism enzymes, which are required to balance source to sink relationships for improved plant growth, development and yield (Mekureyaw et al., 2021; Uematsu et al., 2012). The enhanced activity of the different antioxidant metabolism enzymes and metabolites helps remove excess ROS enzymatically and non-enzymatically (Garcia-Lemos et al., 2020; Mekureyaw et al., 2021). Thus a specific role of the ability of G20-18 to produce the plant hormone CK on different aspects of the host plant physiology under well watered conditions could be functionally established.

The RNA sequencing gene expression study of G20-18 inoculated plants revealed the upregulation and down-regulation of 320 and 227 genes in WW plants, respectively, compared with the corresponding non-inoculated controls (Fig. 7). The expression studies of plants inoculated with the CK-deficient CNT1 mutant showed a much lower number of regulated genes compared with the wild type strain G20-18. In WW plants the expression of only 36 genes is upregulated and of 49 genes is downregulated compared with their respective non-inoculated controls. These results further support a specific role of the CK production by the bacterial strain G20-18 for mediating the various beneficial effects. Transcript analysis has shown that the growth promotion effect of *Pseudomonas simiae* WCS417 in Arabidopsis (*Arabidopsis thaliana*) is associated with upregulation of the sugar transporter gene families SUS3 and SUS4 encoding for sucrose synthase, and CWIN6 encoding for cwInv (Desrut et al., 2020).

4.2. Ecophysiological changes under drought stress

The wild type strain G20-18 and the CK-deficient mutants increased leaf chlorophyll content in both WW and DS plants, including after DS recovery. There were no consistent significant differences between the effects of G20-18 and the mutant strains, indicating that CK production is not involved in this specific response. Other *Pseudomonas* strains were also shown to increase chlorophyll content under DS, as shown for *Pseudomonas* sp. (AG-54) in wheat (Mutumba et al., 2018), and hence improve the plant defence mechanisms against DS (Brilli et al., 2019; Farooq et al., 2009; Mukhtar et al., 2020). ABA plays a pivotal role in regulating stomatal movement and mediating responses under DS

Table 4
Pathways represented by significantly upregulated and downregulated differentially expressed genes annotated for their function in the Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY database.

G20-18 WW vs control WW (upregulated)		
KEGGID	Description	GeneRatio
sly00100	Steroid biosynthesis	11|81
sly00900	Terpenoid backbone biosynthesis	6|81
sly03010	Ribosome	12|81
G20–18 DS vs control DS (upregulated)		
no gene		
G20–18 DS_R vs control DS_R (upregulated)		
sly00195	Photosynthesis	29|310
sly00196	Photosynthesis - antenna proteins	15|310
sly00710	Carbon fixation in photosynthetic organisms	25|310
sly01200	Carbon metabolism	47|310
sly00860	Porphyrin and chlorophyll metabolism	18|310
sly00630	Glyoxylate and dicarboxylate metabolism	22|310
sly00920	Sulfur metabolism	10|310
sly00051	Fructose and mannose metabolism	13|310
sly00260	Glycine, serine and threonine metabolism	13|310
sly00030	Pentose phosphate pathway	11|310
sly01230	Biosynthesis of amino acids	28|310
sly00010	Glycolysis/Gluconeogenesis	18|310
sly00906	Carotenoid biosynthesis	7|310
sly00910	Nitrogen metabolism	6|310
sly00970	Aminoacyl-tRNA biosynthesis	9|310
G20–18 WW vs control WW (downregulated)		
sly00500	Starch and sucrose metabolism	9|59
sly00592	alpha-Linolenic acid metabolism	5|59
sly04712	Circadian rhythm - plant	5|59
sly00591	Linoleic acid metabolism	3|59
sly00860	Porphyrin and chlorophyll metabolism	4|59
G20–18 DS vs control DS (downregulated)		
sly00195	Photosynthesis	33|475
sly00196	Photosynthesis - antenna proteins	17|475
sly00860	Porphyrin and chlorophyll metabolism	21|475
sly00710	Carbon fixation in photosynthetic organisms	22|475
sly00630	Glyoxylate and dicarboxylate metabolism	20|475
sly00906	Carotenoid biosynthesis	11|475
sly01200	Carbon metabolism	45|475
sly00360	Phenylalanine metabolism	11|475
G20–18 DS_R vs control DS_R (downregulated)		
sly03008	Ribosome biogenesis in eukaryotes	11|131
sly04712	Circadian rhythm - plant	6|131
CNT1 WW vs control WW (upregulated)		
no gene		
CNT1 DS vs control DS (upregulated)		
sly00940	Phenylpropanoid biosynthesis	14|134
sly00100	Steroid biosynthesis	7|134
sly00941	Flavonoid biosynthesis	6|134
sly00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	5|134
sly00592	alpha-Linolenic acid metabolism	6|134
CNT1 DS_R vs control DS_R (upregulated)		
sly00710	Carbon fixation in photosynthetic organisms	27|281
sly01200	Carbon metabolism	46|281
sly00195	Photosynthesis	19|281
sly00860	Porphyrin and chlorophyll metabolism	17|281
sly00630	Glyoxylate and dicarboxylate metabolism	20|281
sly00196	Photosynthesis - antenna proteins	8|281
sly00010	Glycolysis/Gluconeogenesis	20|281
sly00051	Fructose and mannose metabolism	12|281
sly00910	Nitrogen metabolism	7|281
sly01230	Biosynthesis of amino acids	26|281
sly00030	Pentose phosphate pathway	10|281
sly00970	Aminoacyl-tRNA biosynthesis	10|281
CNT1 WW vs control WW (downregulated)		
sly04712	Circadian rhythm - plant	2|6
CNT1 DS vs control DS (downregulated)		
sly00195	Photosynthesis	30|268
sly00196	Photosynthesis - antenna proteins	12|268
sly00860	Porphyrin and chlorophyll metabolism	18|268
sly00710	Carbon fixation in photosynthetic organisms	15|268
sly00630	Glyoxylate and dicarboxylate metabolism	14|268
sly01200	Carbon metabolism	31|268
sly03010	Ribosome	28|268
sly00906	Carotenoid biosynthesis	7|268

Table 4 (*continued*)

G20-18 WW vs control WW (upregulated)		
CNT1 DS_R vs control DS_R (downregulated)		
sly04712	Circadian rhythm - plant	6|92
sly00941	Flavonoid biosynthesis	5|92

KEGGID represents the unique identification ID of the KEGG database. Description represents the function description of pathway and GeneRatio represents the ratio of differentially expressed genes to all genes concerned with this pathway.

(Kapoor et al., 2020; Saradadevi et al., 2017). The present study showed that ABA content increased significantly in inoculated DS plants, with no significant difference between plants inoculated with G20-18 and the CK-deficient mutants. After DS, plants inoculated with G20-18 and the CK-deficient mutants showed decreased stomatal conductance compared with non-inoculated plants whereas the effect was more pronounced in the CNT1 and CNT2 mutants. The increased ABA content in inoculated DS plants coincided with decreased stomatal conductance, coordinating the plant response for DS tolerance (Danish et al., 2020; Kumari et al., 2016; Vurukonda et al., 2016). As seen for the bacterial impact on chlorophyll content, the effect on ABA content and was independent of CK production, whereas the CK production apparently contributes to more pronounced effect on stomata conductance which thus seem to be ABA independent.

4.3. Cell physiological changes under drought conditions

In contrast to the above findings for ecophysiological parameters, bacterial CK production had even more pronounced effects on plant carbohydrate and antioxidative metabolism enzymes. To the best of the authors' knowledge, such complex biosignatures for the response of tomato to CK producing beneficial microbes during growth, development and abiotic stress have not previously been described. The observed distinct effects on the activities of several enzymes of carbohydrate and antioxidative metabolisms support a broad and systemic relevance of bacterial CK production on plant holobiont physiology, the physiology that reflect the responses of a plant in combination with their associated microbes.

Central carbohydrate metabolism enzymes are involved in different pathways of the plant, capturing photosynthetic energy and providing the carbon needed to produce new tissues and to also sustain growth of associated microbes (Johnson and Alric, 2013). In DS plants, G20-18 inoculation significantly increased the activities of PFK, UGPase and cytInv, and after DS recovery AGPase, UGPase, cwInv and cytInv activities increased. This study reveals that plants inoculated with G20-18 or mutants with reduced CK production showed an increased activity of the glucose sensor HXK (Granot et al., 2013). Catalysing the irreversible reaction of glucose phosphorylation, HXK is important for regulating plant metabolism and sugar signalling (Pérez-Díaz et al., 2021). In DS plants, the wild type G20-18 increased the activity of PFK, an enzyme that catalyses the phosphorylation of fructose-6-phosphate to fructose-1, 6-bisphosphate (Mustroph et al., 2013). Phosphorylation of hexose monomers in glycolytic pathways is one of the key reactions in sucrose catabolism in plants. The activity of AGPase, which catalyses the rate-limiting step of starch biosynthesis, is increased by G20-18 and CK-deficient mutants after DS and also DS recovery (Saripalli and Gupta, 2015). The increased AGPase activity not only enhances starch synthesis, but also increases the final grain yield in transgenic cereals (Hannah et al., 2012; Lin et al., 1988; Wang et al., 2007). There were also the same pronounced effects on the activities of SuSy and UGPase in DS inoculated plants. UGPase catalyses the interconversion of starch and sucrose responsible for the synthesis of UDP-glucose, which is an important precursor in the biosynthesis and regulation of plant cell wall components, including cellulose, hemicellulose and pectin (Baron et al., 2016).

Invertases are key metabolic enzymes regulating sink activity through the hydrolytic cleavage of sucrose into hexose monomers, thus playing a crucial role in plant growth and development (Roitsch and Gonzalez, 2004). The present study showed that plant adaptation to DS can be markedly improved in tomato by G20-18 and CK-deficient CNT mutants. The increase in sink strength through cwInv activity in plants is a general response under DS conditions (Albacete et al., 2015). Thus, cwInv works as a pivotal enzyme at the integration point of metabolic, hormonal and stress signals (Albacete et al., 2015). Higher invertase activity of young tomato fruits, and increased sucrose import into them, could contribute to their heat tolerance by increasing sink strength and sugar signalling activities (Li et al., 2011). Among enzymes that hydrolyse sucrose and alter carbohydrate partitioning, the present results showed that invertases are affected during plant DS and recovery. As DS causes large yield losses in crops due to a reduction in starch accumulation (Zhang et al., 2016), increasing the activities of different carbohydrate metabolism enzymes will help balance growth and plant defence. Sucrose cleavage and subsequent phosphorylation of hexoses will be facilitated by those enzymes that are increased due to bacterial inoculation in DS tomato plants.

The induction of ROS-scavenging enzymes is the most common mechanism for detoxifying ROS synthesised during DS (Sarker and Oba, 2018). G20-18 significantly decreased DHAR activity and significantly increased the activities of GR and CAT before DS treatment. However, for DS plants, inoculation with G20-18 significantly increased the activities of POX, SOD and CAT, and after DS recovery GR, CAT and DHAR activities were increased. GR plays a key role in the defence system by sustaining the reduced status of glutathione (GSH) (Farooq et al., 2009; Hasanuzzaman et al., 2019). The present study shows that tomato plants inoculated with G20-18 and CK-deficient mutants increased the activities of POX and cwPOX. Previous studies also report that increased activities of POX and cwPOX catabolise excess H_2O_2 and are linked to lignification and thickening of the cell wall as well as plant defences against DS (Santos et al., 2018).

The major breakdown product of SOD is H_2O_2, which is a toxic lipid peroxidant, but it can be eliminated by the activities of CAT and POX (Hasanuzzaman et al., 2020). APX is the key antioxidant enzyme in the ascorbate-glutathione redox cycle, which plays an important role in scavenging ROS and chloroplast protection, and there is significant increased activity by CNT2 (Hasanuzzaman et al., 2019). To maintain the antioxidative capacity of AsA (Asada–Halliwell pathway), rapid regeneration of AsA is regulated by DHAR and MDHAR (Xie et al., 2019). Activation of DHAR in these stress responses has been observed in different plants (Hasanuzzaman et al., 2019; Xie et al., 2019). In transgenic tobacco, overexpression of DHAR protects plants against DS (Mukhtar et al., 2020). SOD and CAT activities are increased and H_2O_2 and lipid peroxidation levels are decreased in DS cowpea plants inoculated with *P. graminis* (Santos et al., 2018). Brilli et al. (2019) reported that *P. chlororaphis* inoculated tomatoes show enhanced tolerance to DS with increased activity of SOD and CAT. Generally, the coordinated activities of different antioxidative enzymes help either detoxify or scavenge ROS and mitigate their deleterious effects. During DS, increased source activity explained by changes in metabolic fluxes and hormonal and redox status suggest that the physiological mechanisms regulated by invertase play a key role in drought stress adaptation in inoculated plants.

In the present study, increased phenol, flavonoid and anthocyanin contents in wild type G20-18 and CK-deficient mutant-inoculated tomato increasing the potential to protect the plant from oxidative stress by eliminating excessive ROS in a non-enzymatic manner. Increases in phenolic accumulation are essential for maintaining the osmotic potential of plant tissues and thereby protecting them from the negative impacts of dehydration during DS (Isah, 2019). Anthocyanin accumulation has also been associated with the production of different osmolytes that are known to contribute to the detoxification of ROS under stress (Cirillo et al., 2021). For comparison, Lingua et al. (2013) reported that inoculation of plant growth-promoting bacterial strains and mycorrhizal fungi on strawberry plants led to high levels of anthocyanin. Silva et al. (2014) show that aqueous extracts of pepper leaves, after bacterial inoculation with the rhizobium strain TVP08, result in a significant increase in flavonoids. Singh et al. (2018) show that buckwheat inoculated with *Azospirillum* spp. and *Azotobacter* spp. produces increased concentrations of flavonoid and phenolic compounds. G20-18 and CK-deficient mutants support the tomato plant to increase the total antioxidant capacity (TEAC), which can have a cumulative effect against ROS produced during DS. Bacterial CK apparently are negatively affecting this process, since the effect was more pronounced in the CK deficient CNT1 and CNT2 mutants. Similar findings have recently been reported, i.e. a higher antioxidant capacity in *Pseudomonas fluorescens* WCS417 and *Bacillus amyloliquefaciens* GB03 inoculated peppermint plants compared with non-inoculated controls under DS (Chiappero et al., 2019).

The PCA global overview showed a distinct clustering of G20-18 and CK-deficient mutant treatments after DS and DS recovery. This confirmed that G20-18 as well as the two mutant strains had distinct and unique effects on plant cell physiological responses to progressive DS. Many of the carbohydrate metabolism enzymes tested were positively correlated with the different ecophysiological parameters measured after DS and DS recovery, further supporting the general impact of the presence of the bacteria. The positive and negative correlation of enzymatic and non-enzymatic antioxidant mechanisms with different ecophysiological parameters, e.g. ABA, stomatal conductance and SPAD, indicate the impact of both the wild type and CNT mutant strains against progressive DS in tomato.

4.4. The ability of G20-18 to produce CK contributes to prime for a more pronounced global transcriptional regulation in response to drought stress

The present study employed Illumina RNA sequencing technology to profile the transcriptome changes in the leaf tissue of G20-18 inoculated and non-inoculated *S. lycopersicum* plants before drought, after DS and after DS recovery. The genome-wide gene expression analyses led to the identification of distinct qualitative and quantitative differences in relation to the sets of genes in the PGPR-inoculated and non-inoculated control plants (Figs. 7 and 8). It was evident that the most pronounced differential impact of G20-18 was under drought stress, with an increase from 547 regulated genes under well-watered conditions by ca. 700 % to 3823 regulated genes under drought. After one week of DS recovery, the number of regulated genes declined by more than 54 %. This result indicated that inoculation with G20-18 primed the plant for a fast and transient response to possible abiotic stress such as drought, and thus contribute to resource-efficient plant resilience. Major differences in the number genes regulated by G20-18 and its CK-deficient mutant were roughly halved under drought conditions (3823 versus 1609 regulated genes). These results indicated that the ability to produce this plant hormone contributes to the beneficial effects of G20-18 under stressed conditions. This specific interkingdom signalling role of the ability of the plant beneficial bacterium to produce the plant hormone cytokinin to mediate the plant abiotic stress response was also evident from specific cell and ecophysiological parameters. Carbohydrate metabolism is central for catabolism and anabolism. Changes in the expression of genes involved in the carbohydrate metabolism thus contribute to sustain drought stress responses (Min et al., 2016). In the present study, genes encoding starch biosynthesis and degradation showed decreased transcript abundance in G20-18 inoculated plants, while steroid and terpenoid biosynthesis showed increased transcript abundance. Steroids regulate antioxidant enzyme activities, chlorophyll content, photosynthetic capacity and carbohydrate metabolism to increase plant growth under stress (Anwar et al., 2018). Flavonoid content changes with the perception of both biotic and abiotic stresses. Bearing this in mind, flavonoid synthesis, transport and allocation could be assumed to be hallmarks of an adaptive metabolism to exert protective and modulatory

effects (Petrussa et al., 2013). In the present study, genes encoding flavonoid biosynthesis enzymes showed increased transcript abundance in CNT1-inoculated drought-stressed plants compared with non-inoculated controls. Since flavonoids have antioxidant properties, these findings suggested that tomato responded to a water deficit in the presence of PGPR by increasing its antioxidant capacity, but further exploration of the pathway for regulating flavonoid biosynthesis under drought stress is required. Phenylpropanoid biosynthesis has been demonstrated to contribute to various aspects of plant abiotic responses and synthesis of phenylpropanoid-based polymers, such as lignin and flavonoids (Vogt, 2010). The drought-induced alteration in the phenylpropanoid biosynthesis pathway in *A. mongolicus* leaves contributes to cell wall modification by affecting lignin biosynthesis, regulates anthocyanin biosynthesis by influencing anthocyanidins formation, and promotes plant cuticle formation by providing cutin (Gao et al., 2015). Lastly, further verification of the functional relevance of the candidate genes identified in this study and their regulatory circuitry will help to identify additional factors essential for the outcome of interactions between plants and PGPR. In the longer term, these novel insights should help develop innovative and environmentally-friendly PGPR-based strategies for crop protection and productivity improvement, and contribute to more widespread sustainable agricultural production.

5. Conclusions

The results of the present study show that *P. fluorescens* G20-18 root inoculation had a significant positive impact on growth promotion in tomato. They reveal that G20-18 can contribute to also a broad spectrum of stress responses that are also effective against DS. The results in DS tomato plants showed that root colonisation with G20-18 induced both physiological and metabolic changes, notably a) a higher chlorophyll content, b) decreased stomatal conductance, c) systemic alteration of the ABA content, d) increased activity of carbohydrate metabolism enzymes, e) increased antioxidant metabolism enzymes, f) increased total antioxidant capacity and accumulation of secondary metabolites, and g) profound quantitative and qualitative changes in gene expression. G20-18 primed tomato plants for enhanced tolerance to DS by improving the antioxidant enzyme activity that avoids excessive accumulation of ROS. In addition, upon DS, they increased ABA levels to a greater extent, thus allowing more efficient modulation of stomatal closure. Distinct differences between the impact of the wild type G20-18 and the CK-deficient CNT1 and CNT2 mutants demonstrate a functional role of the bacterial production of the plant hormone cytokinin and thus interkingdom hormone signalling in mediating the beneficial effects. The most pronounced differences were evident from the global transcriptome analyses. The RNAseq analyses revealed that the majority of profound changes in gene expression under both WW and DS conditions depended on the ability to produce CK by wild type G20-18 compared with the CK-deficient mutants CNT1. Differences between the impact of G20-18 and the CK mutants CNT1 and CNT2 were also evident from the stimulation of growth and specific cell- and ecophysiological parameters. Thus, these findings extend the previous finding that CK produced by G20-18 is not only relevant for conferring pathogen resistance (Akhtar et al., 2019; Grosskinsky et al., 2016), but also important in mediating abiotic stress responses via metabolic and gene regulation. The integration of cell physiological phenotyping via the semi-high-throughput determination of enzyme activity signatures (Fimognari, Lorenzo et al., 2020; Großkinsky et al., 2015, 2018; Jammer, Alexandra et al., 2015) and antioxidant potential in a 96-well microtitre plate format with ecophysiological measurements proved to be suitable for linking growth and stress responses to the underlying metabolic processes within a holistic phenomics approach (Großkinsky et al., 2015, 2018). Future research should focus on investigating the role of PGPR in priming plant stress resilience further and specifically studying the interaction of these bacteria with tomato plants deficient in the above-mentioned enzymatic and non-enzymatic production.

Funding source

The work of MF, ON and TR was supported by the European Union's Horizon 2020 research and innovation programme (MiRA project) under grant agreement No. 765290. The work of CP and TR was supported by NordForsk (NordPlant project) under grant agreement No. 84597. The work of TR was supported by the Ministry of Education, Youth and Sports of the Czech Republic as part of the National Sustainability Programme I (NPU I), grant number LO1415.

CRediT authorship contribution statement

Mengistu F. Mekureyaw: Methodology, Investigation, Writing – original draft, review & editing. **Chandana Pandey:** Methodology, Investigation, Writing – original draft, review & editing. **Rosanna C. Hennessy:** technical advice, Writing – review & editing. **Mette H. Nicolaisen:** technical advice, Writing – review & editing. **Fulai Liu:** Supervision, Writing – review & editing. **Ole Nybroe:** Conceptualization, Supervision, Writing – review & editing. **Thomas Roitsch:** Conceptualization, Supervision, Writing – review & editing.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jplph.2022.153629.

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